JUSTUS-LIEBIG-
UNIVERSITÄT
GIESSEN

Investigating the Role of Microbiologicals (*Bacillus Species*) in the Biodegradation of Residues of Chemical Fungicides

Dissertation zur Erlangung des Doktorgrades (Dr. rer. nat.) im Fachbereich Agrarwissenschaften, Ökotrophologie und Umweltmanagement der Justus-Liebig-Universität Gießen,
durchgeführt am Institut für Phytopathologie

vorgelegt von:
M. Sc. Farnaz Loew

Justus-Liebig-Universität
Gießen, Juli 2023

Mit Genehmigung des Fachbereichs Agrarwissenschaften, Ökotrophologie und Umweltmanagement der Justus-Liebig-Universität Gießen

Prüfungskommission:

1. Gutachter(in):	Prof. Dr. Patrick Schäfer
2. Gutachter(in):	Prof. Dr. Philip Lane
Prüfer(in):	Prof. Dr. Sylvia Schnell
Prüfer(in):	Prof. Dr. Rolf-Alexander Düring

Erklärung gemäß der Promotionsordnung des Fachbereichs 09 vom 07. Juli 2004 § 17 (2)

„Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe.

Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht.

Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.“

_____ _____

Ort, Datum Unterschrift der Doktorandin/des Doktoranden

Acknowledgment

I would like to express my deepest appreciation to Prof. Dr. Lane, my mentor and supervisor throughout my master's and PhD studies. Without his generous provision of knowledge and expertise, I would not have been able to embark on this journey. His unwavering commitment to high standards has undoubtedly played a crucial role in shaping me into a better researcher.

I would also like to extend my thanks to Prof. Dr. Kogel, the former head of the Phytopathology Department, for granting me the opportunity to work in this Institute and for the continuous support provided throughout this research.

I am sincerely grateful to BASF for their financial support of this project, as well as my industrial supervisors at BASF for their invaluable advice and guidance. Their involvement has greatly enriched my research experience.

Lastly, I would be remiss in not mentioning my family, especially my parents and spouse. Their belief in my abilities has been a constant source of encouragement, keeping my spirits high and my motivation unwavering throughout this challenging process.

I am truly grateful to all those who have contributed to my academic and personal growth. Their support and guidance have also played an invaluable role in shaping my journey, and for that, I am thankful.

Summary

This work investigated the feasibility of finding bacteria able to degrade chemical fungicides and also act as biofungicides. Accordingly, bacterial isolates from samples of plant material from sites where fungicide trials had been carried out with multiple sprays of one fungicide were cultured.

Enhanced growth of isolates in the presence of the fungicide was sought in screening tests. This resulted in six isolates from Revysol® sites and 9 from boscalid sites being selected for identification and further characterization. All the strains identified were from the Genus *Bacillus (B. megaterium, B. velenzensis, and B. licheniformis)* and all were in the lowest risk group.

Following the selection of isolates for identification, the growth dynamics of the selected isolates in the absence of any fungicide were examined, and the growth curves obtained were attributed to one of the three typical curves. The isolates segregated into these growth curve types consistent with the species identification.
Despite the initial assumption, the degradation ability of the bacterial isolates was not fungicide specific. Furthermore, the enhanced growth of the isolates was restricted to a certain range of fungicide concentrations, with an optimum of 5 ppm for all four fungicides.

The selected isolates identified as *B. velenzensis* functioned particularly well as biofungicides, significantly inhibiting the growth of all three fungal pathogens (*Botrytis*, *Cercospora*, and *Sclerotinia*) tested. Furthermore MBI 600 the commercial biofungicide, in addition to showing biofungicide activity on all three pathogens as expected also demonstrated the capacity to degrade all four of the fungicides used in this study.

Zusammenfassung

In dieser Studie wurde die Machbarkeit untersucht, Bakterien zu finden, die sowohl in der Lage sind, chemische Fungizide abzubauen als auch als Biofungizide zu wirken. Hierfür wurden bakterielle Isolate aus Pflanzenmaterialproben von Standorten kultiviert, an denen Fungizidversuche mit mehreren Sprühungen eines bestimmten Fungizids durchgeführt wurden.

In Screening-Tests wurde nach einer erhöhten Wachstumsrate der Isolate in Gegenwart des Fungizids gesucht. Aufgrund dieser Tests wurden sechs Isolate von Revysol® Standorten und neun Isolate von Boscalid-Standorten für die Identifizierung und weitere Charakterisierung ausgewählt. Alle identifizierten Stämme gehörten zur Gattung Bacillus (*B. megaterium, B. velenzensis und B. licheniformis*) und wurden als geringes Risiko eingestuft.

Nach der Auswahl der Isolate zur Identifizierung wurde die Wachstumsdynamik der ausgewählten Isolate in Abwesenheit jeglicher Fungizide untersucht. Die erhaltenen Wachstumskurven wurden einem der drei typischen Kurventypen zugeordnet, um die Isolate entsprechend ihrer Art zu klassifizieren. Entgegen der anfänglichen Annahme zeigte sich, dass die Abbaufähigkeit der bakteriellen Isolate nicht spezifisch für bestimmte Fungizide war. Darüber hinaus war das gesteigerte Wachstum der Isolate auf einen bestimmten Bereich von Fungizidkonzentrationen beschränkt, wobei eine optimale Konzentration von 5 ppm für alle vier Fungizide festgestellt wurde.

Die als *B. velenzensis* identifizierten Isolate erwiesen sich als besonders effektive Biofungizide, da sie das Wachstum aller drei getesteten Pilzpathogene (*Botrytis, Cercospora* und *Sclerotinia*) signifikant hemmten. Zusätzlich zeigte das kommerzielle Biofungizid MBI 600 neben der erwarteten biofungiziden Wirkung auf alle drei Pathogene auch die Fähigkeit, alle vier in dieser Studie verwendeten Fungizide abzubauen.

TABLE OF CONTENTS

1. Introduction and Objective

1.1. Population Growth and Food Demand

In 1962 the population growth reached the peak with the annual growth rate of 2.1 %, which has slowed subsequently to 1.1% by 2015. It will grow until 2100 however the rate of growth has been estimated to drop to 0.1% annually. The world's population reached 7.8 billion in 2020 reaching eventually to 11.2 billion by 2100 (Fig. 1)

The increased population will be accompanied by a need for increased food and thus an increase in agricultural production. The FAO in 2012 estimated that an increase in agricultural production of 60% of the production in 2005/7 would be required to feed the expected population of 9.1 billion in 2050 (in value terms). The global population mid-2006 was 6.6 billion (Statista, PRB Population data sheet). With this as a base the population increase by 2050 would be ca. 38%. The higher rate of increase in food production in relation to the population increase is a result of increased urbanisation of the population, improved living standards and dietary changes following improvements in income to a level many multiples of today's earnings (Food and Agriculture Organization (FAO)).

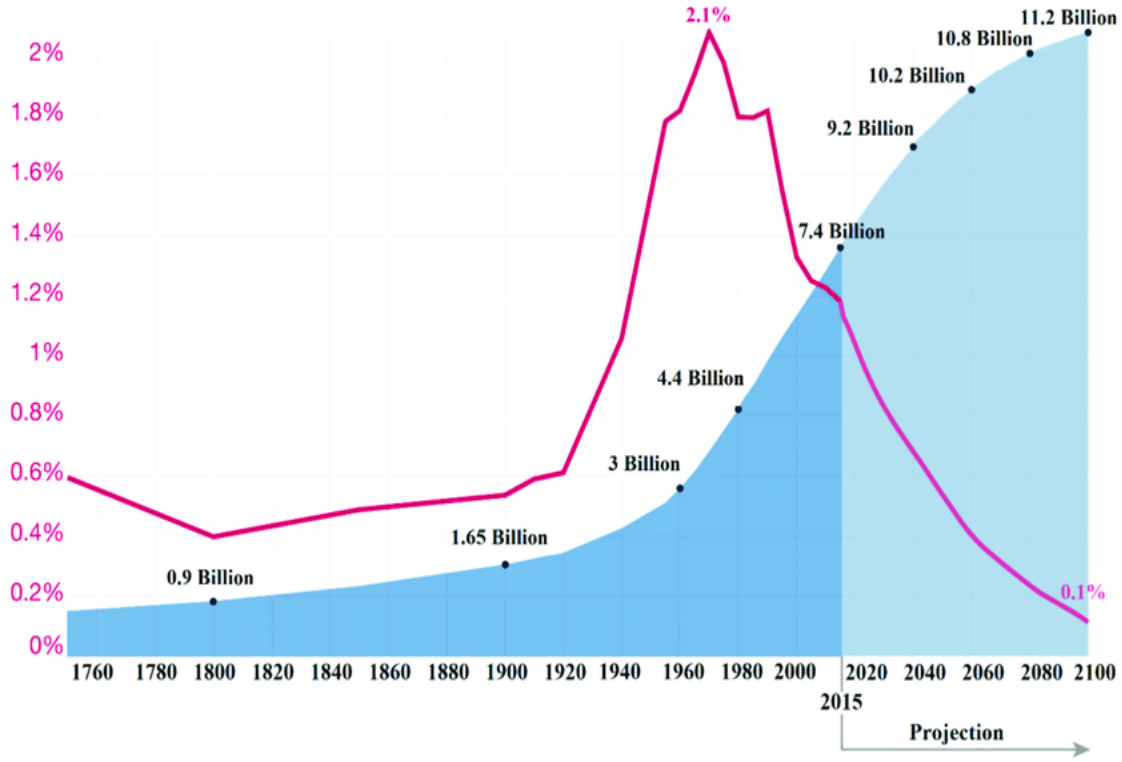


Figure 1.1. World population and growth rate, 1750–2015, and projections until 2100 (Ákos Mesterházy, 2020).

1.2. Agricultural Productivity Outlook.

1.2.1. Cultivated Area

Globally, agricultural land area is approximately 5 billion hectares, one third of which is used as cropland and the rest is pasture and meadow for livestock (Food and Agriculture Organization (FAO))(Fig. 1.2).

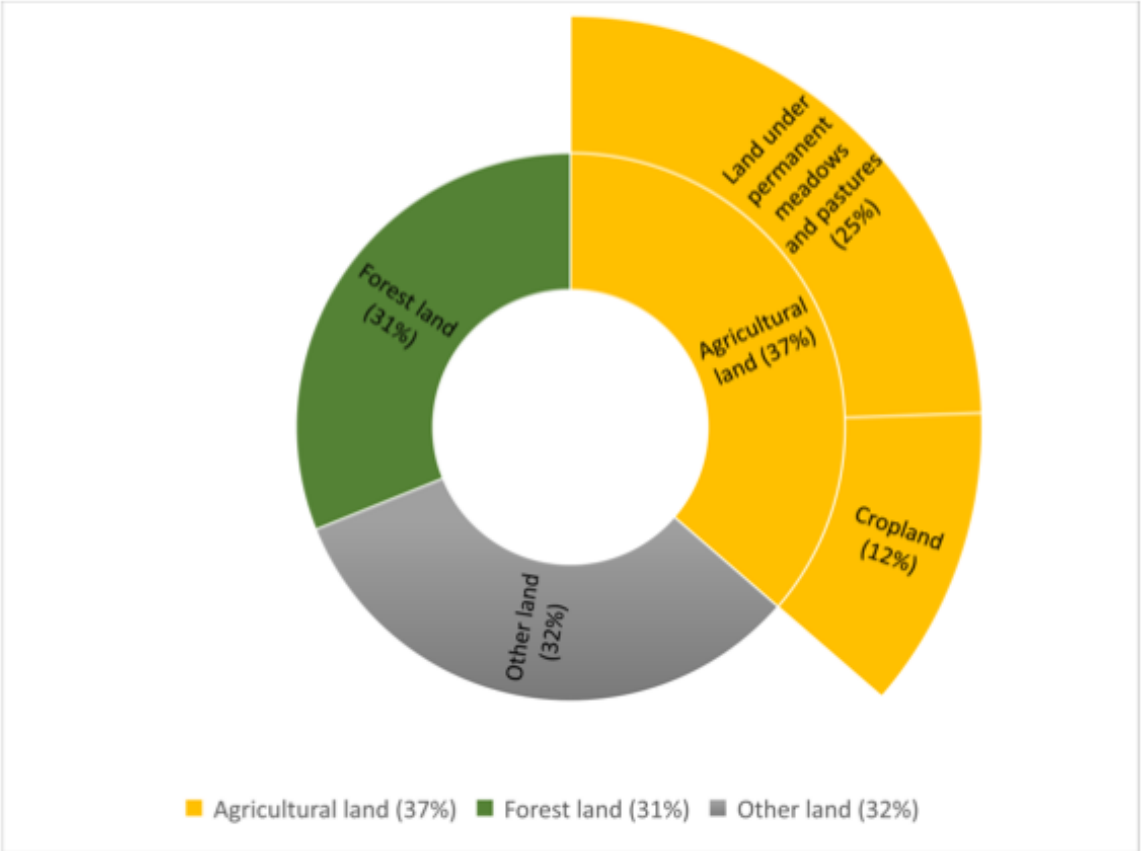


Figure 1.2. Global land area composition in 2019 (Food and Agriculture Organization (FAO)).

Despite an increase in the land used for agricultural production until the early 2000s, overall, between 1990 and 2019, the agricultural land area decreased by 1 percent (Food and Agriculture Organization (FAO)). There is very little available land that will be brought into production between now and 2050. About 100 million hectares in South Saharan Africa could be utilised but 38 million hectares in developed countries are expected to be taken out of production, so overall a net gain of 62 million hectares could be envisaged. Considering 1539 million hectares are in production today, that means there is an increase in available hectares of 4% against a population increase of 38% (Nikos Alexandratos and Jelle Bruinsma, 2012); (FAO).

Considering this in relation to the population, the area of agricultural land per capita decreased by 30% over the past several decades, from ca. 0.9 per capita to ca. 0.6 ha per capita. Roughly

one-third of this land is used for crop production. So clearly with only a 4% increase in the area of land available for crop production and the need to feed a population which will increase by 38% by 2050, then an increase in crop productivity will be necessary (Food and Agriculture Organization (FAO)); (Fig. 1.3).

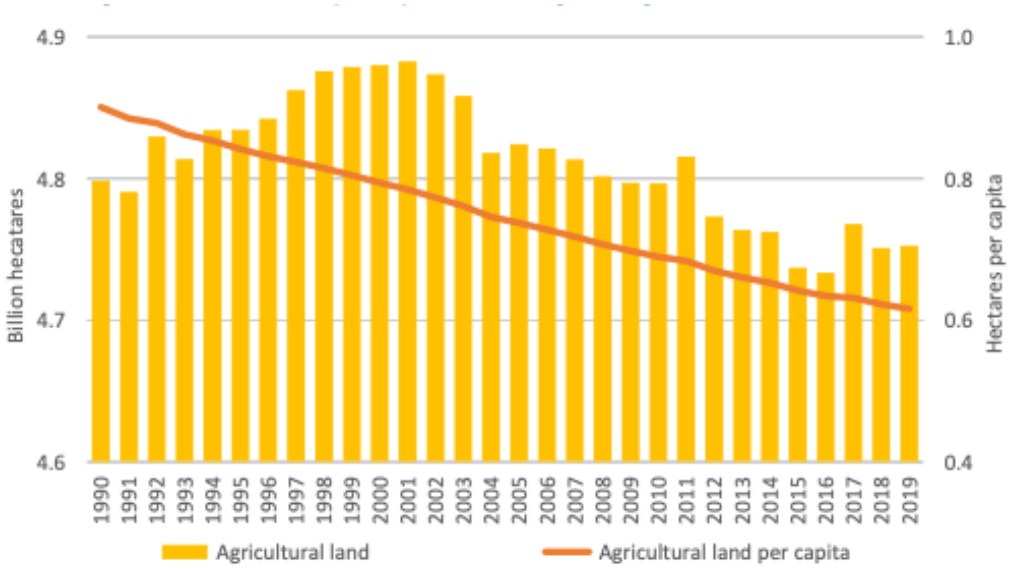


Figure 1.3. Absolute and per capita areas of global agricultural land, 1990–2019 (Food and Agriculture Organization (FAO)).

1.2.2. Crop Productivity

The significant increased demand for more food by 2050 as a result of the estimated 9-billion-plus population is a particular challenge. Providing more food is not easier than in the past. Greater stress on water and land resources, increasing soil degradation and the salinization of irrigated areas together with competition from land uses other than food production are all factors that heighten the challenge. Increases in crop yields have slowed down considerably. For example, world cereal production is projected to grow at 0.9 percent per year from 2005/2007 to 2050, down from the 1.9 percent per year of 1961-2007 and oil crops and their products by 1.9 percent per year from 2005/2007 to 2050. However, even with this reduced rate of increase, world production, which increased by 1,225 million tonnes between 1961/63 and 2005/2007, is projected to increase by another 940 million tonnes in the next 44 years, to reach 3 billion tonnes by 2050 (Fig. 1.4).

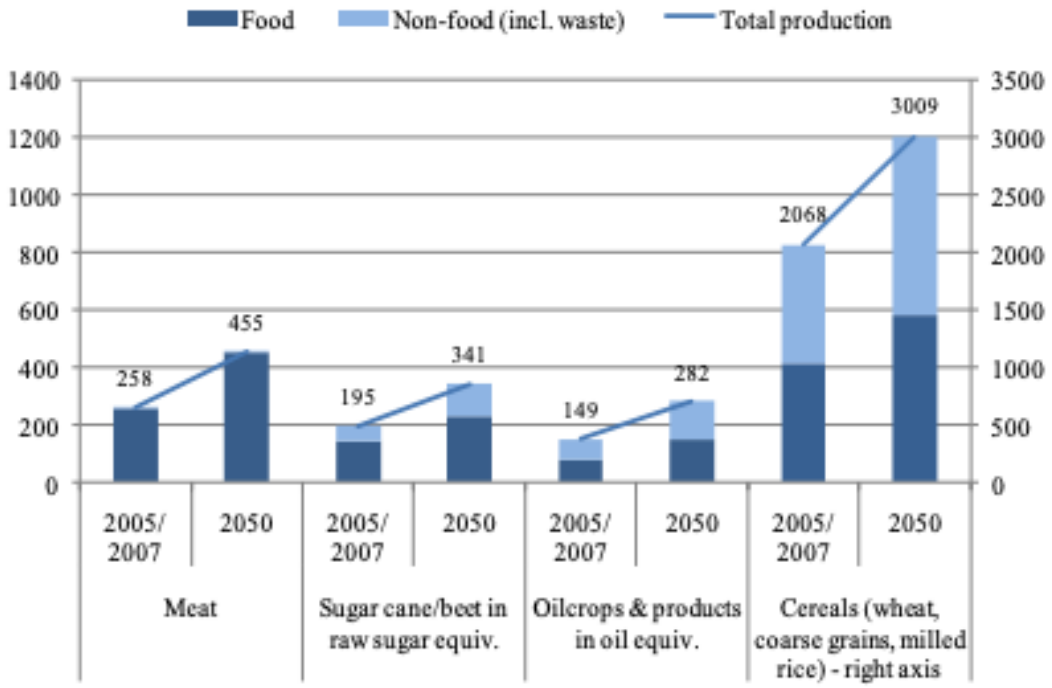


Figure 1.4. World population and use of major agricultural products (million tonnes) (Bruinsma, 2012).

1.2.3. Crop Losses

Crop losses are usually defined as the reduction in either quantity or quality of yield (Schein, 1979)and these may be caused by abiotic and biotic factors, leading to the reduction in crop productivity through lower yields (Oerke, 2006) (Fig. 1.5).

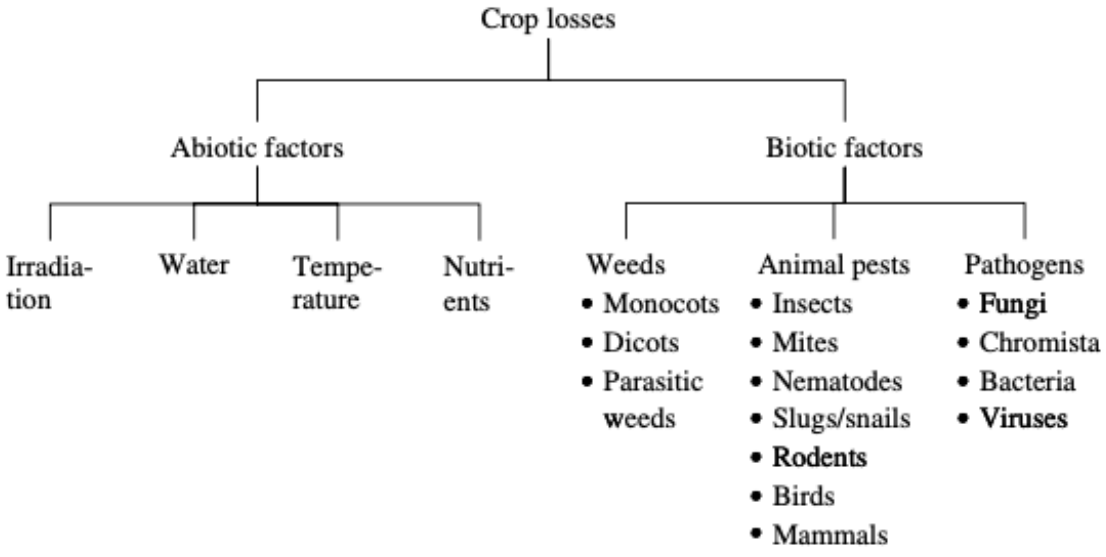


Figure 1.5. Abiotic and biotic factors causing crop losses (Oerke, 2006).

Losses can occur at any stage of crop production in the field (preharvest) or even during storage (postharvest) (Oerke, 2006). One significant cause of yield loss however is that caused by

pathogens, animals (insects) and weeds which together are responsible for 20–40% loss (Ákos Mesterházy, 2020) (Fig. 1.6).

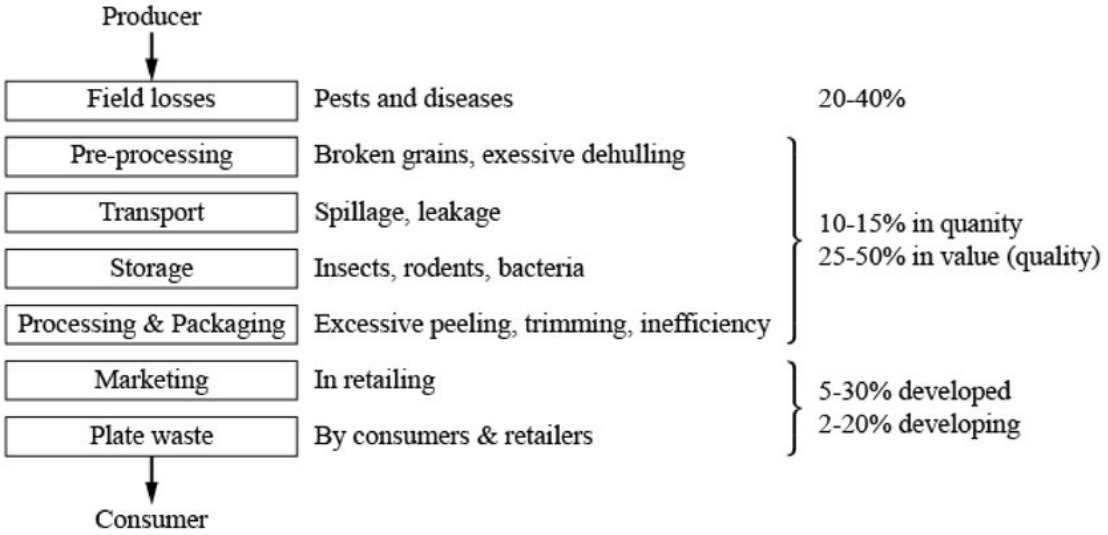


Figure 1.6. Losses along the food chain (Ákos Mesterházy, 2020).

Oerke (2006). estimated the potential and actual losses in the current crop protection practices for wheat, rice, maize, potatoes, soybeans, and cotton for the period 2001–03 on a regional basis (19 regions) as well as for the global total. He found that, the total global potential loss due to pests varied from about 50 % of the potential yield in wheat to more than 80 % in cotton (Fig. 1.7) (Elkhouly, 2021).

Crop protection measures reduced these losses to 26–29 % of the potential yield for soybean, wheat and cotton, and 31, 37 and 40 % for maize, rice and potatoes, respectively. Overall, weeds produced the highest potential loss (49.8 %), with animal pests and pathogens responsible for losses of 18 and 16%. Weed control can be managed mechanically or chemically, therefore worldwide efficacy was considerably higher than for the control of animal pests or diseases, which rely heavily on synthetic chemicals. Oerke estimated that crop protection measures reduced these potential losses by 74, 39 and 32% for weed competition, insect, and disease damage respectively (Elkhouly, 2021).

Clearly if agriculture is challenged with increasing its production per unit area to feed the ever-increasing population in the future, minimising crop losses to weed competition, insects and diseases is critical to this goal. As illustrated above crop protection measures based largely on chemicals are able to reduce these losses considerably. Nonetheless we are still losing over 10% of the potential yield to each of these biotic factors and furthermore there is considerable pressure on the use of agrochemicals the predominant means of controlling such losses (Oerke, 2006).

Crop	Attainable production [M t]	Crop losses [%][1] due to									
		Weeds		Animal pests		Pathogens		Viruses		Total	
		Potential	Actual	Potential	Actual	Potential	Actual	Potential	Actual	Potential	Actual
Wheat	785·0	23·0 (18–29)	7·7 (3–13)	8·7 (7–10)	7·9 (5–10)	15·6 (12–20)	10·2 (5–14)	2·5 (2–3)	2·4 (2–4)	49·8 (44–54)	28·2 (14–40)
Rice	933·1	37·1 (34–47)	10·2 (6–16)	24·7 (13–26)	15·1 (7–18)	13·5 (10–15)	10·8 (7–16)	1·7 (1–2)	1·4 (1–3)	77·0 (64–80)	37·4 (22–51)
Maize	890·8	40·3 (37–44)	10·5 (5–19)	15·9 (12–19)	9·6 (6–19)	9·4 (8–13)	8·5 (4–14)	2·9 (2–6)	2·7 (2–6)	68·5 (58–75)	31·2 (18–58)
Potatoes	517·7	30·2 (29–33)	8·3 (4–14)	15·3 (14–20)	10·9 (7–13)	21·2 (20–23)	14·5 (7–24)	8·1 (7–10)	6·6 (5–9)	74·9 (73–80)	40·3 (24–59)
Soybeans	244·8	37·0 (35–40)	7·5 (5–16)	10·7 (4–16)	8·8 (3–16)	11·0 (7–16)	8·9 (3–16)	1·4 (0–2)	1·2 (0–2)	60·0 (49–69)	26·3 (11–49)
Cotton	78·5[2]	35·9 (35–39)	8·6 (3–13)	36·8 (35–41)	12·3 (5–22)	8·5 (7–10)	7·2 (5–13)	0·8 (0–2)	0·7 (0–2)	82·0 (76–85)	28·8 (12–48)

[1] Figures in parentheses indicate variation among 19 regions.
[2] Seedcotton.

(Elkhouly, 2021)
Fig.1.7. Estimated loss potential of weeds, animal pests (arthropods, nematodes, rodents, birds, slugs, and snails), pathogens (fungi, bacteria) and viruses, and actual losses due to pest groups in six major crops worldwide, in 2001–03.

1.3. Chemical Crop Protection

In 1960 the crop production market valued less than $10 bn with one hundred active ingredients. Today its value is estimated at $50 bn with six hundred active ingredients (Phillips McDougall Evolution of the Crop Protection Industry since 1960, November 2018). Over the years many types of chemical crop protection products have been developed so that today's products come from 40 different chemical groups each with different modes of action which are crucial where resistance to particular modes of action has developed.

However, although the number of new approved products has decreased recently, R&D investment remains high, and companies have been introducing innovation to chemical production process in addition to integrated crop solutions, application technology, and precision agriculture (Phillips McDougall, 2018) (Fig. 1.8).

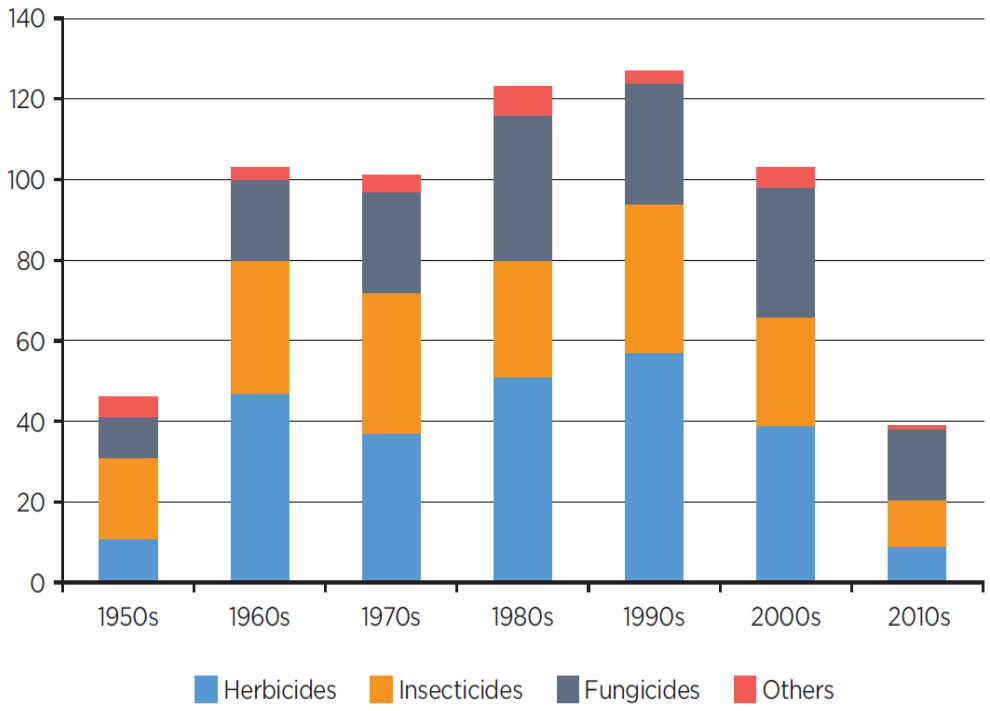


Figure 1.8. Number of new active ingredients introduced per decade: 1950s to present day. (Phillips McDougall, 2018).

To keep up with increasing safety regulations, many products have been withdrawn from the market because they were either banned or were not supported during a re-registration process. This can be observed by comparing the top ten chemical crop production products in 1994 and those that are approved in 2016 in the US. (Fig. 1.9).

Top 10 products in 1968	Top 10 products in 2016
Atrazine	Glyphosate
Toxaphene – ***banned***	Metolachlor
DDT – ***banned****	Pyraclostrobin
2,4-D	Mesotrione
Methyl parathion – ***banned***	Thiamethoxam
Aldrin – ***banned***	Acetochlor
Trifluralin	Azoxystrobin
Propachlor	Atrazine
Dinoseb – ***banned***	Abamectin
Chloramben – ***banned***	Clothianidin

Source: Fernandez-Cornejo et al; Phillips McDougall

*DDT is banned as an agricultural and household pesticide, but it is still allowed for vector control in some countries when locally safe, effective and affordable alternatives are not available.

Figure 1.9. Top 10 products used in major US crops by volume in 1968 and 2016 (Phillips McDougall, 2018).

In the EU there are more stringent data requirements. The introduction of hazard cut-off criteria, and the application of complex procedures for risk assessment have resulted in fewer registered products in the EU and many of those already on the market might never reach the new demanded standards.

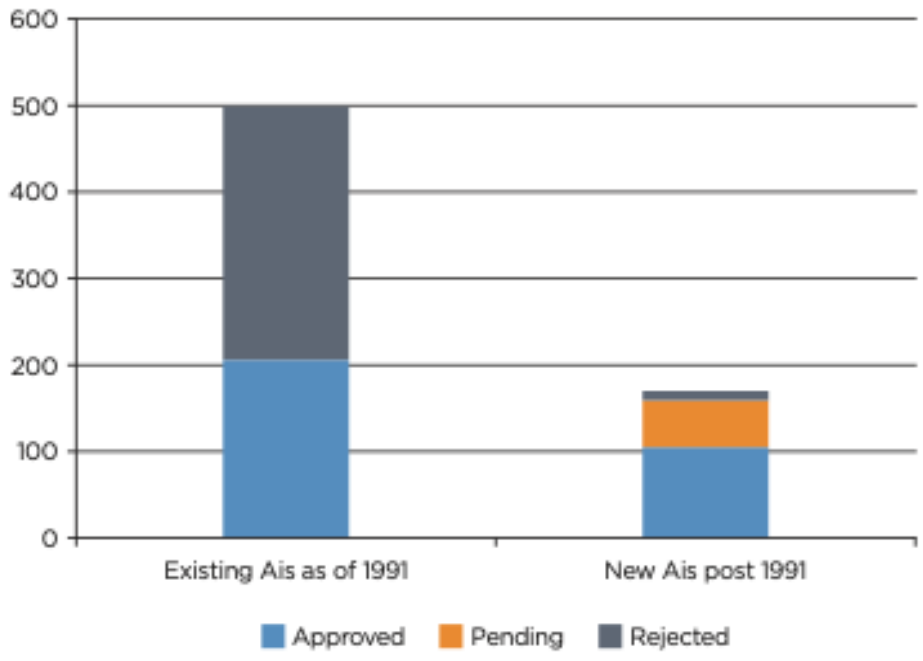


Figure 1.10. Impact of EU re-registration on active ingredients (Phillips McDougall, 2018).

The industry has focused on producing the crop production ingredients with the improved efficacy and effectiveness of product so that now there has been a significant reduction in

application rate. Therefore, the farmer needs lower doses of the product to achieve to the same efficacy (Fig. 1.10).

Despite the increased regulatory aspects required for pesticide registrations, their efficacy is noteworthy. Pesticides offer a fast-acting and highly effective way to prevent yield loss. Sometimes chemical control is the only current option against a particular pest. Moreover, without crop protection chemicals, the food production would decline, many fruits and vegetables would be in short supply and prices would rise. Pesticides also bring high-quality foods with no pest contamination to the customer. However, overuse or continuous use of pesticides causes resistant pathogens to develop and if their resistance is genetically based, it can be carried to the next generation so that the population will be more resistant over the time. Also, whether real or imagined, public concern over risk posed by pesticides and other chemicals used in food production can translate into very real effects in the marketplace.

In general, there are three underlying dimensions of pesticide attitudes. Two very clear dimensions reflecting attitudes toward the "safety" and the "necessity'' of pesticide used and a less distinct dimension is "trust" in the food industry's use of pesticides. As mentioned before, a considerable number of regulatory rules have been introduced over time, which makes the registration more complicated than before but introduces safe products into the market. However, the pessimistic public point of view about the residue of chemical crop products in food and the resistance development of pathogens resulted from being subject to the crop protection products over time were two factors influencing the adoption of a new research area focusing on residue-free applications such as biopesticides.

1.4. Biologicals

According to the Environmental Protection Agency (EPA), biopesticides are defined as naturally occurring pest control substances. They are classified into three groups (S.R.Joshi, 2006).

a. Microbial pesticides: in which a microbial living organism (bacteria, fungi, viruses, protozoans) is the active control agent.

b. Plant pesticides: pesticidal substances produced by plants from introduced genetic material (plant incorporated protectants).

c. Biochemical pesticides: naturally occurring substances that control pests by nontoxic mechanisms. These include substances that interfere with growth or mating such as pheromones

1.4.1. Microbiological Controls

These are derived from microorganisms including bacteria, fungi, and viruses.

Over the last decade, extensive research activities on microbial biopesticides have led to the discovery and development of a considerable number of biopesticides and have paved the way for their marketability (Luca Ruio, 2008).

The first bacteria to be used as a commercial insecticide was *Bacillus thuringiensis* in France in 1938 and the USA in the 1950's. More effective products were developed in the 1960's which led to increasing prominence and increased usage over the next 3 decades. Microbials continued to be produced and sold by small companies into the early 2000s.

In 2009, for the first time the idea of the integrated use of biological and chemical fungicides in fruit and vegetable crops was publicly voiced by BASF (Lane, 2009). BASF also acquired the right to market Serenade® in a licensing agreement with Agraquest in the same year. In 2011 Bayer successfully launched Votivo to control nematodes in corn, soya bean and cotton. Syngenta also gained distribution for MBI's biofungicide Regalia outside of North America. Also, FMC and Christian Hansen announced a research co-operation. In 2012, Syngenta gained rights to distribute for Novozyme's biofungicide Teagro. In the same year even, bigger steps were taken than acquiring marketing rights to biological products with Bayer buying Agraquest and BASF Becker Underwood.

As one of main advantages of biologicals, their safety to non-target organisms such as pollinators, natural enemies, and workers plays an important role. It has caused the low pre-harvest and re-entry intervals for biologicals. They decompose quickly and using them causes less need for pesticides. As a result, there is reduced registration requirements for biologicals. Such advantages have also induced the positive public view towards biologicals. There is a strong public belief that synthetic substances or man-made products impact on nature, man, and the environment adversely. This thinking is fueled by the experience with DDT and its severe side effects on the environment and humans. It is believed that synthetic substances have a harsher effect due to their "unnatural" nature and thus means of metabolizing and denaturing them are often not readily present in the organisms in the environment. On the other hand, natural substances do not produce synthetic residues and as they are "native" to the environment, mechanisms to degrade and establish a good healthy equilibrium within the environment are already present. However, applying only biologicals in order to control diseases is still not effective enough.

Presumably, the aim in applying microbiologicals is establishing a living colony of the organism on the root, leaf, or rhizophore. Currently, there are still challenges with establishing live organisms

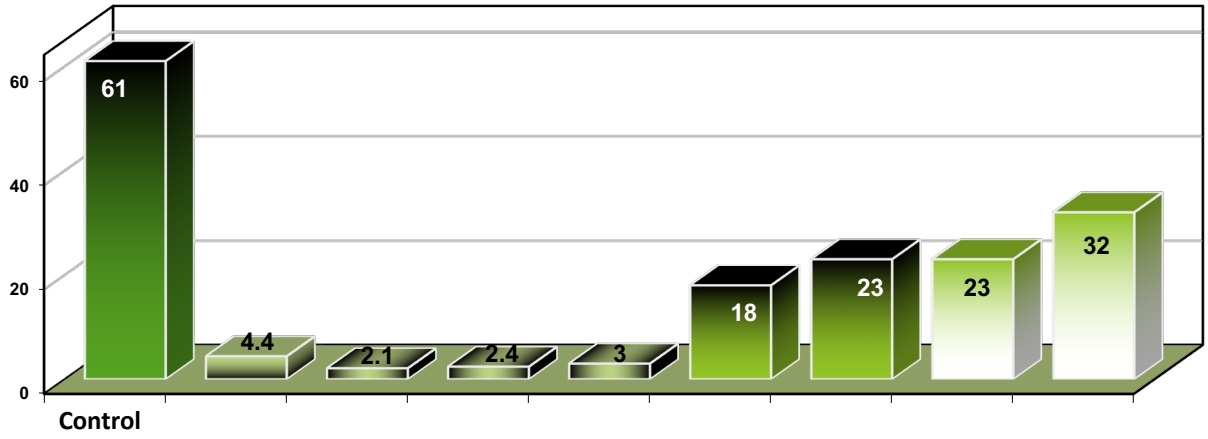
on the target but nevertheless, there are many biologicals in the market at this point in time which are either used in the field close to the harvest time (pre-harvest and harvest time), when the disease pressure is low or in an integrated program with chemical crop production products. Since in terms of cost performance biologicals are not attractive, if other potentials of biologicals could be used in an integrated program with chemicals it may justify their cost for the farmer and increase their attractiveness.

One promising use of microorganisms is to harness their ability to remove residues of pollutants from contaminated areas as an alternative treatment strategy that is effective with minimal hazards, economical, and environment-friendly (Nahila Anjum, 2022) (Nitschke, 2011) (Fig. 1.11). Investigating microbial diversity in contaminated areas followed by studying the microbial ecology, physiology, and evolution due to the biotransformation of organic contaminants in natural environments (Jitendra Kumar, 2021) led to the identification of several genes and enzymes for biodegradation of organic compounds and offers the potential of employing genetic engineering to enhance the microbial degradation of the residues of chemical treatments (Sabine Grundmann, 2004).

On the other hand, with respect to crop protection uses, the superior control obtained with chemical crop products has led to a lack of prominence for microbiologicals in the agricultural market. Many of these microbiological products are not interesting for farmers, firstly because they are not as effective on the target pests in their field and secondly, their high specificity makes them -impractical in a field confronting different pests. In this situation multiple biologicals would be required (Jitendra Kumar, 2021).

However, these biological products are well suited to situations where supermarkets have introduced secondary standards for their suppliers. These secondary standards are related to the levels of residues of chemical crop protection products and are considerably more rigorous than those set and deemed to be safe by the regulatory authorities.

Many authors have suggested that microorganisms may play a significant role in the degradation of the active substance. It's fascinating and logical to consider the use of effective microorganisms (EM) to biodegrade fungicide since they are widely employed in crop cultivation, vegetable farming, and animal husbandry. EM consists of selected microorganisms, such as lactic acid bacteria, yeasts, low-density photosynthetic bacteria, actinomyces, and other natural microorganisms. These microorganisms are harmonious and can coexist in liquid culture. (Elżbieta Wołejko, 2016)



Chemical		Sequential		Full season			
0.3	0.3	0.3 + 8	0.3 + 4	8	4	4	2
Signum	Signum	Signum	Signum	Ser. ASO		Ser. MAX	
Signum	Signum	Signum	Signum	Ser. ASO		Ser. MAX	
	Signum	Ser. ASO	Ser. MAX	Ser. ASO		Ser. MAX	
		Ser. ASO	Ser. MAX	Ser. ASO		Ser. MAX	

Figure 1.11. The integrated program with the chemical crop production product (Signum) and biologicals (Serenade®) to control Alternaria solani on tomato (Lane, 2009).

A good illustration is the strobilurin fungicides that have been utilized in agricultural fields for several decades. While these pesticides are intended to manage fungal pathogens, their broad-spectrum mode of action may also have unintended consequences. Researchers have identified and characterized strobilurin-degrading microbes and microbial communities in numerous reports. These microbes include *Bacillus, Pseudomonas, Klebsiella, Stenotrophomonas, Arthrobacter, Rhodanobacter, Cupriavidus, and Aphanoascus* (Yanmei Feng, 2020)

Another recent research study investigated the fungicide tolerance and bioremoval potential of *Trichoderma* species found in the living leaf tissues of wild Rubiaceae (coffee family) plants. The study aimed to determine the ability of these fungi to remove harmful substances such as azoxystrobin, chlorothalonil, cyproconazole, and *trifloxystrobin* from the plants (Efraín Escudero-Leyva, 2022).

1.4.1.1. The Mode of Action of Microbial Pesticides

Microbial fungicides and bactericides misfunction the target organism in diverse ways mostly through inhibiting or disrupting protein synthesis (Kimberly M.Parker, 2019). For instance,

they bind to ribosome 50S in prokaryotes to prevent the transfer of peptides and inhibit the chain elongation or in another way, they can bind to tRNA to stop the translation (Barbara S Schuwirth, 2006).They can also target the plasma membrane permeability and cause leakage of substances (amino acids and electrolytes), thereby causing the cell death. For instance, Serenade® a biofungicide product contains *Bacillus subtilis* which produces lipopeptides which insert into the cell membrane causing perforations through which the cell contents leak out leading to the death of the fungal cells.

Another example is *Bacillus thurengiensis* a prominent bioinsecticides which produces a crystalline inclusion body during sporulation (Frankenhuyzen, 2009), (Unzue, 2022). The crystal proteins (Cry proteins) are toxic to many insects and are defined as endotoxins (Bt toxin) that are encoded by bacterial plasmids (Jose M. Gonzalez, 1980) (Anusha Delanthabettu, 2022). The toxicity of the protein depends on the presence of receptor sites in the insect's body. The different crystal proteins are responsible for the specificity of bioinsecticides because each has a specific receptor in the target organism.

However, despite many advantages of biologicals, they have not been as widespread as expected for many reasons: The process of application of biologicals is not yet optimized. Rather they are currently applied in the same way as chemical products, which may not be optimal.

Presumably the goal is to establish a living colony of the organism but where? On the leaf, root, or rhizosphere? How can the success be measured? Currently, no method has been developed to monitor the establishment and development of the microbial population.

The fermentation process is different to normal fermentation processes because spores and living organisms are required rather than the products of the microbe's metabolism. Hence, not only efficient facilities are required to optimise the process in order to get sufficient spores or living organisms, but also the legitimate storage condition is crucial to produce the applicable end-product. The microorganism, whether in spore form or vegetative cell, should be dilutable with water in order to spray on the field.

Another "unknown" is where is the target destination for biologicals, should it penetrate into the plant? If yes, where should it then go and what happens in the plant? There are still doubts about the optimal doses that are required to produce the desired effect.

Currently little is known about these aspects and further research is required to provide answers to these questions. Nevertheless, biologicals have clearly showed strong potential in Integrated Pesticide Management (IPM) strategies, where the use of biologicals reduces the use of chemicals significantly (André Luiz Meleiro Porto, 2011).

1.5. Biotechnology

Another type of farming system with respect to sustainability is transgenic or biotechnological agriculture, based on the use of genetically modified organisms (GMO). GM plants arose intense debates especially in Europe. Initially, transgenic agriculture was considered advantageous by reducing the environmental pressure resulting from chemical application while maintaining high productivity. However, over time global debates have started about possible unknown effects on the environment and human health across countries ((Morris, 2001), ((EFSA), Report From The Commision To The council And The European Parlament, 2005)).

1.5.1. Environmental Hazard

The gene flow between species and then unconscious hybridisation has been documented a long time ago by Darwin (1876) (Rowe, 2004). Later Ellstrand found out that the gene flow happens between the cultivated crops and their wild types frequently (Danny A. P. Hooftman, 2005).Therefore, it was considered there might always be the possibility of gene flow between genetically modified plants and their weed relatives. This means the resistance of weeds to pests could also be fostered. This can lead to the development of a “superweed” resistant to herbicides. For instance, transgenic toxic Bt persists in the soil for eighteen months and can also transfer its gene to wild plants which might create mutant weeds resistant to pests (EFSA, 2004) . For instance, one study in UK showed that one genomic modified oilseed rape had long distance cross-fertilization with its wild type which resulted in a tough herbicide-resistant strain (Yann Devos, 2004)

1.5.2. Risks to Human Health

There may be allergenic effects - especially in people who are predisposed to allergies or other adverse effects on human health (Charu Verma, 2011). The concerns come from the lack of large-scale long-term epidemiological studies that lead to safe conclusions about the allergenic effects of genetically modified plants, which makes some researchers skeptical about the use of genetically modified products. in other words, the introduction of a gene that expresses a non-allergenic protein does not mean that it will produce a product without allergenic

action. (Dimitrios T. Karalis, 2020). Because of such evidence there are concerns that GM crops can invade environments without a possible solution to manage it.

1.5.3. Benefits of using Genetically Modified Products

A series of extensive and long-term research has shown that the benefits of growing genetically modified crops in the fight against global food shortages and hunger have been significant (Steier, 2017). The steady increase in the global population has led researchers to focus on the benefits of developing genetically modified products, rather than the potential risks they pose each time (Herring, Transgenics and the poor : biotechnology in development studies, 2013).
A number of studies show the economic benefits of using genetically modified products. Between 1996 and 2011, farmers' income worldwide increased by $92 million from the use of genetically modified crops. Part of the revenue is due to the more efficient treatment of weeds and insects, while another part is due to lower overall production costs (P.J. Jones, 2017)
However, it has been observed that although modified species have developed through conventional agriculture they do not tend to survive for long. In general, the probability of modified species becoming invasive and tolerant to herbicides tolerance is not different between GM and non-GM plants. The simple solution is to avoid cultivating the transgenic crops close to their sexually compatible wild relatives and the crop rotation (J. D. Arthington, 2003).In addition, specific timing and applicable tillage system are recommended (Cesare Pacini, 2003). Nevertheless, even if resistance occurs it would be for one specific herbicide, so there are always other possible ways to apply other plant protection products (Andow, F2 Screen for Rare Resistance Alleles, 1998).

1.6. Objective

Chemical and microbial fungicides have similar costs in the marketplace, but the inferior efficacy of microbial fungicides is a considerable competitive disadvantage. If other benefits of microbial fungicides could be identified or developed, this could address this disadvantage. One such benefit could be the ability of the biological fungicide to degrade the residues remaining from chemical fungicide application. It is well established that microbes can degrade chemical fungicides and indeed other types of crop protection product in the soil. (Junaid Ahmad Malik, 2023) (Nahila Anjum, 2022).

In order to investigate the feasibility of this, isolates from samples of plant material from sites where fungicide trials had been carried out with multiple sprays of one fungicide were cultured. Using a previously developed method (Soltani, 2019), the growth of the isolates in the presence of the fungicides used during the trials was examined. The hypothesis was that if the isolates were able to utilise the fungicide as an additional source of nutrients, enhanced growth in the presence of the fungicide should be observed. If the fungicide was being utilised by the isolate as a nutrient source, it was assumed that the fungicide would be degraded. The isolates that showed the greatest growth in these screens would then be further characterised, including their potential as biological fungicides in order to select the optimal isolates.

This work would serve as a basis for further progress towards the ultimate goal of developing a bacterial fungicide that is capable not only of controlling plant diseases but also of degrading residues of chemical fungicide applications. This could be distinctly advantageous in an integrated (chemical and biological) spray program, particularly in fruit and vegetable crops where chemical residues are viewed particularly negatively by the consumer and supermarkets consequently set the farmer chemical residue levels well below the legal level, so called secondary standards. Biological fungicides that can degrade chemical fungicide residues in and on the plant in this way may be commercially more attractive than those that merely control fungal diseases and could enhance their marketability to the grower.

2. Materials and methods

2.1. Biological Materials

2.1.1. Leaves

Samples of leaves are from apple and strawberry taken from BASF trials with Revysol®, boscalid, pyraclostrobin, fluxapyroxad over 2 field seasons. (Table 2.1.)

Crop	Fungicide	Sampling time after finishing the application
Apple	Revysol®, Bellis	End of June/ Beginning of July, 7 Applications
Strawberry	Fluxapyroxad, Boscalid	Realtime, 7 Applications

Table 2.1. Field Trials and Sampling Time and number of applications.

2.1.2. *Bacillus subtilis* QST713 (Serenade®)

Few droplets of the biological product (Serenade®) were pipetted on the solid agar medium in the Petri dishes placed in the incubator at a temperature 27 to 30 °C for two to three days in order to culture the bacterial strain in this product *(Bacillus subtilis* QST713). Thereafter, the samples were re-cultured through the streaking method and placed in the incubator under the same condition in order to achieve single colonies.

2.1.3. *Bacillus subtilis* DMS10

(Standard Laboratory Strain allocated by Dr. Gläser from the microbiology department in the Justus Liebig University).

2.1.4. MBI600

A culture of *Bacillus amyloliquefaciens* (strain MBI600), was supplied by BASF.

2.2. Chemical Materials

All fungicides were supplied by BASF as technical material.

2.2.1. Pyraclostrobin

Chemical name: (Methyl-N-{2-[1-(4-chlorphenyl)-1H-pyrazol-3-yloxymethyl] phenyl} (N-methoxy) carbamate (content (W/W): >= 97.5 %)
Form: melt, petrified
Colour: dark brown
Solubility in acetone: 500 g/l

2.2.2. Boscalid

Chemical name: 2-chloro-N-(4'-chloro[1,1'-biphenyl]-2-yl) pyridine-3-carboxamide
Form: crystalline powder
Colour: White, crystalline, powdery solid and odourless
Solubility in acetone: 180 g/l

2.2.3. Fluxapyroxad

Chemical name: 3-(difluoromethyl)-1-methyl-N-(3',4',5'-trifluoro [1,1' biphenyl]-2-yl)-1H-pyrazole-4-carboxamide
Form: White crystalline powdery solid and odourless
Solubility in acetone: 250000 g/l

2.2.4. Mefentrifluconazole (Revysol®)

Chemical name: rac-(2R)-2-(4-(4-chlorophenoxy)-2-(trifluoromethyl) phenyl)-1-(1H-1,2,4-triazol-l-yl) propan-2-ol
Form: crystalline powder
Colour: white
Solubility in acetone: 93.2 g/l

2.2.5. Preparation of Fungicide Solutions

The required amount of each fungicide was dissolved in 10 ml of solvent (usually acetone) to make the highest concentration then the lower concentrations were made by serial dilution with acetone.

2.3. Experimental Instrumentation

2.3.1. Incubators

2.3.1.1. Plate Thermo-Shaker of Biosan

Shaking with temperature control for 4 standard 96-well microtiter plates per shaker at a temperature 35 °C with a shaker speed of 600 - 700 rpm. Each plate was wrapped in a plastic bag with wet tissue inside to maintain high humidity. In order to prevent condensation on the lid of microtiter plate which could disturb optical density measurements, a solution of 5 percent triton and 20 percent ethanol was used to coat the inside of the lid of the microtiter plate.

2.3.1.2. Glass Tube Thermo-shaker

Incubators HPE and Edmund Bühler GmbH, were used to incubate glass tubes at a temperature 35-37 °C, with a shaker speed of 220 rpm. The tubes were maintained in racks at an angle of approximately 25% off the vertical.

2.3.2. Microplate Reader

The ELx808 Absorbance Microplate Reader set on OD600 nm was used to measure the optical densities of the medium in the microtiter plates at the desired assessment intervals either every 3 to 4 hours, 4 times a day, or for the 30-minute tests every 30 minutes over two to three days.

2.3.3. Spectrometer used to Measure Optical Density

The optical density (OD) at the wavelength 600 nm was measured by an Eppendorf UV/visible BioSpectrometer basic, 230 V/50-60 Hz. For measuring, 1 ml of the sample was pipetted into either a plastic or glass cuvette. Glass cuvettes were rinsed with distilled water after each measurement. Should the bacterial growth in the tube be too high to allow a reading to be taken, successive 50:50 dilutions with distilled water were conducted until the readings were once again within range and a value could be determined.

2.3.4. HPLC-FLD

The Agilent 1200 HPLC System equipped with a fluorescence detector (FLD) (also from the Agilent compony).

2.4. Culture Medium

2.4.1. Salt Medium

Double the stated amount of each of the groups of substances 1-5 (Table 2.2.) were dissolved in 200ml of distilled water. The amount of glucose needed for 1000 ml was dissolved in 500 ml distilled water. Each of the resulting 6 solutions were autoclaved for an hour. After autoclaving, groups 1 to 5 were mixed together to give a volume of 1000 ml. 500ml of the 1000ml solution was then mixed with 500ml of the glucose solution to form the salt solution containing glucose as a carbon source. All mixings of the solutions were carried out in sterile conditions under a laminar flow bench.

Minimal Salt Medium supplemented with Glucose (MS+G)	
Group 1	
2.6 g/L	NH4)2SO4
1.0 g/L	NH4Cl
0.5 g/L	NaCl
15.0 g/L	Na2HPO4·12 H2O
3.0 g/L	KH2PO4
Group 2	
50.0 mg/L	FeCl3·6 H2O
65.0 mg/L	EDTA Na2
Group 3	
1.8 mg/L	ZnSO4·7 H2O
1.8 mg/L	CuSO4·5 H2O
1.2 mg/L	MnSO4·H2O
1.8 mg/L	CoCl2·6 H2O
Group 4	
2.0 mM	MgSO4
Group 5	
0.2 mM	CaCl2
Group 6	
20 mM	Glucose

Table 2.2. Minimal Salt Medium composed of 5 groups of chemicals supplemented with glucose.

2.4.2. Agar Preparation

To prepare agar Petri dishes, 15 g Agar-agar, 5 g peptone, 3 g meat extract, and 10 mg manganese (2) sulfate were mixed in 1-liter distilled water and then autoclaved for one hour. After autoclaving, after 30 minutes cooling, the agar was poured into petri dishes.

2.5. Preparation of Biological Materials

2.5.1. Strain isolation

An isolation procedure was established based on methods available in published literature (Kefi, 2015). A heat shock treatment was applied to restrict the isolation to organisms possessing resistant spores. Leaf samples in plastic bags were placed into a small beaker half filled with distilled water at a temperature 80 °C for 10 minutes. After the heat shock, surface sterilization was carried out with 1% NaOCl and 70% ethanol under the clean bench to focus on the isolation of heat resistant spore forming microorganims. The samples were immersed in the solution for 2 to 3 minutes. Then the leaves were cut into small pieces of similar size with a scalpel and placed on agar petri dishes. The agar petri dishes were incubated in the oven at 27

to 30 °C for a week. During the incubation time, whenever any colony appeared on the agar plate, it was taken and re-cultured on a new agar plate. The re-culturing was repeated at least 3 times to ensure pure colonies. Through this step around 400 isolates were established for further investigation.

2.5.2. Isolate Storage

For long term storage, salt medium supplemented with glucose was made by taking colonies from the agar culture plate using an inoculation loop and transferring the inoculum to 5 mls salt medium with glucose in the 10 ml-glass tube. The tube was then incubated at 37 °C with a shaker speed of 220 rpm for 17 to 24 hours. At the end of this period, 1.8 mls of the culture medium and -and the same volume of glycerol were placed in a 2-ml Eppendorf tube. The tube is then immersed in a liquid nitrogen and then transferred into a freezer maintained at - 80 °C.

2.6. Screening Method

To monitor the modification in the growth of isolated samples in the presence of fungicides, a two-staged screening method has been developed

2.6.1 Primary Screening

In the first primary screening stage, 4 isolates were tested in each 96 well microplate. Each isolate was tested with 3 different doses of fungicide, 10, 5, and 2.5 ppm, each with 6 replicates. The culture medium contained 150 microliters salt medium, 3 microliters of an acetoneic solution of the fungicide, and 1 microliter of bacterial inoculum. In the control, instead of the fungicide containing solution, the 1 microliter of acetone was added into each cell (Table 2.3). The ODs were measured and then the plates were incubated in the Plate Thermo-Shaker for 3 to 5 days. OD readings were taken, 4 times per day between 08:00 and 18:00.

a.

	SM+G (150µl)
Fungicide Doses (3µl) Prepared in acetoneic solution (ppm)	10 5 2.5
Bacterial Solution (1µl)	OD: 0.3
Replicates	6

b.

	Column 1-6		Column 7-12	
A	Strain 1 (ppm)	10	Strain 3 (ppm)	10
B		5		5
C		2.5		2.5
D	Control		Control	
E	Strain 2 (ppm)	10	Strain 4 (ppm)	10
F		5		5
G		2.5		2.5
H	Control		Control	

Table 2.3. The set-up of the primary screening test, a. the Treatments used *b.* the arrangement of the treatments in the 96 well microtiter plate.

2.6.2. Secondary Screening

Isolates selected from the primary screening were evaluated in a second stage referred to as secondary screening. There, the selected isolates were evaluated with a broader range of doses; 50, 25, 10, 5, 2.5, 1.25, and 0.625 ppm. Furthermore, a parallel set of uninoculated treatments were included in order to monitor whether the fungicide present were influencing the OD readings and to check that no contamination had occurred. OD measurements were taken according to the same procedure used in primary screening (Table 2.4.).

2.6.3. ‘’30-minute’’ Experiments

In order to follow the growth of the isolates in more detail over the first 24 hours the growth of six selected strains from the Revysol® trials with six doses of Revysol® 100, 50, 25, 12.5, 5, 2.5 ppm were examined in more detail by monitoring optical densities at 30-minute intervals over the first 48 hours and for the remaining 2 days every 3 to 4 hours. Other than this, the set up was the same as for the secondary screens.

a.

	SM+G inoculated (150µl)	SM±G Uninoculated (150µl)
Fungicide Doses (3µl) Prepared in acetoneic solution (ppm)	50 25 10 5 2.5 1.25 0.625	50 25 10 5 2.5 1.25 0.625
Bacterial Solution(1µl)	OD: 0.3	OD: 0.3
Replicates	5-6	5-6

b.

Column 1-6 inoculated (ppm)	Column 7-12 uninoculated (ppm)
50	50
25	25
10	10
5	5
2.5	2.5
1.25	1.25
0.625	0.625
0	0

Table 2.4. The treatments used in the secondary screening test, a. the Treatments used b. the arrangement of the treatments in the 96 well microtiter plate.

2.6.4. Cross Screening

Up to this point, the growth of all strains had been evaluated in the presence of fungicides applied to the trial sites that they were isolated from. To investigate whether some of these strains were also able to grow more strongly in the presence of other fungicides, a set of tests was set up based on the microtiter plate technique used for the primary and secondary screens, with selected strains from secondary screening (Table. 2.5). Also included was the strain from the BASF biological fungicide "Serifel®" and a strain isolated from the Bayer product "Serenade®". Three dose rates (25, 5 and 1 ppm) of the fungicides Revysol®, boscalid, pyraclostrobin and fluxapyroxad were used, and the growth compared to the controls where no fungicide was present.

2.7. Statistical Analysis

The data generated in the primary and secondary screening, 30 minute and cross screening tests were subjected to a single factor analysis of variance at each sampling date in Excel and 5%, 1%, and 0.05% LSDs determined to compare treatment with control values. Growth curves were plotted for each treatment based on the mean OD values for each treatment at each assessment time. Furthermore, the area under the curve was calculated using the trapezium method to calculate the incremental changes between each sampling time and then summating them over the whole experimental period. This procedure was later adapted to take into account any effects of the fungicide on optical density by subtracting the optical density at time zero (immediately after setting up the experiment) for each treatment from subsequent optical density readings and then calculating the "area under the curve" again using the trapezium method. This latter assessment is referred to as "growth". The area under the curve and the

"growth" measurements were then also subjected to a single factor analysis of variance in Excel and 5%, 1%, and 0.05% LSDs determined to compare treatment with control values.

2.8 Biofungicidal Activity

Quantitative agar plate bioassays developed in our department (Schmidt 2021) were used to examine the fungicidal activity of the leading isolates. There we examined the isolates with 3 fungi, *Cercospora*, *Botrytis*, and *Sclerotinia*. In each bioassay a 6mm plug taken from a solid PDA plate culture of the pathogen using a cork borer was placed in the center of new PDA petri dish. A bacterial solution of the tested strain in distilled water was adjusted to an OD of 0.3. and 6 microliters of the bacterial solution placed on 3 pieces of sterilized filter paper (6 mm in diameter) place equidistant from the canter of the plate and 3.5 cm from each other.
The plates were then maintained in the dark at room temperature The distance between the growth zone of bacteria and fungi was measured every day for three to five days.

2.9. Confirmation of Fungicide Degradation accompanying the Enhanced Growth in the Presence of the Fungicide.

2.9.1. Direct Measurement of UV-VIS Absorbance in the Culture Medium

1 ml of culture medium containing fungicide was put directly into a cuvette and measured in a single-cell UV-Vis spectrophotometer (Thermo Scientific UV Visible Spectrophotometer, One-Position Cell Holder). Wavelengths used were 202nm, 207nm and 203nm for Revysol®, boscalid and fluxapyroxad respectively.

a.

	SM+G (150µl)
Fungicide Doses (3µl) Prepared in acetoneic solution (ppm)	25 5 2.5
Bacterial Solution(1µl)	OD: 0.3
Replicates	6

b.

	Column 1-6		Column 7-12	
A	Inoculated Treat. 1 Boscalid in ppm	25	Inoculated Treat. 2 Revysol® in ppm	25
B		5		5
C		1		1
D	Inoculated Treat. 3 Pyraclostrobin in ppm	25	Inoculated Treat. 4 Fluxapyroxad in ppm	25
E		5		5
F		1		1
G	Control			
H	SM+G			

	Column 1-6		Column 7-12	
A	Inoculated Treat. 1 Boscalid in ppm	25	Inoculated Treat. 2 Revysol® in ppm	25
B		5		5
C		1		1
D	Inoculated Treat. 3 Pyraclostrobin/ Fluxapyroxad in ppm	25	Wasser	
E		5	Wasser	
F		1	Wasser	
G	Control			
H	SM+G			

Table 2.5. The treatments used in the cross screening test, a. the Treatments used b. the arrangement of the treatments in the 96 well microtiter plate.

2.9.2. HPLC Analysis of the Fungicide Concentrations

The experiment was set up to analyse the data from reader machine using an HPLC method supplied by BASF. (BASF, 1998). However, some modifications were made to the chromatographic conditions. Specifically, the mobile phase consisted of 60% acetonitrile and 40% water, deviating from the original 50:50 ratio and eliminating the use of H_2SO_4. Additionally, the flow rate was reduced from 1 ml/min to 0.3 ml/min, and a smaller injection volume of 40 µl was used instead of the previous 1 or 5 ml volume.

3. Results

3.1. Isolation

From 400 isolates taken from leaf samples, 168 were evaluated in primary screens, 79 from Revysol® sites, 34 from boscalid sites, 25 from fluxapyroxad sites and 30 from Bellis sites. All isolated strains from Bellis were evaluated with boscalid and 26 of them with pyraclostrobin. Focusing on the greater growth in the presence of glucose, 10 (12%), 38 (59%), 9 (34%) and 1 (4%) of the isolates were found to have enhanced growth in the presence of Revysol®, boscalid, pyraclostrobin, and fluxapyroxad respectively (Table 3.1.). (Note that the fluxapyroxad® isolates all were taken from irrigated strawberry trial sites and the wash off that was caused by the irrigation might be the reason for the lower success rate). The active strains in the primary screens were considered as potential secondary screening candidates.

Fungicide	Isolates tested in Primary Screen	Isolates tested in Secondary Screen	Selected for Identification
Revysol®	79	10	6
Boscalid	64	38	9
Pyraclostrobin	26	9	0
Fluxapyroxad	25	1	0

Table 3.1. The number of isolates tested in the primary and secondary screening tests.

3.2. Screening

3.2.1. Primary Screening

3.2.1.1. Revysol® Group

79 strains were isolated from leaf samples treated with Revysol® and tested with three Revysol® doses, 1.25, 2.5 and 5 ppm. In this case the area under the curve was calculated and used as the main indicator of the growth occurring.7 strains showed increased growth of between 10 and 65% . All of the selected strains have shown positive growth in the treatment with 2.5 ppm of between 12 and 50% of the control., and 60 % of these increases were statistically significant 57% of the selected strains showed enhanced “growth” at all 3 doses.
Although with strains 45 and 61 there is no significantly enhanced growth, consistently increased growth was observed in all 3 treatments. Although there is neither a significant difference nor consistently higher growth with all 3 doses, isolate 80 was also selected due to the large growth increases observed at two of the rates (Table 3.2.).

Revysol®	Integrated ODs as % of control				0 ppm	5% LSD
Strain ID	1.25 ppm	2.5 ppm	5 ppm	Average%		
1	109.8	100.9	124.5	111.72	45.4	24.23
2	103.1	96.2	75.3	91.52	44.3	12.70
3	83.6	81.1	74.8	79.81	42.7	8.08
4	74.4	86.3	58.7	73.14	64.5	14.31
6	103.9	96.1	85.1	95.05	31.0	7.66
7	96.0	98.6	72.9	89.16	43.6	7.17
8	101.3	89.2	49.8	60.07	63.3	9.20
9	57.8	60.7	70.3	62.94	53.8	23.64
10	96.6	84.2	81.3	87.38	36.1	6.96
12	84.1	94.5	72.4	83.67	41.8	17.32
13	117.4	103.5	98.4	106.41	26.6	5.62
14	94.4	112.2	103.5	103.39	38.4	8.76
15	80.2	84.7	78.4	81.12	39.6	11.77
16	87.7	80.5	52.4	73.52	43.2	11.87
17	93.4	96.1	89.4	92.99	44.5	3.07
20	76.4	76.8	66.7	73.31	48.8	9.68
23	85.0	83.9	68.6	79.17	50.0	10.05
24	103.7	101.8	92.3	99.25	41.9	3.58
25	86.2	92.0	84.2	87.50	34.5	6.84
26	99.7	99.9	88.6	96.11	44.2	2.49
27	83.3	76.2	76.9	78.82	45.2	5.02
28	101.2	105.6	96.2	101.01	46.2	3.80
30	111.4	111.9	80.6	101.30	30.1	6.55
33	85.0	74.9	52.3	70.72	54.0	9.65
34	100.6	82.8	74.4	85.90	50.6	15.99
36	99.1	67.9	56.2	74.40	58.8	15.70
38	81.0	82.1	66.7	76.61	55.4	15.02
42	104.4	110.1	98.2	104.22	28.0	8.50
43	97.1	94.9	78.4	90.14	44.3	5.66
43	96.5	101.1	87.0	94.87	36.9	5.83
44	98.6	98.7	91.4	96.25	38.0	3.57
45	109.1	148.1	124.9	127.33	27.9	18.81
46	97.6	99.3	80.0	92.30	67.0	11.82
48	104.9	99.0	90.2	98.05	11.7	3.06
51	92.9	96.2	56.1	81.73	49.6	6.95
52	94.7	98.6	80.4	91.24	34.0	5.20
53	125.5	84.6	65.7	91.93	44.5	14.77
53	111.7	124.6	97.7	111.35	66.4	15.31
54	116.1	75.2	72.4	87.89	24.2	9.84
56	103.8	103.7	98.8	102.09	37.1	7.94
57	71.6	78.2	57.3	69.03	28.3	20.08
58	90.8	91.3	76.0	86.04	81.4	13.10
60	102.7	105.3	85.4	97.82	32.7	6.57
61	95.0	89.6	87.3	90.66	72.6	9.23
61	129.3	111.7	120.1	120.35	37.3	15.81
62	58.9	78.8	63.9	67.21	62.4	12.79

Revysol®	Integrated ODs as % of control				0 ppm	5% LSD
Strain ID	1.25 ppm	2.5 ppm	5 ppm	Average%		
65	89.1	85.6	73.1	82.61	48.6	7.54
66	131.9	146.1	144.9	140.98	12.5	5.06
68	76.1	90.2	70.2	78.84	48.5	7.58
70	81.0	74.7	83.1	79.58	33.1	18.81
71	86.4	76.2	64.8	75.80	42.4	15.12
73	93.0	105.5	97.3	98.60	35.7	5.33
80	89.8	121.5	165.7	125.67	25.3	17.25
81	88.5	90.4	52.7	77.25	56.8	6.32
90	97.3	112.4	90.9	100.21	39.7	11.25
91	91.6	75.4	35.6	67.56	82.7	25.73
92	79.8	69.1	65.9	71.61	79.8	11.14
93	96.6	109.1	109.6	105.13	78.2	14.89
94	97.9	87.5	85.2	90.19	83.4	10.26
95	100.4	100.9	96.1	99.12	104.5	3.47
96	103.4	112.1	115.2	110.25	107.2	9.47
97	97.4	112.9	107.9	106.06	49.6	8.80
98	67.7	59.7	60.7	62.72	52.3	7.46
99	53.7	46.7	31.9	44.09	99.3	21.72
100	98.2	63.1	60.8	74.04	99.2	35.19
101	86.5	90.1	83.8	86.81	33.4	4.15
102	133.0	126.5	95.9	118.47	68.7	46.32
103	106.2	107.2	76.6	96.70	82.4	7.91
104	86.1	97.2	80.7	88.02	44.4	9.94
110	96.4	92.3	80.1	89.61	62.7	15.74
110	102.7	105.7	95.8	101.40	38.4	2.11
111	104.4	107.9	103.9	105.39	37.6	3.35
113	93.2	93.0	82.7	89.60	99.6	5.74
114	104.9	111.6	104.0	106.82	40.8	5.27
115	94.0	97.5	96.3	95.92	40.1	3.93
118	97.3	99.3	89.3	95.30	40.3	4.79
120	78.9	80.8	60.0	73.21	59.9	20.45
123	90.2	105.1	86.8	94.03	48.2	7.88
124	121.2	137.1	123.0	127.12	35.1	8.51

Table 3. 2.The primary screening results with the isolates from the Revysol® group tested with Revysol®.

Statistically significant increase compared to the control at the 5% level.

NO Indicates a large but nor statistically significant increase.

3.2.1.2. Boscalid Group

Observing the "growth" of the 64 strains isolated from leaf samples treated with 3 doses 1.25, 2.5, and 5 ppm of either boscalid or Bellis, 13 isolates showed enhanced" growth", 184, 189, 194, 195, 201, 203, 205, and 209 in the presence of boscalid (Table 3.3) and 249, 286a, 280, 284, and 285 in the presence of Bellis table (Table 3.4). In order to have a comparison with a commercial biological fungicide, Serenade® a biofungicide containing *Bacillus subtilis* QST 713 was included in addition to the "standard laboratory strain" DSM10, also a *Bacillus subtilis*.

Boscalid	"Growth" as % of control				0 ppm	5% LSD
Strain	1.25 ppm	2.5 ppm	5 ppm	Average %		
DMS10	320.8	52.4	18.9	130.7	0.6	102.1
Serenade®	82.3	90.6	66.7	79.9	56.5	30.46
53	87.0	64.7	81.9	77.9	102.92	38.97
96	114.3	127.7	110.9	117.6	79.25	37.86
96	124.1	142.3	113.3	126.6	64.16	48.15
180	83.2	95.0	27.4	68.5	88.2	36.53
182	245.5	346.2	129.5	240.4	6.6	425.43
183	94.3	103.4	82.9	93.5	73.5	27.07
184	229.2	209.5	188.1	208.9	35.5	82.37
185	-46.0	-132.4	-193.2	-123.9	-0.5	-600.58
186	79.6	0.2	-0.2	26.5	94.4	28.35
187	84.5	20.4	23.0	42.6	71.0	43.93
187	104.7	104.9	97.5	102.4	71.3	5.76
188	80.7	26.9	65.6	57.7	29.7	37.98
189	100.4	125.1	122.8	116.1	47.5	17.62
189	67.7	48.9	73.7	63.4	37.9	41.83
190	67.9	32.3	49.8	50.0	85.6	47.16
191	100.2	50.9	57.8	69.7	26.4	58.55
194	110.5	24.4	15.3	50.0	0.6	110.33
195	116.7	130.5	120.0	122.4	52.9	19.93
196	60.9	26.7	80.4	56.0	46.4	38.55
197	35.6	-9.4	1424.4	483.5	0.4	2266.59
200	100.6	101.0	91.6	97.7	100.7	6.86
201	107.7	112.9	111.4	110.7	73.0	6.85
202	98.1	99.9	91.1	96.4	63.7	10.29
203	107.1	116.8	106.9	110.3	50.8	13.75
204	110.8	111.3	76.4	99.5	48.2	30.09
205	125.2	124.4	115.0	121.5	32.8	15.52
206	102.1	102.7	58.1	87.6	89.3	32.69
207	98.7	98.8	96.4	98.0	87.0	8.53
208	106.0	108.5	93.6	102.7	75.6	6.91
209	170.7	73.6	39.5	94.6	1.5	121.62
239	102.3	110.2	63.8	92.1	44.7	41.38
240	107.0	-0.5	-0.6	35.3	38.1	7.67
241	111.1	115.1	33.1	86.4	64.2	43.39
242	84.6	89.6	75.7	83.3	48.1	24.30
243	72.8	88.6	82.6	81.3	109.9	22.32
244 A	88.5	56.0	43.2	62.5	54.2	34.33
244 B	115.3	16.8	83.5	71.9	1.1	67.48
245	81.4	58.2	37.1	58.9	9.9	44.58
246	99.4	97.7	76.3	91.1	52.9	26.37
247	-10.1	-25.5	5.9	-9.9	2.1	52.60

Table 3.3. The primary screening results with the isolates from the boscalid group.

Statistically significant increase compared to the control at the 5% level.

NO Indicates a large but nor statistically significant increase.

Significantly enhanced growth was observed with 92% of the selected strains in the treatment with boscalid 2.5 ppm. Although the enhanced growth with strain 286a was not statistically significant, the strain was selected because of substantially enhanced growth at all 3 doses. 38, 38, and 23% of the selected strains have shown positive growth with one, two and all three doses respectively.

Bellis/Boscalid	"Growth" as % of control				0 ppm	5% LSD
Strain	2.5 ppm	5ppm	10 ppm	Average %		
248	100.3	98.5	103.7	100.8	69.7	6.66
249	102.0	108.3	99.3	103.2	69.6	3.94
250	101.2	101.2	97.1	99.9	88.3	6.51
250!	92.8	92.7	87.5	91.0	70.9	8.39
251	102.9	105.8	64.7	91.1	97.2	18.65
252	89.3	94.6	84.8	89.5	48.4	4.85
253	97.6	101.3	96.4	98.4	68.4	6.11
255	107.4	105.4	94.5	102.4	62.7	18.74
257	100.2	105.6	103.7	103.2	84.0	5.69
258	101.7	99.2	93.9	98.3	81.2	12.31
259	101.7	109.4	110.8	107.3	28.4	23.13
261	93.7	92.5	91.2	92.5	50.4	17.24
262	84.8	80.0	79.5	81.4	49.8	7.05
265	80.4	101.7	93.4	91.8	42.3	23.63
266	107.6	106.9	100.2	104.9	75.0	9.66
274	96.1	98.5	95.1	96.6	65.0	9.45
275	104.9	113.3	104.0	107.4	73.5	8.71
276	107.4	106.3	92.2	102.0	75.8	18.42
277	97.2	104.3	99.8	100.5	82.8	7.90
278	101.1	100.6	96.0	99.2	88.9	4.69
280	99.4	106.2	107.3	104.3	59.5	5.27
281	92.2	99.7	84.3	92.1	69.8	11.09
282	86.9	86.4	107.0	93.4	79.5	99.81
283	100.1	109.0	108.4	105.8	53.5	9.38
284	113.4	125.2	122.4	120.3	44.4	17.70
285	119.3	126.2	128.9	124.8	39.2	16.58
286	97.7	97.0	90.2	95.0	61.7	8.44
286a	113.5	129.8	116.3	119.8	52.5	30.95
287	81.9	103.2	123.7	102.9266	2.0	50.07
289	101.8	111.7	124.3	112.6	50.4	15.04

Table 3.4. The primary screening results with the isolates from the Bellis group tested with boscalid.
Statistically significant increase compared to the control at the 5% level.
NO Indicates a large but nor statistically significant increase.

The remaining isolates were not selected either because there was insufficient "growth" enhancement or the growth of the isolate during the experimental period was, compared to the other strains, relatively low indicating that the strain might be difficult to culture and thus less

desirable for future work. Strain 53 and 96 isolated from the Revysol® trials were also tested for growth enhancement in the presence of boscalid in the primary screen. Although strain 53 showed increased (10 -27%) "growth" at all 3 doses, the increased growth was not statistically significant.

3.2.1.3. Pyraclostrobin Group

Bellis/ Pyraclostrobin	"Growth" as % of control				0 ppm	5% LSD
Strain	2.5 ppm	5ppm	10 ppm	Average %		
248	97.02	105.42	85.66	96.03	80.53	14.63
249	95.39	91.93	70.52	85.95	59.20	20.56
250	105.11	115.25	117.40	112.59	57.56	6.98
250!	104.73	106.08	94.17	101.66	54.78	16.96
251	102.00	99.46	81.01	94.16	84.81	5.85
252	113.16	132.42	136.91	127.50	41.10	7.46
253	94.81	95.44	71.42	87.22	62.12	10.01
255	99.66	99.76	78.44	92.62	65.39	9.69
257	97.02	105.42	85.66	96.03	80.53	14.63
258	104.71	115.21	100.98	106.97	89.75	6.78
259	109.30	110.46	97.29	105.68	48.14	9.52
261	125.67	142.17	144.85	137.57	38.23	25.15
262	113.98	126.22	126.04	122.08	44.62	9.41
265	86.33	96.26	91.32	91.30	42.23	9.16
266	102.70	100.98	95.94	99.87	90.39	11.58
276	96.20	99.86	94.70	96.92	84.33	11.45
277	98.36	101.25	91.89	97.16	85.61	5.20
278	101.73	101.85	96.92	100.16	90.55	3.46
280	106.15	120.08	113.31	113.18	42.82	18.06
281	97.51	95.44	76.51	89.82	54.36	53.38
282	87.09	104.22	95.96	95.76	74.84	18.47
283	105.36	109.64	104.50	106.50	56.04	9.91
284	111.01	119.81	94.01	108.28	50.10	20.24
285	85.32	91.88	79.12	85.44	57.01	10.90
286	81.60	72.42	68.85	74.29	67.00	13.25
289	93.02	94.58	80.47	89.35	62.13	13.60

Table 3.5. The primary screening results with the isolates from the Bellis group tested with pyraclostrobin.
Statistically significant increase compared to the control at the 5% level.

Since Bellis is the combination of pyraclostrobin and boscalid the isolates derived from these trial sites were also tested in the presence of pyraclostrobin. 8 strains; 250, 252, 258, 259, 261, 262, 280, and 284 out of 26 showed statistically higher "growth" with 2.5ppm pyraclostrobin. 23, 38, and 38 % of selected isolates had significantly positive "growth" at 3, 2, and only 1 dose

respectively. The remaining 26 isolates were not considered of further interest since no sufficient "growth" increase was observed (Table 3.5).

3.2.1.4. Fluxapyroxad Group

Fluxapyroxad	"Growth" as % of control				0 ppm	5% LSD
Strain	1.25 ppm	2.5ppm	5 ppm	Average %		
DSM10	49.77	59.55	66.27	58.53	43.90	72.17
53	36.61	57.83	84.93	59.79	44.84	91.75
61.a	0.49	38.31	36.62	25.14	18.85	97.99
61.b	130.25	114.42	128.61	124.43	93.32	24.57
80	100.46	132.27	109.31	114.01	85.51	29.26
124	114.75	126.09	72.60	104.48	78.36	36.88
185	77.76	81.34	71.55	76.89	57.67	0.98
186	66.18	17.07	6.46	29.90	22.43	96.49
190	73.84	85.14	62.07	73.68	55.26	82.26
210	87.71	127.58	151.65	122.31	91.74	2.82
211	103.43	64.98	78.01	82.14	61.61	94.85
212	82.08	60.99	156.89	99.99	74.99	3.11
213	268.91	155.21	176.32	200.15	150.11	2.35
214	93.09	80.93	161.59	111.87	83.90	6.52
215	105.17	82.39	60.07	82.54	61.91	8.98
216	2.29	7.26	85.92	31.83	23.87	4.97
217	171.31	165.14	136.53	157.66	118.25	3.90
218	442.04	501.41	483.79	475.75	356.81	14.19
219	49.87	23.68	12.50	28.68	21.51	100.99
220	44.78	72.63	41.26	52.89	39.67	93.78
222	96.32	80.87	55.53	77.57	58.18	92.96
222	94.46	97.49	98.60	96.85	72.64	92.46
223	99.12	101.65	89.25	96.67	72.51	63.21
225	71.84	109.44	85.05	88.77	66.58	66.84
226	196.42	181.03	180.90	186.11	139.59	0.41
227	92.63	115.40	104.10	104.05	78.03	2.68
228	0.83	0.40	0.86	0.70	0.52	61.36
229	27.30	6.30	9.04	14.21	10.66	65.39
230	39.71	0.57	0.85	13.71	10.28	71.10
231	44.20	39.43	50.19	44.61	33.45	1.06
233	-913.17	-790.73	-2768.78	-1490.89	-1118.17	-0.02
234	95.82	82.42	78.56	85.60	64.20	39.02
235	20.84	7.10	29.11	19.01	14.26	2.37
238	96.27	105.47	95.66	99.13	74.35	2.29

Table 3.6. The primary screening results with the isolates from the fluxapyroxad group tested with fluxapyroxad. Statistically significant increase compared to the control at the 5% level.

25 strains have been isolated from leaf samples from fluxapyroxad trials. Strain 218 was selected for secondary screening having a high growth enhancement at all three

doses.Moreover, comparing the bacterial behaviour with the presence of different fungicides, some isolates from other fungicides were also screened with fluxapyroxad, here we applied strains 53, 61, 80, and 124 from Revysol® and 185 and 186 from boscalid. It demonstrates the prompt growth of strain 61.b, 80, and in smaller size strain 124 with the average percent of growth 124, 114, and 104 respectively (Table 3.6).

3.2.2. Secondary Screening

3.2.2.1. Revysol® Isolates tested in the Presence of Revysol®.

Revysol®	"Growth" %									0 ppm	LSD %
Strain ID ppm	0.625	1.25	2.5	5	7.5	10	15	37.5	Avge %		
53	133.4	139.3	94.0	138.0		112.3	84.3		116.9	48.9	70.1
61.a	147.9	103.1	128.7	63.1		37.5	66.3		91.1	40.6	65.6
61.b					110.3		84.1	14.3	69.5	56.8	12.2
80	205.2	200.4	206.8	141.5		122.9	79.7		159.4	22.7	48.5
96	173.3	121.1	70.6	135.7		50.3	10.6		93.6	43.0	57.3
124	159.4	191.9	180.7	217.3		118.6	51.7		153.3	22.7	39.4

Table 3.7. The secondary screening results with the selected isolates from the Revysol® group tested with Revysol®.

Statistically significant increase compared to the control at the 5% level.

NO Indicates a large but nor statistically significant increase.

In the secondary screening, strains were tested with a broader range of doses of each fungicide from 0.625 to 50 ppm. The 6 isolates 53, 61.a, 61.b, 80, 96, and 124 ppm which were identified from the primary screening tests conducted with isolates derived from field trials where the fungicide Revysol® was used, based on the integrated OD value of each treatment over the experimental time (the area under the OD curve).

In the secondary screens with the Revysol® isolates, the greatest effects occurred at the rates between 0.625 and 10 ppm Revysol®. All the selected strains showed (numerically or statistically) greater "growth" than the control in the 0.625ppm treatment, 80% with the 1,25 and 5 ppm treatments, and 60% with the 2.5 ppm treatment. At 10 ppm and higher rates, inhibition occurred. So, in the 10 ppm treatment only the "integrated" ODs of the isolate 80 was significantly better than the control and in the 15 and 37.5 ppm all of isolates the integrated ODs were significantly lower than the control. These findings are in agreement with the primary screening where 100% of the selected isolates showed markedly enhanced growth with the 2.5 ppm treatment (the lowest rate). Among the selected isolates, 124 and 80 showed the integrated

OD significantly increased with a broad range of Revysol® rates compared to the control (Table 3.7).

3.2.2.2. Boscalid and Bellis Isolates tested in the Presence of Boscalid.

In the secondary screening with isolates from boscalid field trials, the initial OD values were subtracted from the OD values throughout the experiment to take into account any differences of the medium composition between the treatments. The integrated parameter resulting is referred to as the "growth" resulting from the treatment. 23 isolates were selected also on the basis of "growth" for secondary screening. Again, the rates used were from 0.625 to 50 ppm.
In 53% of the tests markedly enhanced "growth" occurred in the presence of boscalid and 32% (8) of the isolates have shown significantly higher "growth" than the control at all rates between 1.25 and 25 ppm. Among the best were the isolates 184, 201, 250, 260a, and 284 which were tested on at least two occasions. These strains were thus selected for identification and further evaluation.
The isolates (184, 189, 197, 203, 208, 249, 258, 259, 262, and 281) were tested in the secondary screen due to some different behaviour in the primary screens. For instance, the isolate 203 showed significantly greater "growth" compared to the control but the level of "growth" in the control was very low (Table 3.3), In the secondary screen, although the isolate grew well, there was no appreciable change in the treatments compared to the control. The isolates 197, 259, also showed a very low "growth" not only in the primary but also in the secondary screening. With the isolates 208 and 269 the "growth" of the treatments was slightly higher than the control in the primary screening. However, no significant increase in "growth" was observed in the secondary screen. With the isolate 249, in the primary screening only the "growth" with the 2.5 ppm treatment bordered on statistical significance and in the secondary screening again only one treatment, 1.25 ppm, reached a statistically significant level. A similar situation occurred with the isolate 258, where a slight increase in "growth" was observed in the primary screening and in the secondary screen only the "growth" of the 1.25 ppm treatment bordered on statistical significance With the isolate 281 there was no positive change in the treatments compared to the control in the primary screen whereas two higher doses (10 and 25 ppm) outside the dose ranges used in the primary screen showed significantly enhanced "growth" compared to the control but the isolate grew very little in both of the secondary tests (Table 3.8).

Strain	"Growth" as % of control								Control	LSD %
Boscalid ppm	0.625	1.25	2.5	5	10	25	50	Mean %		
180	107.3	112.4	104.2	106.9	95.6	38.9	34.2	85.6	97.32	15.7
184	102.0	109.6	109.3	111.4	113.3	113.3	104.6	109.1	92.14	3.3
184	100.7	106.6	103.5	107.8	108.1	109.5	98.3	104.9	85.41	2.5
189	92.1	100.0	97.9	95.2	96.6	100.1	91.2	96.2	61.16	5.0
195	100.5	107.4	107.7	109.2	105.3	106.8	104.5	105.9	63.35	3.3
196	101.8	106.7	101.7	111.5	109.6	109.5	105.4	106.6	64.62	3.9
196	97.5	105.5	100.2	100.9	97.4	100.7	95.5	99.7	27.38	2.6
196	102.2	110.6	114.9	122.1	104.8	36.6	15.1	86.6	53.24	16.9
197	124.4	94.4	87.2	57.8	107.4	134.7	98.2	100.6	3.62	43.8
201	108.7	119.9	118.8	126.2	124.9	119.4	103.3	11 7.3	61.08	10.6
201	115.0	125.0	126.3	126.0	122.5	129.0	126.6	124.4	73.58	8.9
201	89.7	96.8	92.3	100.3	95.0	102.5	104.3	97.3	33.02	6.6
201	112.1	114.7	117.8	118.8	118.1	106.0	38.9	103.8	82.79	18.4
203	100.5	103.5	90.1	100.3	72.9	72.2	78.3	88.3	80.16	7.0
205	97.6	101.7	101.5	103.0	102.2	110.3	92.7	101.3	53.38	12.9
208	98.5	102.3	102.6	104.7	104.6	102.1	97.8	101.8	65.42	4.9
249	89.4	105.0	103.7	105.7	110.9	118.2	92.9	103.7	59.87	11.6
250	101.9	105.8	104.1	106.8	107.6	109.8	101.6	105.4	79.99	3.0
250	105.1	116.2	112.4	118.2	117.3	117.8	101.3	112.6	45.09	6.1
250	101.8	106.8	111.2	114.1	113.5	36.3	15.8	85.6	71.03	17.5
258	96.3	103.3	97.9	100.3	99.1	100.8	95.9	99.1	86.04	2.9
259	98.6	101.9	104.2	105.7	104.4	109.0	124.2	106.9	39.02	7.9
260a	110.9	118.7	109.5	110.9	102.8	112.1	91.7	108.1	78.95	5.1
260a	98.9	107.9	98.6	98.6	98.2	96.7	94.4	99.0	42.53	3.9
260a	106.4	115.3	109.9	114.4	106.5	104.5	88.0	106.4	75.09	8.1
260a	103.7	101.5	97.0	99.8	100.7	108.8	104.6	102.3	66.47	3.9
260	110.0	114.7	109.8	104.0	99.5	27.3	32.2	85.3	89.20	19.4
261	92.7	95.2	98.7	96.9	102.5	102.8	90.7	97.1	20.06	6.4
261	101.0	111.9	112.5	118.5	123.4	124.4	110.9	114.7	41.54	4.8
261	104.7	105.9	107.1	106.7	121.7	129.1	103.0	111.2	48.77	7.8
262	96.7	102.9	100.5	103.2	104.1	104.6	99.8	101.7	69.46	5.7
280	95.4	99.9	94.4	100.9	106.2	114.2	96.1	101.0	59.45	10.6
280	101.0	107.9	102.1	108.0	112.3	20.7	51.1	86.1	55.75	15.6
281	94.7	103.9	91.6	100.6	102.5	112.9	90.8	99.6	22.65	8.8
281	98.7	95.9	100.8	100.4	101.7	100.5	100.1	99.7	31.19	5.2
284	101.5	110.7	110.6	111.1	111.9	108.3	105.7	108.6	26.17	4.1
284	100.1	100.9	102.1	111.2	104.8	97.2	95.1	101.6	65.75	8.4
284	112.8	116.6	116.2	123.9	133.8	133.9	118.8	122.3	47.18	7.3
284	101.5	106.3	106.5	133.6	96.7	30.1	32.9	86.8	37.46	23.7
285	100.0	109.7	103.9	111.2	108.9	106.1	102.9	106.1	60.69	7.2
285	101.5	108.2	103.9	109.0	85.6	41.2	14.6	80.6	46.80	19.2

Table 3.8. The secondary screening results with the selected isolates from the boscalid group tested with Revysol®.

Statistically significant increase compared to the control at the 5% level.

NO Indicates a large but nor statistically significant increase.

3.2.2.3. Bellis Isolates tested in the Presence of Pyraclostrobin

Strain	"Growth" %									
Pyraclostrobin ppm	0.625	1.25	2.5	5	10	25	50	Mean %	Control	LSD %
249	95.74	100.88	95.70	100.71	100.46	92.82	68.59	93.6	70.93	2.8
250	97.03	105.01	106.39	108.12	105.56	90.68	69.56	97.5	79.37	3.9
258	102.86	108.05	102.69	107.44	108.30	108.06	94.87	104.6	76.76	7.8
259	92.09	96.02	83.60	81.78	70.36	56.50	28.71	72.7	48.57	5.4
261	108.03	109.99	92.94	101.91	100.28	98.47	92.21	100.5	55.78	14.9
262	106.26	109.28	100.76	96.23	73.53	70.01	65.16	88.7	68.75	34.6

Table 3.9. The secondary screening results with the selected isolates from the Revysol® group tested with pyraclostrobin.

Statistically significant increase compared to the control at the 5% level.

NO Idicates a large but nor statistically significant increase.

Six isolates obtained from the Bellis (a mixture containing the fungicides boscalid and pyraclostrobin) trials, were also tested in the secondary screen with pyraclostrobin with rates ranging from 0.625 to 50 ppm Isolates 258 and especially 250 had significantly greater "growth" in the presence of pyraclostrobin with the doses between 1.25 and 10 ppm and in the case of 258 also at 25ppm. Despite the significantly enhanced "growth" of the isolate 258, it was not selected for further investigation because it only showed enhanced "growth" in the presence of pyraclostrobin and the main focus of the project in the latter stages was boscalid.

3.2.2.4. Secondary Screening Dose Rates

The extended range of doses in the secondary screening tests of the Revysol® group didn't result in better growth in the doses lower than 2.5ppm except with the isolate 96. Rates higher than 10 ppm inhibited the "growth". In the boscalid group, rates of 10 ppm or higher didn't have an inhibitory effect on the "growth", but the "growth" enhancement did not increase further with increasing doses. The 50 ppm treatment tended to inhibit the "growth" which was also observed with pyraclostrobin, Doses lower than 2.5 ppm didn't show a stronger enhancement of "growth" and the 50 ppm treatment tended to inhibit the "growth" of the isolates.

The best performing strains from these secondary screens (6 from Revysol® sites and 9 from boscalid sites) were then selected for identification and further characterisation. As can be seen from (Table 3.9.) all the strains identified were from the Genus *Bacillus* and all were in the lowest risk group.

Fungicide	Isolate Number	Species	Risk Group
Revysol®	I8V801050053	*Bacillus megaterium*	1
Revysol®	I8V801050061.a	*Bacillus megaterium*	1
Revysol®	I8V801050061.b	*Bacillus subtilis / velenzensis*	1
Revysol®	I8V801050080	*Bacillus subtilis / velenzensis*	1
Revysol®	I8V801050096	*Bacillus megaterium*	1
Revysol®	I8V801050124	*Bacillus licheniformis*	1
Boscalid	I8V801050180	*Bacillus megaterium*	1
Boscalid	I8V801050195	*Bacillus megaterium*	1
Boscalid	I8V801050196	*Bacillus simplex*	1
Boscalid	I8V801050201	*Bacillus megaterium*	1
Boscalid	I8V801050250	*Bacillus megaterium*	1
Boscalid	I8V801050260	*Bacillus megaterium*	1
Boscalid	I8V801050280	*Bacillus subtilis*	1
Boscalid	I8V801050284	*Bacillus licheniformis*	1
Boscalid	I8V801050285	*Bacillus licheniformis*	1

Table 3.10. the identified bacteria isolated from leaves treated with fungicides Revysol® and boscalid or Bellis in field trials.

3.2.3. “30 minute” Experiments

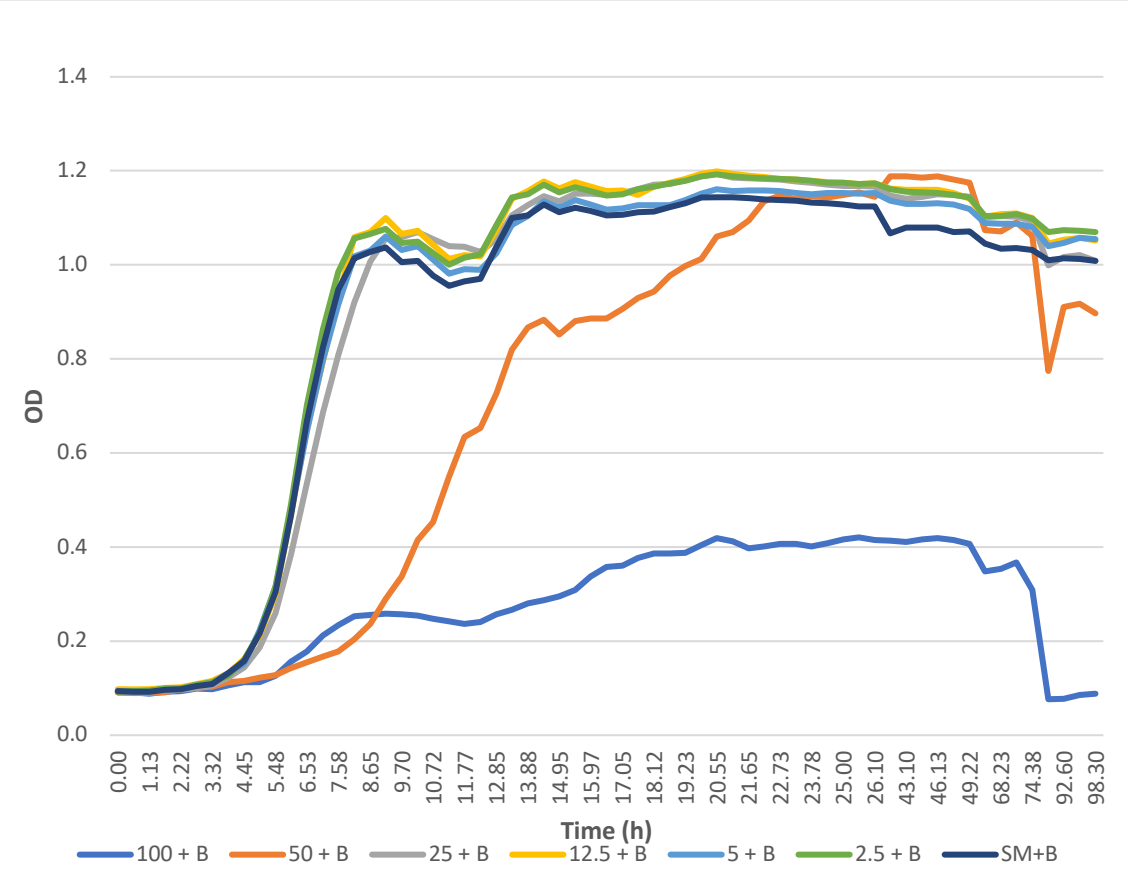


Figure 3.1. The growth curve of isolate 53 in the presence of different concentrations of Revysol® over 4 days.

The growth of isolate 53, an isolate from the Revysol® trials is monitored in treatments with different doses of Revysol® from 2.5 to 100 ppm over 4 days through measuring the OD at 600nm. During the first 48 hours, measurements are taken every 30 minutes and then 4 times per day every 3 to 4 hours between 8:00 and 17:00.

The growth of isolate 53 initiated with the lag phase over the first four hours and then the exponential phase begun in the control and treatments simultaneously. The first peak occurred 8 to 9 hours after the incubation started then after 5 hours living in the stationary phase a higher peak occurred. From then until the end of experiment the ODs remained at a similar level (Fig. 3.1). Exceptions to this are 50 and 100 ppm where the isolate had delayed exponential growth. With 100 ppm the isolate grew very little from 3 hours on, so the OD values were markedly less than that of in the control. With the 50 ppm treatment the isolate showed a delay in the onset of exponential growth until 8 hours after which the isolate grew exponentially until 43 hours when the highest OD was recorded. Although the peak value with 50 ppm is the highest value numerically it is not significantly higher than other treatments or the control.

In general, the responses of two highest Revysol® doses 100 and 50 ppm and to a lesser extent 25 ppm differ from the other three doses (Table 3.11).

Ass. (h)	100+B	50+B	25+B	12.5+B	5+B	2.5+B	SM+B	P-value	5% LSD	1% LSD	0.1% LSD
0.00	0.091	0.092	0.093	0.098	0.093	0.093	0.093	3.1E-07	0.004	0.005	0.007
0.62	0.091	0.090	0.093	0.098	0.093	0.093	0.092	2.4E-14	0.004	0.005	0.006
1.13	0.088	0.090	0.092	0.098	0.094	0.095	0.093	3.6E-13	0.004	0.005	0.006
1.70	0.093	0.091	0.095	0.100	0.099	0.099	0.096	3.5E-13	0.005	0.006	0.008
2.22	0.094	0.097	0.097	0.102	0.100	0.100	0.098	3.9E-09	0.005	0.007	0.009
2.80	0.099	0.100	0.101	0.109	0.108	0.107	0.104	6.0E-17	0.006	0.008	0.010
3.32	0.098	0.104	0.107	0.115	0.112	0.112	0.109	8.7E-25	0.005	0.007	0.009
3.90	0.106	0.113	0.122	0.132	0.132	0.126	0.132	6.0E-29	0.009	0.011	0.015
4.45	0.113	0.116	0.144	0.162	0.152	0.161	0.157	6.1E-38	0.011	0.014	0.018
4.98	0.113	0.122	0.186	0.212	0.224	0.217	0.216	1.4E-39	0.019	0.024	0.031
5.48	0.126	0.128	0.259	0.299	0.315	0.318	0.305	4.1E-43	0.029	0.038	0.048
6.02	0.157	0.142	0.384	0.468	0.473	0.490	0.467	2.9E-45	0.047	0.061	0.079
6.53	0.178	0.156	0.536	0.651	0.646	0.702	0.669	1.5E-48	0.064	0.084	0.108
7.07	0.213	0.167	0.685	0.795	0.797	0.863	0.824	3.1E-47	0.085	0.111	0.143
7.58	0.233	0.178	0.809	0.932	0.917	0.986	0.948	3.2E-48	0.097	0.127	0.163
8.07	0.254	0.205	0.921	1.060	1.018	1.057	1.014	5.5E-49	0.104	0.137	0.176
8.65	0.256	0.236	1.007	1.070	1.031	1.066	1.028	1.8E-49	0.105	0.138	0.177
9.12	0.259	0.289	1.057	1.100	1.061	1.077	1.038	3.7E-51	0.102	0.134	0.172
9.70	0.257	0.337	1.059	1.066	1.033	1.047	1.006	8.8E-51	0.100	0.132	0.169
10.18	0.254	0.415	1.070	1.073	1.040	1.050	1.008	3.9E-49	0.105	0.138	0.178
10.72	0.248	0.453	1.056	1.042	1.011	1.026	0.978	4.1E-48	0.106	0.139	0.178
11.30	0.243	0.550	1.040	1.013	0.982	1.000	0.956	9.0E-48	0.105	0.137	0.176

Ass. (h)	100+B	50+B	25+B	12.5+B	5+B	2.5+B	SM+B	P-value	5% LSD	1% LSD	0.1% LSD
11.77	0.237	0.635	1.039	1.021	0.991	1.015	0.965	1.4E-45	0.113	0.148	0.190
12.32	0.240	0.653	1.028	1.018	0.989	1.022	0.971	5.4E-44	0.119	0.155	0.200
12.85	0.257	0.726	1.055	1.075	1.025	1.084	1.038	9.1E-43	0.130	0.171	0.220
13.33	0.266	0.820	1.105	1.141	1.086	1.144	1.099	1.3E-43	0.135	0.177	0.228
13.88	0.281	0.868	1.127	1.157	1.104	1.150	1.106	1.3E-43	0.137	0.180	0.232
14.38	0.287	0.884	1.147	1.178	1.135	1.171	1.128	1.5E-44	0.136	0.179	0.230
14.95	0.296	0.852	1.136	1.162	1.122	1.155	1.113	1.8E-44	0.135	0.177	0.227
15.42	0.309	0.881	1.152	1.176	1.138	1.166	1.122	3.9E-46	0.130	0.170	0.218
15.97	0.338	0.887	1.152	1.166	1.129	1.158	1.115	4.3E-46	0.129	0.169	0.217
16.55	0.358	0.886	1.149	1.157	1.118	1.148	1.105	2.9E-45	0.131	0.172	0.221
17.05	0.361	0.907	1.154	1.159	1.120	1.150	1.107	3.4E-45	0.132	0.173	0.222
17.58	0.377	0.930	1.161	1.149	1.127	1.162	1.112	3.9E-45	0.133	0.174	0.224
18.12	0.387	0.944	1.171	1.167	1.127	1.166	1.113	1.2E-44	0.135	0.177	0.228
18.68	0.386	0.977	1.172	1.174	1.127	1.174	1.123	2.5E-44	0.138	0.181	0.233
19.23	0.388	0.997	1.180	1.183	1.139	1.179	1.131	1.2E-44	0.138	0.181	0.233
19.77	0.404	1.013	1.189	1.194	1.152	1.189	1.143	6.9E-45	0.138	0.181	0.233
20.55	0.419	1.060	1.194	1.199	1.161	1.193	1.144	1.4E-45	0.136	0.178	0.230
21.07	0.413	1.070	1.186	1.194	1.158	1.189	1.144	2.3E-47	0.129	0.169	0.218
21.65	0.397	1.095	1.184	1.190	1.158	1.186	1.142	2.9E-49	0.123	0.161	0.206
22.20	0.402	1.136	1.183	1.188	1.158	1.184	1.140	1.3E-49	0.122	0.159	0.205
22.73	0.407	1.148	1.181	1.184	1.157	1.183	1.138	1.1E-49	0.121	0.159	0.205
23.28	0.407	1.140	1.178	1.181	1.153	1.181	1.137	7.1E-50	0.120	0.158	0.203
23.78	0.401	1.146	1.175	1.179	1.150	1.179	1.133	7.7E-50	0.120	0.158	0.203
24.37	0.409	1.144	1.171	1.177	1.152	1.175	1.131	4.5E-49	0.123	0.161	0.207
25.00	0.416	1.149	1.169	1.175	1.153	1.174	1.128	2.8E-48	0.126	0.165	0.212
25.55	0.421	1.155	1.167	1.173	1.152	1.172	1.125	5.3E-48	0.127	0.166	0.213
26.10	0.416	1.145	1.166	1.173	1.152	1.173	1.124	4.2E-48	0.126	0.165	0.212
41.30	0.413	1.189	1.148	1.163	1.137	1.162	1.067	1.7E-47	0.126	0.165	0.213
43.10	0.411	1.188	1.141	1.160	1.131	1.156	1.079	6.9E-48	0.125	0.163	0.210
44.78	0.416	1.186	1.146	1.159	1.130	1.155	1.080	5.2E-48	0.124	0.163	0.209
46.13	0.419	1.188	1.151	1.160	1.132	1.154	1.079	8.0E-48	0.125	0.163	0.210
47.68	0.415	1.182	1.150	1.153	1.128	1.149	1.070	8.7E-48	0.124	0.163	0.209
49.22	0.407	1.175	1.145	1.140	1.119	1.143	1.072	1.8E-48	0.121	0.159	0.204
65.30	0.349	1.074	1.102	1.103	1.089	1.105	1.045	2.2E-55	0.095	0.124	0.159
68.23	0.354	1.072	1.103	1.109	1.088	1.105	1.035	2.8E-55	0.095	0.124	0.160
69.97	0.368	1.090	1.103	1.109	1.087	1.108	1.036	1.3E-54	0.097	0.127	0.163
74.38	0.309	1.062	1.093	1.099	1.081	1.099	1.032	4.2E-58	0.087	0.114	0.146
89.53	0.077	0.774	0.999	1.046	1.040	1.070	1.011	3.2E-53	0.095	0.124	0.160
92.60	0.077	0.910	1.017	1.053	1.047	1.074	1.015	3.0E-63	0.072	0.095	0.122
95.60	0.085	0.917	1.021	1.058	1.057	1.073	1.013	3.4E-64	0.071	0.093	0.119
98.30	0.085	0.917	1.021	1.058	1.057	1.073	1.013	1.0E-59	0.080	0.104	0.134

Table 3.11. The mean OD values for each treatment at each assessment time for isolate 53 (B) in treatments with 100, 50, 25, 12.5, 5, and 2.5 ppm Revysol® and in the control (SM+B) over the experimental period. An analysis of variance was conducted at each assessment time and significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and in significantly decreased ODs highlighted in shades of orange.

Treatment	"Growth"
100 + B	20.59
50 + B	83.86
25 + B	92.93
12.5 + B	94.08
5 + B	92.54
2.5 + B	94.89
SM+B	89.04
LSD 5%	7.211
LSD 1%	9.591
LSD 0.1%	12.151

Table 3.12. The mean "growth" of isolate 53 (B) in treatments with 100, 50, 25, 12.5, 5, and 2.5 ppm Revysol® and in the control (SM+B) over the experimental period. Significantly increased growth at the 5, 1, and 0.1 % LSD highlighted in shades of green and significantly decreased growth in shades of orange.

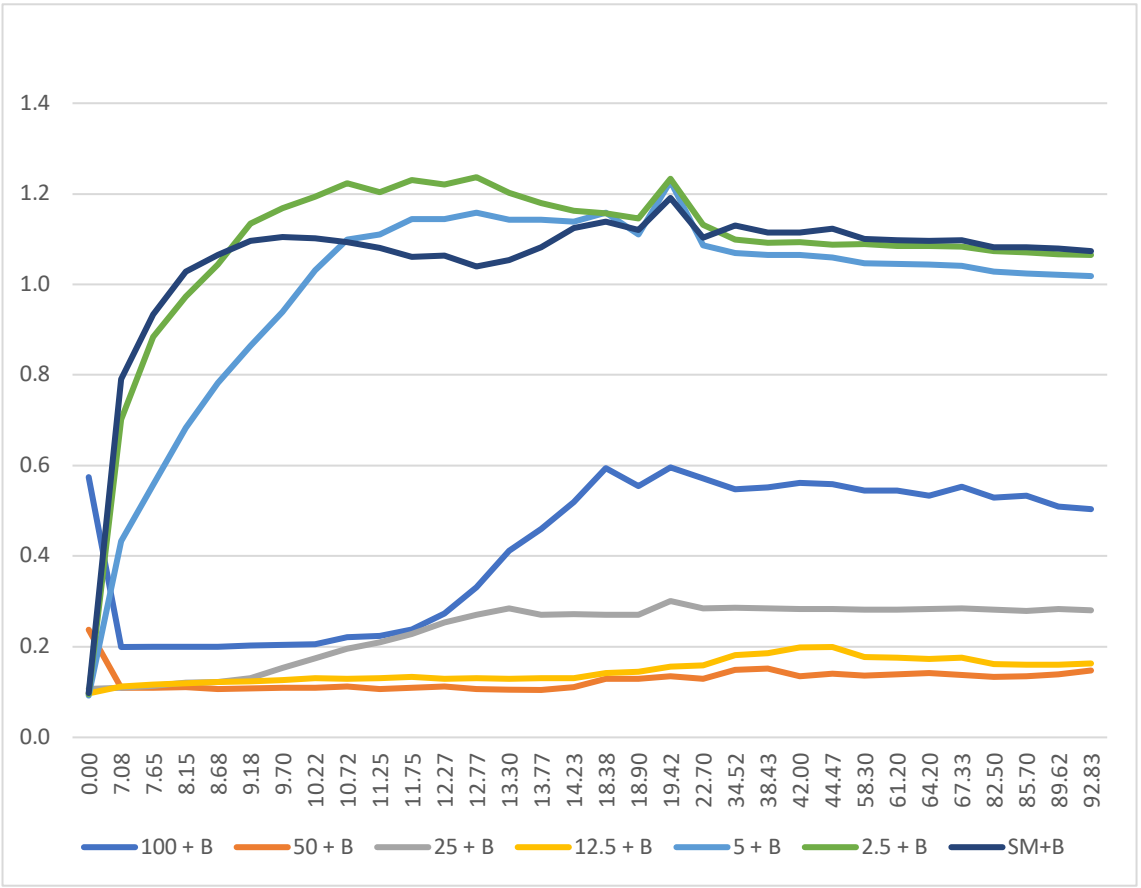


Figure 3.2. The growth curve of isolate 61.a in the presence of different concentrations of Revysol® over 4 days.

The growth of 61.a an isolate form Revysol® trials is monitored in treatments with different doses of Revysol® from 100 to 2.5 ppm through measuring the OD at 600nm within 4 days. With 2.5, 5 ppm and the control, the isolate started exponential growth immediately. Initially the ODs of the control were significantly higher than those for the 2, 5, and 5 ppm treatments but after 8 to 9 hours the 2.5 ppm treatment grew significantly more than the control until 13 hours after which the control and two lower concentrations were not statistically

distinguishable.as the growth curves plateaued. With the 5ppm treatment the ODs were somewhat lower than those of the 2.5ppm and control treatments throughout the experiment (Fig. 3.2).

The 12.5, 25, 50, and 100 ppm treatments showed a different pattern. The ODs of all these treatments were significantly smaller (mostly at the 0.1% level) than those of the control (Table 3.14). All treatments and the control plateaued out more or less after 14 hours and stayed in this phase until the end of experiment (Fig. 3.2).

Ass. (h)	100+B	50+B	25+B	12.5+B	5+B	2.5+B	SM+B	P-value	5% LSD	1% LSD	0.1% LSD
0.00	0.574	0.238	0.107	0.097	0.092	0.095	0.098	P-value	0.021	0.027	0.035
7.08	0.200	0.110	0.109	0.112	0.433	0.702	0.791	2.2E-70	0.034	0.045	0.057
7.65	0.200	0.109	0.114	0.117	0.558	0.884	0.933	2.2E-69	0.039	0.051	0.066
8.15	0.200	0.111	0.121	0.120	0.683	0.973	1.028	2.3E-72	0.036	0.047	0.061
8.68	0.200	0.107	0.122	0.122	0.783	1.044	1.065	3.1E-79	0.030	0.040	0.051
9.18	0.202	0.108	0.130	0.123	0.864	1.134	1.096	1.3E-87	0.032	0.042	0.054
9.70	0.204	0.109	0.154	0.127	0.939	1.168	1.105	2.1E-87	0.036	0.048	0.061
10.22	0.205	0.110	0.174	0.131	1.031	1.193	1.101	3.3E-84	0.049	0.065	0.083
10.72	0.221	0.113	0.196	0.130	1.099	1.223	1.094	3.7E-74	0.062	0.081	0.105
11.25	0.224	0.107	0.210	0.131	1.110	1.204	1.081	7.7E-67	0.071	0.093	0.120
11.75	0.238	0.110	0.229	0.134	1.144	1.231	1.060	9.0E-62	0.085	0.111	0.143
12.27	0.274	0.113	0.253	0.129	1.144	1.221	1.063	5.6E-56	0.100	0.131	0.168
12.77	0.331	0.106	0.271	0.131	1.159	1.237	1.040	3.4E-50	0.114	0.149	0.192
13.30	0.412	0.106	0.284	0.130	1.143	1.202	1.054	1.2E-45	0.125	0.163	0.210
13.77	0.460	0.105	0.271	0.131	1.142	1.179	1.082	2.8E-42	0.129	0.169	0.217
14.23	0.519	0.111	0.272	0.131	1.138	1.163	1.124	1.7E-41	0.139	0.183	0.235
18.38	0.595	0.129	0.271	0.141	1.158	1.157	1.138	2.0E-39	0.149	0.195	0.250
18.90	0.555	0.129	0.271	0.145	1.111	1.145	1.120	1.3E-37	0.150	0.196	0.252
19.42	0.596	0.135	0.301	0.156	1.228	1.234	1.191	1.4E-36	0.165	0.217	0.279
22.70	0.571	0.129	0.285	0.159	1.086	1.132	1.104	5.9E-36	0.161	0.211	0.271
34.52	0.548	0.149	0.287	0.182	1.070	1.099	1.130	9.7E-34	0.153	0.201	0.258
38.43	0.552	0.152	0.284	0.186	1.064	1.092	1.115	3.9E-35	0.156	0.204	0.263
42.00	0.561	0.136	0.284	0.199	1.064	1.093	1.114	2.9E-34	0.156	0.204	0.263
44.47	0.559	0.140	0.283	0.200	1.060	1.088	1.123	2.7E-34	0.157	0.206	0.264
58.30	0.544	0.137	0.281	0.178	1.047	1.089	1.100	3.9E-34	0.151	0.197	0.254
61.20	0.545	0.139	0.282	0.177	1.046	1.085	1.098	5.3E-35	0.151	0.198	0.255
64.20	0.534	0.141	0.284	0.173	1.045	1.085	1.096	9.6E-35	0.152	0.199	0.256
67.33	0.554	0.138	0.284	0.175	1.042	1.083	1.098	1.7E-34	0.151	0.197	0.254
82.50	0.529	0.133	0.282	0.162	1.028	1.074	1.082	8.4E-35	0.150	0.196	0.252
85.70	0.533	0.136	0.280	0.161	1.024	1.071	1.083	1.5E-34	0.149	0.195	0.251
89.62	0.510	0.140	0.283	0.161	1.021	1.066	1.079	8.9E-35	0.150	0.197	0.253
92.83	0.504	0.148	0.281	0.163	1.018	1.065	1.074	3.0E-34	0.151	0.198	0.255

Table 3.13. The mean OD values for each treatment at each assessment time for isolate 61.a (B) in treatments with 100, 50, 25, 12.5, 5, and 2.5 ppm Revysol® and in the control (SM+B) over the experimental period. An analysis of variance was conducted at each assessment time and significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and in significantly decreased ODs highlighted in shades of orange.

Treatment	"Growth"
100 + B	-5.42
50 + B	-8.94
25 + B	13.83
12.5 + B	6.02
5 + B	82.80
2.5 + B	87.90
SM+B	88.09
LSD 5%	12.092
LSD 1%	16.083
LSD 0.1%	20.376

Table 3.14. The mean "growth" of isolate 61.a (B) in treatments with 100, 50, 25, 12.5, 5, and 2.5 ppm Revysol® and in the control (SM+B) over the experimental period. Significantly increased growth at the 5, 1, and 0.1 % LSD highlighted in shades of green and significantly decreased growth in shades of orange.

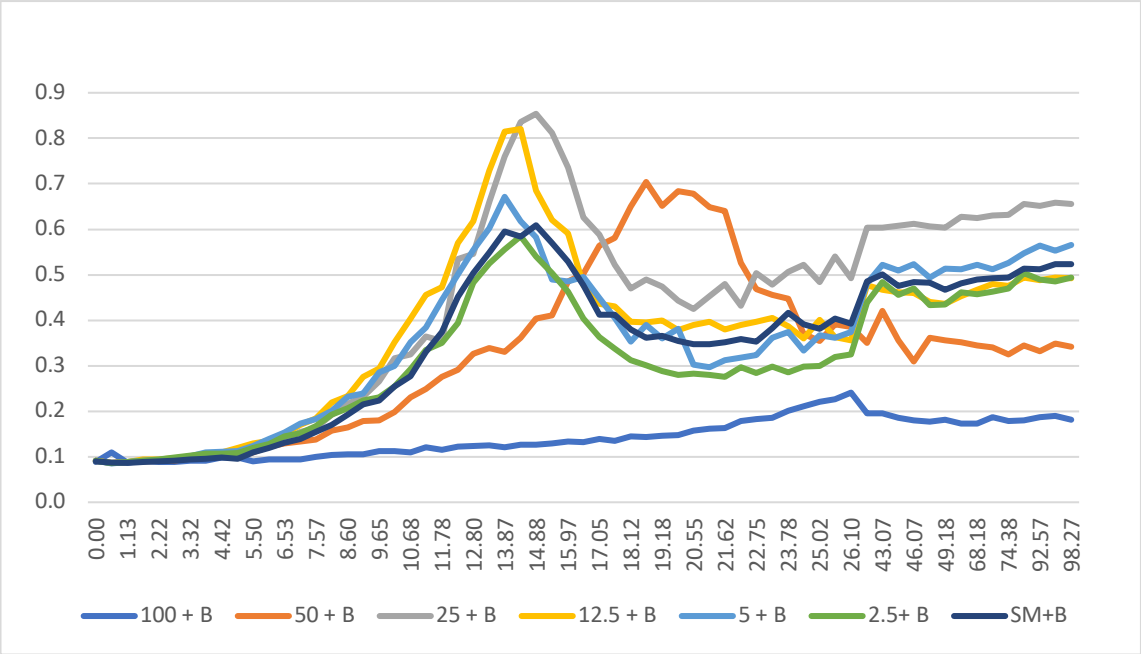


Figure 3.3. The growth curve of isolate 61.b in the presence of different concentrations of Revysol® over 4 days.

Isolates 61.b and 80 indicated a very similar growth pattern, started with a lag phase and then after 4 to 5 hours exponential growth began. With the exception of the 50 and 100ppm treatments, the isolates reached peak values in the treatments and the control after 13 to 15 hours. With isolate 61.b, the 3 biggest peaks were observed with the 25, 12.5, and 5 ppm treatments from the biggest to the smallest size respectively, although with isolate 80, the highest peaks belonged to 12.5, 5, and 2.5 ppm respectively. At the higher doses (50 and 100 ppm) there is a delay in the exponential phase starting, peak values occur later and are much smaller than with the other treatments. The effect is more sever with the 100 ppm treatment where relatively little growth occurred treatments and control dropped down (Fig. 3.3). Therefore, there was the significant greater growth compared to the control at this specific time frame (Table.3.15).

Ass. (h)	100+B	50+B	25+ B	12.5+ B	5+B	2.5+ B	SM+B	P-value	5% LSD	1% LSD	0.1% LSD
0.00	0.088	0.092	0.090	0.091	0.091	0.091	0.091	9.0E-09	0.003	0.004	0.005
0.62	0.110	0.087	0.087	0.086	0.085	0.086	0.087	2.5E-06	0.008	0.010	0.013
1.13	0.087	0.088	0.088	0.089	0.089	0.088	0.087	3.7E-06	0.002	0.003	0.004
1.70	0.092	0.089	0.089	0.094	0.091	0.091	0.088	3.1E-05	0.004	0.006	0.007
2.22	0.088	0.091	0.091	0.095	0.093	0.094	0.090	8.9E-12	0.003	0.004	0.005
2.80	0.089	0.092	0.093	0.097	0.096	0.098	0.092	6.5E-13	0.003	0.004	0.006
3.32	0.091	0.098	0.099	0.100	0.102	0.103	0.094	1.0E-15	0.005	0.006	0.008
3.90	0.091	0.101	0.104	0.105	0.109	0.108	0.096	1.7E-25	0.005	0.006	0.008
4.45	0.099	0.109	0.103	0.110	0.111	0.106	0.099	2.1E-19	0.006	0.008	0.011
4.98	0.098	0.100	0.107	0.119	0.112	0.108	0.096	1.4E-18	0.007	0.010	0.012
5.48	0.090	0.110	0.116	0.129	0.124	0.119	0.109	4.1E-22	0.009	0.012	0.016
6.02	0.095	0.123	0.125	0.135	0.139	0.128	0.119	2.5E-22	0.011	0.015	0.019
6.53	0.094	0.129	0.139	0.150	0.154	0.145	0.131	2.2E-24	0.014	0.018	0.024
7.07	0.094	0.133	0.155	0.170	0.173	0.152	0.139	4.9E-27	0.016	0.021	0.027
7.58	0.100	0.138	0.163	0.185	0.183	0.168	0.155	1.9E-26	0.019	0.025	0.033
8.07	0.105	0.158	0.193	0.219	0.202	0.193	0.170	1.8E-27	0.024	0.031	0.040
8.65	0.106	0.164	0.217	0.234	0.232	0.207	0.193	6.9E-25	0.030	0.040	0.051
9.12	0.105	0.179	0.232	0.276	0.239	0.224	0.215	3.1E-24	0.037	0.049	0.062
9.70	0.112	0.180	0.266	0.294	0.285	0.230	0.223	7.8E-28	0.037	0.048	0.062
10.18	0.113	0.198	0.317	0.352	0.299	0.255	0.255	8.0E-25	0.050	0.066	0.085
10.72	0.109	0.231	0.325	0.404	0.351	0.292	0.278	3.3E-25	0.057	0.075	0.097
11.30	0.120	0.248	0.364	0.456	0.384	0.335	0.331	5.6E-25	0.069	0.090	0.116
11.77	0.116	0.276	0.355	0.473	0.445	0.350	0.374	2.8E-23	0.081	0.106	0.136
12.32	0.123	0.292	0.534	0.570	0.501	0.394	0.451	7.2E-24	0.101	0.132	0.170
12.85	0.124	0.326	0.546	0.618	0.555	0.483	0.504	1.1E-23	0.113	0.148	0.191
13.33	0.126	0.340	0.660	0.729	0.603	0.525	0.549	8.8E-24	0.131	0.172	0.222
13.88	0.121	0.331	0.759	0.815	0.671	0.555	0.595	7.4E-24	0.148	0.194	0.249
14.38	0.126	0.361	0.836	0.821	0.618	0.585	0.583	2.2E-23	0.153	0.200	0.258
14.95	0.126	0.404	0.854	0.686	0.582	0.540	0.609	5.0E-22	0.154	0.202	0.259
15.42	0.129	0.410	0.813	0.621	0.490	0.503	0.569	8.5E-21	0.148	0.194	0.250
15.97	0.134	0.485	0.736	0.590	0.486	0.462	0.529	3.7E-20	0.142	0.186	0.239
16.55	0.133	0.502	0.626	0.477	0.496	0.404	0.477	4.4E-20	0.125	0.164	0.210
17.05	0.139	0.565	0.588	0.437	0.449	0.363	0.412	3.9E-21	0.112	0.147	0.189

Ass. (h)	100+B	50+B	25+ B	12.5+ B	5+B	2.5+ B	SM+B	P-value	5% LSD	1% LSD	0.1% LSD
17.58	0.136	0.581	0.522	0.431	0.406	0.337	0.412	1.4E-21	0.105	0.138	0.177
18.12	0.145	0.651	0.471	0.396	0.354	0.312	0.380	1.5E-22	0.099	0.129	0.166
18.68	0.144	0.704	0.490	0.395	0.390	0.301	0.361	2.7E-24	0.097	0.128	0.164
19.23	0.147	0.651	0.474	0.399	0.360	0.288	0.366	8.6E-24	0.094	0.123	0.158
19.77	0.148	0.684	0.443	0.379	0.382	0.280	0.355	1.2E-24	0.092	0.120	0.155
20.55	0.158	0.679	0.425	0.390	0.303	0.282	0.347	4.6E-24	0.091	0.119	0.153
21.07	0.161	0.648	0.453	0.397	0.297	0.279	0.347	1.1E-21	0.098	0.128	0.164
21.65	0.163	0.640	0.479	0.379	0.312	0.275	0.351	6.3E-22	0.097	0.127	0.163
22.20	0.179	0.527	0.432	0.390	0.318	0.297	0.359	2.7E-19	0.095	0.125	0.161
22.73	0.183	0.469	0.504	0.396	0.324	0.284	0.353	3.4E-20	0.093	0.121	0.156
23.28	0.185	0.457	0.478	0.405	0.362	0.298	0.383	1.6E-17	0.103	0.135	0.173
23.78	0.202	0.447	0.506	0.387	0.374	0.286	0.416	2.4E-15	0.117	0.153	0.197
24.37	0.212	0.370	0.522	0.361	0.334	0.299	0.391	4.8E-13	0.122	0.160	0.205
25.00	0.221	0.354	0.484	0.401	0.367	0.300	0.382	1.7E-13	0.119	0.156	0.201
25.55	0.227	0.391	0.541	0.363	0.361	0.320	0.404	1.1E-14	0.120	0.158	0.203
26.10	0.241	0.386	0.493	0.355	0.375	0.326	0.393	3.6E-13	0.123	0.162	0.208
41.30	0.196	0.350	0.604	0.477	0.481	0.439	0.485	6.2E-15	0.146	0.192	0.247
43.10	0.195	0.421	0.604	0.468	0.522	0.484	0.501	6.1E-15	0.154	0.201	0.259
44.78	0.186	0.356	0.608	0.462	0.509	0.455	0.476	3.9E-15	0.147	0.192	0.247
46.13	0.180	0.310	0.612	0.461	0.524	0.469	0.483	1.8E-15	0.146	0.191	0.245
47.68	0.177	0.361	0.606	0.441	0.495	0.433	0.483	1.2E-15	0.142	0.185	0.238
49.22	0.181	0.356	0.604	0.436	0.513	0.435	0.467	7.5E-16	0.139	0.182	0.234
65.30	0.173	0.352	0.627	0.452	0.512	0.461	0.481	1.4E-15	0.146	0.191	0.246
68.23	0.173	0.345	0.625	0.467	0.522	0.458	0.489	1.7E-15	0.147	0.193	0.248
69.97	0.186	0.340	0.630	0.479	0.512	0.462	0.493	2.1E-15	0.148	0.194	0.250
74.38	0.178	0.326	0.632	0.476	0.526	0.470	0.494	5.9E-16	0.147	0.192	0.247
89.53	0.180	0.345	0.656	0.494	0.547	0.503	0.513	1.9E-15	0.157	0.206	0.264
92.60	0.186	0.332	0.651	0.488	0.564	0.489	0.513	1.4E-15	0.155	0.202	0.260
95.60	0.190	0.348	0.659	0.493	0.553	0.485	0.523	1.2E-15	0.156	0.204	0.263
98.30	0.190	0.348	0.659	0.493	0.553	0.485	0.523	2.1E-15	0.158	0.207	0.266

Table.3.15. The mean OD values for each treatment at each assessment time for isolate 61.b (B) in treatments with 100, 50, 25, 12.5, 5, and 2.5 ppm Revysol® and in the control (SM+B) over the experimental period. An analysis of variance was conducted at each assessment time and significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and in significantly decreased ODs highlighted in shades of orange.

Treatment	"Growth"
100+B	8.33
50+B	24.73
25+B	45.17
12.5+B	32.98
5+B	35.56
2.5+B	30.45
SM+B	33.77
LSD 5%	11.689
LSD 1%	15.546
LSD 0.1%	19.696

Table 3.16. The mean "growth" of isolate 61.b (B) in treatments with 100, 50, 25, 12.5, 5, and 2.5 ppm Revysol® and in the control (SM+B) over the experimental period. Significantly increased growth at the 5, 1, and 0.1 % LSD highlighted in shades of green and significantly decreased growth in shades of orange.

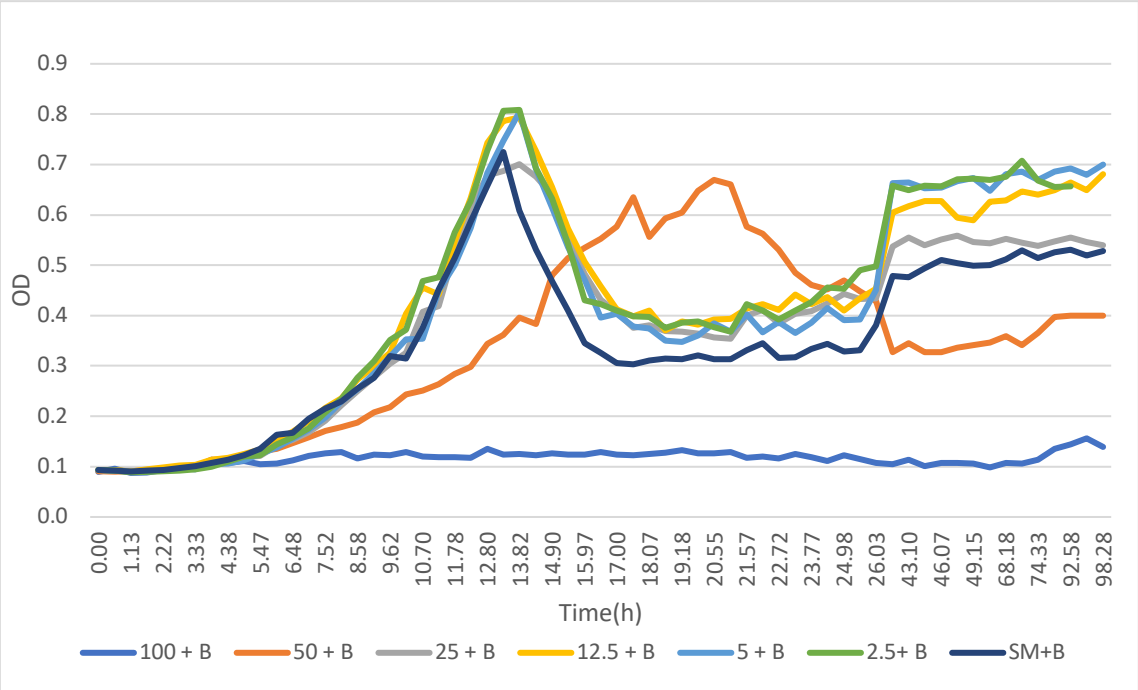


Figure 3.4. The growth curve of isolate 80 in the presence of different concentrations of Revysol® over 4 days.

Considering both the individual assessment times and the "average growth" over the experimental period, the strong inhibitory effect of the 100ppm Revysol® treatment can be seen. The average growth of the isolate over the 4-day experiment. period shows that only the 25 ppm treatment is significantly greater than the control. (Table. 3.16)

Isolate 80 in all treatments and the control entered exponential growth after 4 to 5 hours of the lag phase and then first the control followed by 12.5, 5, and 2.5 ppm reached a peak after 13 to 14 hours. The isolate had almost no growth in the treatment with 100 ppm. The 12.5, 5 and 2.5 ppm treatments showed consistently higher ODs than the control during this period. Following the occurrence of the peak values, first the ODs of the control and then the treatments started

to decrease. After 7 hours the ODs once more began to increase until about 43 hours when the OD values plateaued out until the end of the experiment. The exception to this was the 50 ppm treatment where delayed growth occurred and the peak values were reached when the other treatments had declined from their peaks (Fig. 3.4).

Ass. (h)	100+B	50+B	25+ B	12.5+B	5 + B	2.5+ B	SM+B	P-value	5% LSD	1% LSD	0.1%LSD
0.00	0.090	0.091	0.093	0.093	0.093	0.094	0.093	5.7E-13	0.002	0.002	0.003
0.55	0.095	0.090	0.093	0.093	0.092	0.092	0.092	3.9E-01	0.006	0.007	0.009
1.13	0.088	0.091	0.092	0.090	0.090	0.089	0.090	1.7E-19	0.002	0.002	0.003
1.65	0.089	0.093	0.091	0.095	0.091	0.090	0.092	1.8E-13	0.003	0.004	0.005
2.22	0.094	0.096	0.095	0.099	0.092	0.091	0.094	2.5E-20	0.003	0.004	0.005
2.75	0.097	0.098	0.099	0.103	0.096	0.092	0.097	5.5E-23	0.003	0.004	0.006
3.33	0.095	0.100	0.100	0.104	0.097	0.094	0.101	8.0E-23	0.004	0.005	0.007
3.88	0.113	0.113	0.108	0.114	0.104	0.100	0.107	5.1E-21	0.006	0.008	0.011
4.38	0.108	0.115	0.111	0.118	0.107	0.110	0.113	7.0E-15	0.008	0.011	0.014
4.92	0.112	0.124	0.119	0.125	0.113	0.120	0.122	2.7E-30	0.007	0.009	0.012
5.47	0.105	0.131	0.121	0.134	0.125	0.123	0.135	3.6E-39	0.007	0.009	0.011
6.02	0.106	0.136	0.141	0.152	0.137	0.144	0.163	3.7E-48	0.007	0.010	0.013
6.48	0.113	0.147	0.153	0.170	0.155	0.159	0.167	1.7E-41	0.010	0.014	0.017
7.05	0.121	0.158	0.169	0.190	0.173	0.176	0.195	1.4E-49	0.011	0.014	0.018
7.52	0.126	0.171	0.191	0.217	0.198	0.207	0.215	9.7E-48	0.014	0.018	0.023
8.03	0.128	0.179	0.222	0.236	0.230	0.235	0.230	5.8E-48	0.016	0.021	0.027
8.58	0.117	0.187	0.250	0.272	0.254	0.277	0.255	3.6E-51	0.018	0.024	0.031
9.13	0.123	0.208	0.278	0.299	0.285	0.310	0.277	6.8E-59	0.017	0.022	0.028
9.62	0.122	0.218	0.305	0.326	0.318	0.351	0.320	1.6E-50	0.025	0.033	0.042
10.17	0.129	0.244	0.326	0.403	0.353	0.372	0.315	3.2E-55	0.025	0.032	0.041
10.70	0.121	0.251	0.407	0.455	0.354	0.468	0.376	3.2E-49	0.036	0.047	0.061
11.23	0.119	0.264	0.419	0.441	0.449	0.476	0.452	3.9E-52	0.037	0.048	0.062
11.78	0.118	0.285	0.528	0.547	0.501	0.566	0.514	3.0E-61	0.035	0.045	0.058
12.27	0.117	0.299	0.614	0.634	0.574	0.628	0.586	1.1E-53	0.051	0.066	0.085
12.80	0.135	0.344	0.678	0.744	0.684	0.727	0.658	1.4E-44	0.077	0.101	0.131
13.33	0.124	0.362	0.688	0.786	0.747	0.807	0.725	6.9E-37	0.107	0.141	0.181
13.82	0.125	0.396	0.701	0.794	0.804	0.809	0.608	1.2E-31	0.123	0.161	0.208
14.37	0.123	0.383	0.676	0.726	0.691	0.692	0.530	2.2E-27	0.125	0.164	0.210
14.90	0.127	0.480	0.641	0.656	0.615	0.633	0.469	6.2E-23	0.133	0.174	0.223
15.42	0.124	0.514	0.555	0.572	0.537	0.543	0.410	1.1E-21	0.121	0.158	0.203
15.97	0.124	0.535	0.482	0.507	0.471	0.431	0.346	1.1E-20	0.108	0.142	0.183
16.50	0.128	0.553	0.432	0.458	0.397	0.423	0.326	7.5E-19	0.108	0.141	0.182
17.00	0.124	0.577	0.406	0.412	0.403	0.410	0.306	1.8E-18	0.108	0.141	0.181
17.57	0.122	0.635	0.375	0.399	0.378	0.398	0.304	1.2E-20	0.100	0.132	0.169
18.07	0.126	0.556	0.381	0.410	0.374	0.397	0.311	2.3E-23	0.085	0.111	0.143
18.67	0.127	0.593	0.369	0.371	0.350	0.375	0.314	9.3E-24	0.084	0.110	0.141
19.18	0.133	0.605	0.368	0.388	0.347	0.386	0.313	5.6E-25	0.081	0.107	0.137
19.70	0.126	0.648	0.364	0.382	0.361	0.388	0.321	2.4E-26	0.081	0.106	0.137
20.55	0.127	0.670	0.357	0.393	0.384	0.378	0.313	7.9E-27	0.082	0.107	0.138
21.05	0.129	0.660	0.354	0.394	0.369	0.369	0.313	2.6E-27	0.079	0.104	0.134

Ass. (h)	100+B	50+B	25+ B	12.5+B	5 + B	2.5+ B	SM+B	P-value	5% LSD	1% LSD	0.1%LSD
21.57	0.118	0.577	0.400	0.413	0.403	0.423	0.331	4.5E-23	0.091	0.119	0.153
22.13	0.120	0.563	0.411	0.423	0.367	0.410	0.345	1.2E-22	0.091	0.119	0.153
22.72	0.116	0.530	0.385	0.412	0.387	0.393	0.316	3.4E-22	0.088	0.115	0.148
23.23	0.125	0.485	0.403	0.441	0.365	0.410	0.318	4.4E-21	0.090	0.119	0.152
23.77	0.119	0.461	0.409	0.422	0.385	0.426	0.333	2.5E-20	0.093	0.122	0.157
24.32	0.112	0.451	0.423	0.437	0.416	0.456	0.344	1.5E-19	0.101	0.132	0.170
24.98	0.122	0.470	0.443	0.410	0.391	0.453	0.329	1.1E-18	0.102	0.134	0.172
25.53	0.115	0.448	0.433	0.434	0.392	0.490	0.331	1.5E-17	0.109	0.143	0.184
26.03	0.108	0.430	0.434	0.453	0.450	0.498	0.381	1.2E-17	0.115	0.150	0.194
41.25	0.105	0.327	0.538	0.605	0.663	0.658	0.479	5.5E-19	0.145	0.190	0.245
43.10	0.114	0.345	0.555	0.617	0.665	0.650	0.476	1.7E-18	0.149	0.195	0.251
44.73	0.100	0.328	0.540	0.628	0.653	0.658	0.494	1.5E-18	0.149	0.195	0.251
46.07	0.107	0.328	0.551	0.628	0.654	0.657	0.510	1.9E-18	0.152	0.199	0.256
47.70	0.107	0.337	0.559	0.595	0.667	0.671	0.504	1.9E-18	0.152	0.199	0.256
49.15	0.106	0.341	0.546	0.590	0.673	0.672	0.499	5.1E-19	0.147	0.193	0.248
65.25	0.099	0.346	0.544	0.626	0.648	0.670	0.501	1.1E-21	0.134	0.176	0.226
68.18	0.107	0.359	0.552	0.628	0.681	0.676	0.512	5.3E-21	0.141	0.184	0.237
70.00	0.106	0.341	0.545	0.646	0.686	0.708	0.530	1.1E-21	0.141	0.185	0.238
74.33	0.114	0.366	0.538	0.640	0.669	0.668	0.514	3.7E-20	0.143	0.188	0.242
89.50	0.135	0.397	0.547	0.649	0.686	0.655	0.526	2.2E-20	0.144	0.189	0.243
92.58	0.145	0.400	0.555	0.664	0.692	0.657	0.531	5.6E-20	0.148	0.193	0.249
95.53	0.156	0.400	0.546	0.649	0.679	0.669	0.519	5.1E-20	0.145	0.190	0.245
98.28	0.156	0.400	0.546	0.649	0.679	0.669	0.519	8.2E-20	0.150	0.197	0.254

Figure 3.17. The mean OD values for each treatment at each assessment for isolate 80 (B) in treatments with 100, 50, 25, 12.5, 5, and 2.5 ppm Revysol® and in the control (SM+B) over the experimental period. An analysis of variance was conducted at each assessment time and significantly increased ODs compared to the control are highlighted in shades of green and significant decreases in shades of oranges depending on the level of significance.

Treatment	"Growth"
100 + B	2.45
50 + B	26.40
25 + B	38.11
12.5 + B	44.17
5 + B	46.28
2.5 + B	46.85
SM+B	34.44
LSD 5%	10.814
LSD 1%	14.382
LSD 0.1%	18.221

Table 3.18. the mean "growth" of isolate 80 (B) in treatments with 100, 50, 25, 12.5, 5, and 2.5 ppm Revysol® and in the control (SM+B) over the experimental period. Significantly increased growth at the 5, 1, and 0.1 % LSD highlighted in shades of green and significantly decreased growth in shades of orange.

The ANOVA derived analysis showed the isolate started growing in the 12.5 ppm treatment sooner than in the other treatments and the control. After 8 to 10 hours the 2.5, 5, and 12.5 ppm treatments started the to show significantly increased ODs although not consistent at all

assessments. The delay in growth with the 50ppm treatments is also evident from these assessments (Table 3.17).

The average "growth" over the 4-day period indicates significantly enhanced "growth" with the 2.5 and 5 ppm treatments and also with the 12.5 ppm treatment although in this case the effect was not statistically significant (Table 3.18).

The isolate with similar growth pattern to isolate 61.a started its growth with the lag phase for 5 to 6 hours then it continued with the exponential growth simultaneously in all treatments and the control. Up until this stage, the highest growth was observed in the control. After reaching the peak OD value recorded with the control, the OD values remained at or around these levels for the remainder of the experiment. None of the treatments reached the peak OD value of the control except the 12.5 ppm treatment which had the highest peak OD although not significantly higher than the control. With 25, 50, and 100 ppm significantly lower ODs than the control were observed whereas there was hardly any growth of the isolate with the 50ppm treatment. (Fig. 3.5). With The 5 and 2.5 ppm treatments although having ODs numerically lower than those of the control, these differences were not statistically different and they followed a similar growth curve to that of the control (Table 3.19).

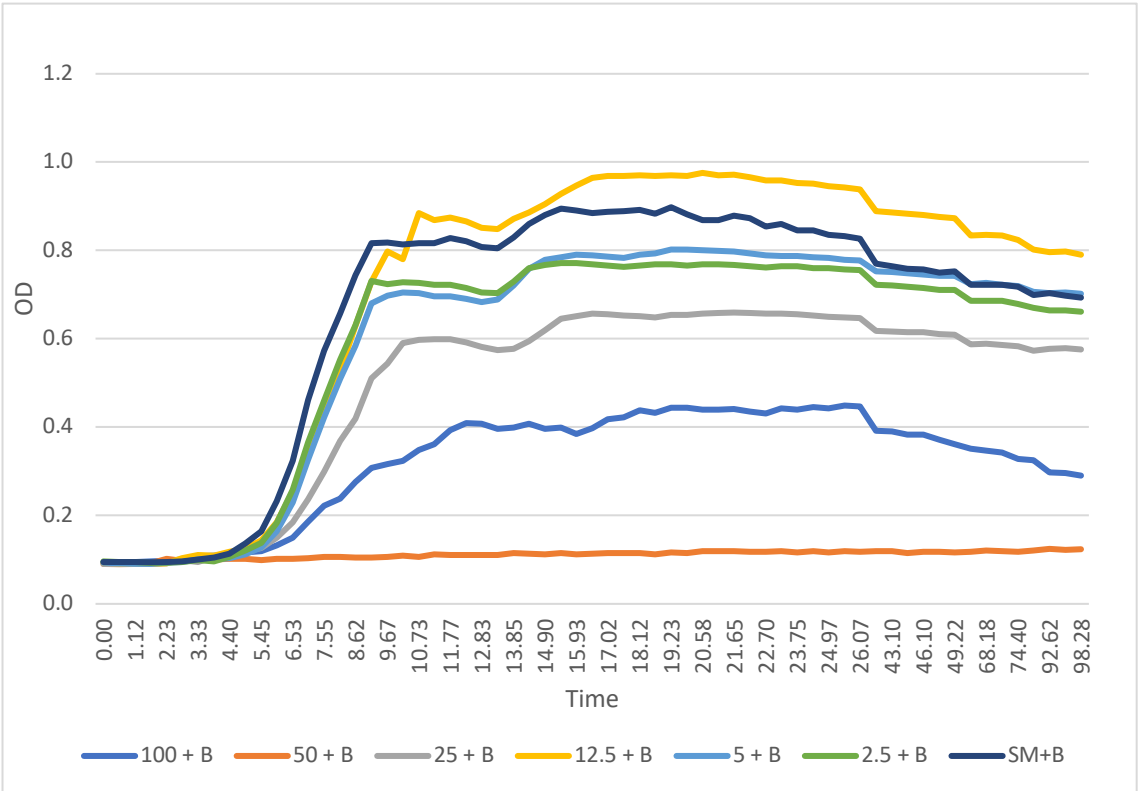


Figure 3.5. The growth curve of isolate 96 in the presence of different concentrations of Revysol® over 4 days.

The average "growth" over the 4-day experimental period shows that the 12.5ppm Revysol® treatment had the highest growth numerically although it was not significantly different from

the control. The 50 ppm and 100ppm treatments showed the greatest decrease in growth which was significant at the 0.1% level. The "growth" of the three remaining Revysol® treatments (25, 5 and 2.5 ppm) while being numerically lower were not statistically distinguishable from the control (Table 3.20).

Ass. (h)	100+B	50+B	25+B	12.5+B	5+B	2.5+B	SM+B	P-value	5% LSD	1%LSD	0.1% LSD
0.00	0.095	0.090	0.091	0.093	0.093	0.096	0.095	1.6E-08	0.003	0.004	0.005
0.55	0.093	0.090	0.091	0.092	0.092	0.094	0.095	1.0E-06	0.002	0.003	0.004
1.12	0.094	0.091	0.091	0.092	0.091	0.094	0.094	3.9E-07	0.003	0.004	0.005
1.65	0.097	0.092	0.091	0.090	0.091	0.093	0.094	9.8E-07	0.003	0.005	0.006
2.23	0.097	0.102	0.093	0.093	0.094	0.094	0.095	8.3E-20	0.003	0.004	0.005
2.77	0.101	0.098	0.099	0.103	0.094	0.095	0.097	9.5E-09	0.007	0.009	0.012
3.33	0.108	0.102	0.095	0.110	0.100	0.100	0.100	1.6E-10	0.008	0.011	0.014
3.87	0.110	0.101	0.106	0.109	0.100	0.096	0.105	9.9E-14	0.008	0.011	0.014
4.40	0.112	0.102	0.110	0.118	0.104	0.107	0.114	6.0E-17	0.009	0.012	0.016
4.95	0.117	0.102	0.117	0.125	0.112	0.121	0.137	9.7E-17	0.014	0.018	0.024
5.45	0.120	0.099	0.127	0.145	0.128	0.138	0.164	1.3E-15	0.022	0.029	0.037
6.02	0.132	0.102	0.150	0.184	0.164	0.183	0.233	1.1E-14	0.041	0.054	0.070
6.53	0.150	0.102	0.185	0.254	0.230	0.259	0.323	1.2E-13	0.072	0.094	0.121
7.02	0.186	0.103	0.237	0.345	0.326	0.366	0.462	1.2E-12	0.119	0.156	0.201
7.55	0.222	0.106	0.300	0.435	0.422	0.460	0.573	5.2E-12	0.162	0.212	0.273
8.07	0.238	0.106	0.369	0.528	0.510	0.553	0.657	1.0E-11	0.199	0.261	0.336
8.62	0.275	0.105	0.420	0.630	0.584	0.632	0.744	2.6E-11	0.238	0.312	0.401
9.12	0.308	0.105	0.511	0.730	0.681	0.731	0.816	2.9E-11	0.275	0.360	0.463
9.67	0.317	0.106	0.545	0.798	0.698	0.723	0.819	2.5E-11	0.281	0.368	0.473
10.18	0.324	0.110	0.591	0.781	0.705	0.729	0.814	3.9E-11	0.284	0.372	0.479
10.73	0.349	0.107	0.598	0.885	0.704	0.727	0.816	1.0E-11	0.285	0.373	0.480
11.27	0.362	0.112	0.599	0.869	0.696	0.722	0.816	1.3E-11	0.284	0.372	0.479
11.77	0.394	0.111	0.599	0.874	0.696	0.723	0.828	1.5E-11	0.287	0.376	0.484
12.28	0.410	0.111	0.592	0.866	0.691	0.715	0.821	2.0E-11	0.286	0.375	0.482
12.83	0.408	0.110	0.582	0.851	0.683	0.704	0.808	2.6E-11	0.283	0.371	0.477
13.35	0.396	0.111	0.575	0.849	0.688	0.704	0.805	1.5E-11	0.279	0.365	0.470
13.85	0.399	0.116	0.577	0.872	0.721	0.730	0.830	2.0E-11	0.290	0.380	0.489
14.38	0.407	0.113	0.595	0.886	0.759	0.760	0.860	2.4E-11	0.303	0.396	0.510
14.90	0.397	0.113	0.620	0.906	0.779	0.768	0.880	2.5E-11	0.311	0.407	0.523
15.45	0.400	0.115	0.645	0.928	0.785	0.772	0.895	2.3E-11	0.316	0.414	0.532
15.93	0.384	0.112	0.652	0.948	0.791	0.772	0.891	2.5E-11	0.318	0.417	0.536
16.50	0.397	0.114	0.657	0.964	0.789	0.769	0.885	2.4E-11	0.318	0.417	0.536
17.02	0.417	0.115	0.656	0.969	0.786	0.766	0.887	2.9E-11	0.321	0.420	0.540
17.53	0.423	0.115	0.653	0.969	0.783	0.763	0.889	2.4E-11	0.319	0.418	0.537
18.12	0.439	0.115	0.651	0.971	0.791	0.766	0.892	2.6E-11	0.321	0.420	0.540
18.65	0.433	0.112	0.648	0.969	0.794	0.769	0.883	3.3E-11	0.321	0.421	0.541
19.23	0.444	0.116	0.655	0.971	0.802	0.768	0.898	2.9E-11	0.323	0.423	0.545
19.72	0.444	0.115	0.655	0.969	0.802	0.766	0.881	4.3E-11	0.323	0.423	0.544
20.58	0.439	0.119	0.657	0.976	0.801	0.769	0.868	5.0E-11	0.323	0.423	0.544
21.03	0.440	0.119	0.658	0.970	0.799	0.768	0.868	4.4E-11	0.321	0.420	0.541

Ass. (h)	100+B	50+B	25+B	12.5+B	5+B	2.5+B	SM+B	P-value	5% LSD	1%LSD	0.1% LSD
21.65	0.441	0.120	0.660	0.971	0.798	0.768	0.878	5.0E-11	0.324	0.424	0.546
22.20	0.435	0.117	0.658	0.966	0.793	0.765	0.873	4.8E-11	0.322	0.422	0.542
22.70	0.431	0.119	0.658	0.958	0.788	0.761	0.854	6.0E-11	0.319	0.418	0.538
23.28	0.443	0.119	0.657	0.959	0.788	0.764	0.860	6.2E-11	0.321	0.420	0.540
23.75	0.440	0.117	0.655	0.953	0.788	0.764	0.845	6.9E-11	0.317	0.415	0.534
24.38	0.445	0.120	0.653	0.952	0.785	0.759	0.846	7.2E-11	0.318	0.417	0.536
24.97	0.442	0.117	0.650	0.946	0.784	0.759	0.835	6.8E-11	0.314	0.411	0.529
25.55	0.449	0.119	0.648	0.943	0.780	0.757	0.832	8.2E-11	0.315	0.413	0.531
26.07	0.447	0.117	0.647	0.939	0.778	0.756	0.827	8.3E-11	0.314	0.411	0.529
41.32	0.392	0.119	0.618	0.889	0.753	0.723	0.770	6.3E-11	0.293	0.383	0.493
43.10	0.391	0.120	0.617	0.887	0.751	0.720	0.764	5.3E-11	0.290	0.380	0.489
44.78	0.383	0.116	0.615	0.884	0.748	0.718	0.759	5.7E-11	0.289	0.378	0.487
46.10	0.383	0.117	0.615	0.880	0.746	0.715	0.757	6.3E-11	0.289	0.378	0.487
47.68	0.372	0.117	0.610	0.876	0.742	0.711	0.750	5.6E-11	0.285	0.374	0.481
49.22	0.362	0.116	0.609	0.873	0.743	0.711	0.753	4.4E-11	0.284	0.372	0.479
65.25	0.351	0.117	0.588	0.834	0.724	0.686	0.722	7.7E-11	0.274	0.359	0.462
68.18	0.347	0.120	0.590	0.835	0.727	0.687	0.723	5.0E-11	0.272	0.356	0.458
70.00	0.342	0.120	0.587	0.834	0.723	0.686	0.723	5.0E-11	0.272	0.357	0.459
74.40	0.328	0.118	0.584	0.825	0.720	0.679	0.717	4.2E-11	0.268	0.352	0.452
89.48	0.325	0.120	0.574	0.802	0.707	0.670	0.699	5.6E-11	0.262	0.344	0.442
92.62	0.298	0.125	0.577	0.797	0.704	0.664	0.703	3.3E-11	0.259	0.340	0.437
95.57	0.297	0.122	0.579	0.797	0.705	0.665	0.697	3.7E-11	0.259	0.339	0.436
98.28	0.297	0.122	0.579	0.797	0.705	0.665	0.697	3.4E-11	0.257	0.337	0.433

Table 3.19. The mean OD values for each treatment at each assessment time for isolate 96 (B) in treatments with 100, 50, 25, 12.5, 5, and 2.5 ppm Revysol® and in the control (SM+B) over the experimental period. An analysis of variance was conducted at each assessment time and significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and in significantly decreased ODs highlighted in shades of orange.

Treatment	"Growth"
100 + B	24.70
50 + B	2.54
25 + B	46.33
12.5 + B	69.65
5 + B	58.25
2.5 + B	55.43
SM+B	60.94
LSD 5%	25.747
LSD 1%	34.243
LSD 0.1%	43.383

Table 3.20. The mean "growth" of isolate 96 (B) in treatments with 100, 50, 25, 12.5, 5, and 2.5 ppm Revysol® and in the control (SM+B) over the experimental period. Significantly increased growth at the 5, 1, and 0.1 % LSD highlighted in shades of green and significantly decreased growth in shades of orange.

The ODs obtained at the start of the experiment with the two highest does are considered to be spurious and may arise from the higher rates of the fungicide used being not maintained in solution. The 2.5 ppm treatment and the control started the analogous exponential growth.

Following a lag period of approximately 7 hours, the exponential phase began. After 14 to 15 hours, the control reached its peak OD value and shortly after started a slow decrease until the end of experiment (Fig. 3.6). The OD values of the 2.5 ppm treatment after reaching the peak OD 2 to 3 hours after the control remained significantly greater than the control during the decline following the peak. until 23 hours. The remaining treatments all showed lower ODs than the control throughout most of the experiment (Table. 3.21).

The analysis demonstrates the inhibitory effect of all doses except 2.5 ppm on the growth. These OD reductions were mostly significant at the 0.1% level. Confirming the growth curve and the ANOVA analysis, the average "growth" over 4 days indicates the 5 Revysol® doses 100,50,25,12.5, and 5 ppm caused the great reduction in the growth. The 2.5 ppm treatment numerically exceeded the "growth" of the control, but the difference was not statistically significant (Table 3.22).

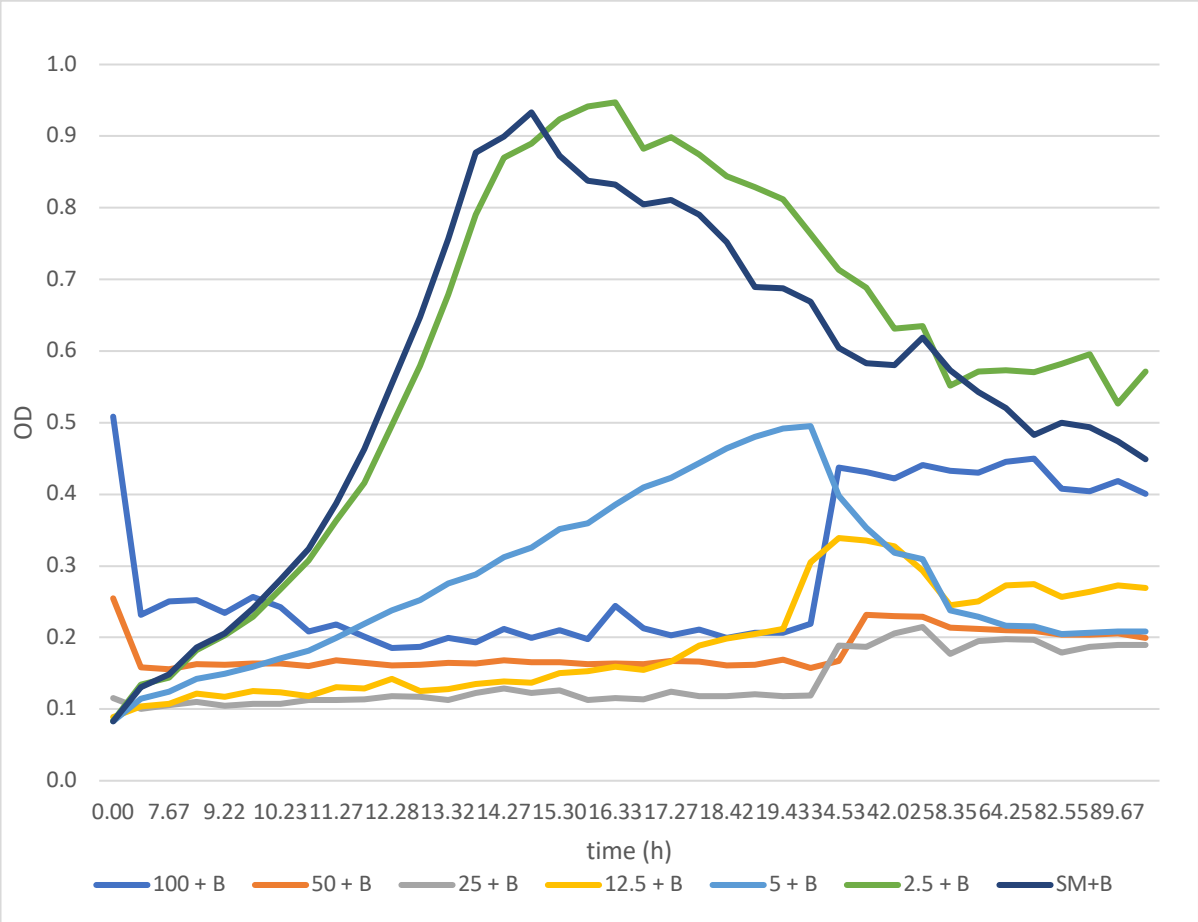


Figure 3.6. The growth curve of isolate 124 in the presence of different concentrations of Revysol® over 4-days. Treatments with different doses and the isolate(B) shown with different colored curves. OD values as X-axis and time intervals of the measurements in hour as Y-axis are illustrated the growth cycle of the isolate was monitored through reading the OD at wavelength 600 nm over 4 days.

Considering the growth curves of the six isolates examined here, three distinct types of growth

curve can be distinguished. First there is the type of curve with a rapid initial increase in the OD in the first twenty hours or so to reach a maximum value and then a very slow decrease throughout the remainder of the experimental period. (Type 1).

Ass. (h)	100+B	50+B	25+B	12.5+B	5+B	2.5+B	SM+B	P-value	5% LSD	1% LSD	0.1%LSD
0.00	0.508	0.255	0.116	0.089	0.083	0.084	0.083	1.8E-58	0.025	0.033	0.042
7.13	0.231	0.158	0.100	0.104	0.114	0.134	0.130	7.9E-24	0.022	0.029	0.037
7.67	0.250	0.156	0.105	0.108	0.124	0.144	0.148	2.7E-18	0.028	0.037	0.047
8.70	0.252	0.163	0.110	0.122	0.143	0.183	0.186	1.1E-19	0.028	0.037	0.047
9.22	0.235	0.162	0.105	0.117	0.150	0.203	0.206	2.5E-22	0.025	0.033	0.043
9.72	0.257	0.164	0.107	0.125	0.159	0.229	0.241	2.5E-26	0.026	0.034	0.044
10.23	0.242	0.164	0.107	0.124	0.171	0.267	0.281	1.1E-30	0.027	0.035	0.045
10.75	0.209	0.161	0.113	0.118	0.181	0.308	0.324	6.1E-38	0.025	0.033	0.043
11.27	0.218	0.168	0.113	0.131	0.199	0.363	0.387	5.9E-41	0.029	0.038	0.049
11.78	0.202	0.164	0.114	0.129	0.219	0.416	0.464	5.7E-50	0.028	0.037	0.047
12.28	0.185	0.161	0.118	0.142	0.238	0.496	0.554	1.2E-55	0.030	0.040	0.051
12.82	0.187	0.162	0.117	0.126	0.252	0.580	0.647	3.7E-60	0.032	0.043	0.055
13.32	0.200	0.165	0.113	0.128	0.275	0.679	0.757	1.4E-60	0.039	0.051	0.066
13.80	0.193	0.164	0.122	0.135	0.288	0.791	0.877	8.3E-68	0.038	0.050	0.064
14.27	0.212	0.168	0.129	0.138	0.312	0.870	0.899	5.0E-69	0.038	0.050	0.064
14.78	0.200	0.165	0.123	0.137	0.325	0.890	0.933	2.5E-70	0.039	0.051	0.065
15.30	0.210	0.166	0.126	0.150	0.351	0.924	0.872	2.7E-66	0.041	0.054	0.070
15.83	0.198	0.163	0.112	0.153	0.359	0.942	0.838	3.4E-60	0.049	0.064	0.082
16.33	0.244	0.164	0.116	0.159	0.385	0.947	0.833	1.6E-58	0.051	0.066	0.085
16.80	0.213	0.163	0.114	0.155	0.410	0.882	0.805	8.8E-60	0.047	0.061	0.079
17.27	0.203	0.168	0.125	0.166	0.423	0.899	0.811	1.6E-54	0.055	0.072	0.092
17.85	0.211	0.166	0.118	0.189	0.444	0.875	0.790	6.7E-54	0.054	0.071	0.091
18.42	0.200	0.161	0.118	0.198	0.464	0.844	0.752	6.8E-45	0.067	0.088	0.113
18.93	0.206	0.162	0.121	0.205	0.480	0.828	0.690	6.5E-51	0.053	0.069	0.089
19.43	0.206	0.169	0.118	0.212	0.492	0.812	0.688	1.4E-45	0.061	0.080	0.103
22.75	0.219	0.157	0.119	0.305	0.495	0.764	0.669	4.3E-30	0.094	0.123	0.159
34.53	0.437	0.167	0.189	0.339	0.398	0.714	0.604	2.9E-17	0.136	0.178	0.228
38.48	0.431	0.232	0.187	0.335	0.353	0.688	0.583	9.4E-17	0.134	0.175	0.225
42.02	0.422	0.230	0.206	0.327	0.318	0.631	0.581	4.5E-15	0.138	0.180	0.232
44.50	0.441	0.229	0.215	0.293	0.309	0.635	0.618	9.6E-16	0.137	0.180	0.232
58.35	0.433	0.214	0.177	0.245	0.238	0.552	0.573	1.4E-16	0.123	0.161	0.208
61.22	0.430	0.212	0.195	0.250	0.229	0.571	0.543	1.6E-16	0.118	0.154	0.199
64.25	0.445	0.210	0.198	0.273	0.217	0.573	0.520	7.8E-14	0.127	0.167	0.214
67.35	0.450	0.209	0.196	0.275	0.215	0.570	0.482	1.9E-14	0.119	0.155	0.200
82.55	0.408	0.204	0.179	0.257	0.205	0.582	0.499	1.9E-18	0.105	0.138	0.177
85.72	0.404	0.204	0.187	0.264	0.207	0.596	0.494	5.9E-16	0.116	0.151	0.195
89.67	0.419	0.206	0.190	0.273	0.209	0.527	0.474	1.7E-14	0.112	0.147	0.190
92.90	0.401	0.200	0.190	0.270	0.209	0.571	0.449	2.3E-15	0.126	0.166	0.213

Table 3.21. The mean OD values for each treatment at each assessment time for isolate 124 (B) in treatments with 100, 50, 25, 12.5, 5, and 2.5 ppm Revysol® and in the control (SM+B) over the experimental period. An analysis of variance was conducted at each assessment time and significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and in significantly decreased ODs highlighted in shades of orange.

The isolates 53, 61.a, and 96 showed this type of curve. The second type of curve consisted of a rapid rise to a peak value again in the first fifteen hours followed by a steep decline until about twenty-five hours and then a gentle increase and an eventual plateauing out around 40 hours (Type 2), Isolates 61.b and 80 showed this type of pattern. The isolate 124 again showed an initial rapid increase in the first 15 hours or so followed by a gradual decline for the remainder of the experiment (Type 3).

It would appear that the main difference between these different types of curves is what happens after the maximum value has been reached – very slow decrease, Type 1, steep decline followed by a gentle increase before plateauing out, Type 2 and a more marked decrease Type 3, although it might also be argued that the isolates showing a Type 1 curve took a little longer to reach the maximum OD value.

If we consider the results of the identification of these six isolates, we find that the taxonomic classification is also consistent with the growth curves observed: 53, 61.a, and 96 were identified as *Bacillus megaterium*, isolates 61.b and 80 as *Bacillus subtilis* and 124 as *Bacillus licheniformis*.

Treatment	"Growth"
100 + B	-11.88
50 + B	-5.00
25 + B	3.78
12.5 + B	13.50
5 + B	15.81
2.5 + B	42.45
SM+B	38.80
LSD 5%	7.087
LSD 1%	9.426
LSD 0.1%	11.942

Table 3.22. The mean "growth" of isolate 124 (B) in treatments with 100, 50, 25, 12.5, 5, and 2.5 ppm Revysol® and in the control (SM+B) over the experimental period. Significantly increased growth at the 5, 1, and 0.1 % LSD highlighted in shades of green and significantly decreased growth in shades of orange.

3.2.4. Cross Screening

3.3.4.1. Isolate 53

Cross screening tests with isolate 53 were run on two occasions. In the first test (Fig. 3.7), the control reached a peak OD after 22 hours with 25 ppm and 5 ppm boscalid reached a peak at the next assessment at 24 hours (24.53 hours) with 1 ppm boscalid there appeared to be something of a peak at the same time as the control (21.53 hours) but after an initial decline the

OD value started to increase again to reach a maximum value at 43 hours, while the control and 25 ppm boscalid declined after reaching their peak values before starting to plateau after 43 hours. With the 1 and 5 ppm treatments high ODs around the peak values were maintained for a longer period before they started to decline after 47.8 hours and then beginning to plateau at 64.62 hours. This is reflected in the significant differences observed between these two treatments and the control at assessments taken between 40 and 64 hours. With 5ppm boscalid, the significantly increased ODs (compared to the control) extended up to the assessment taken at 72.35 hours (Table 3.23). It was these differences that largely led to the significant increase in "growth" observed over the course of the experiment for these two treatments compared to the control (Table 3.25).

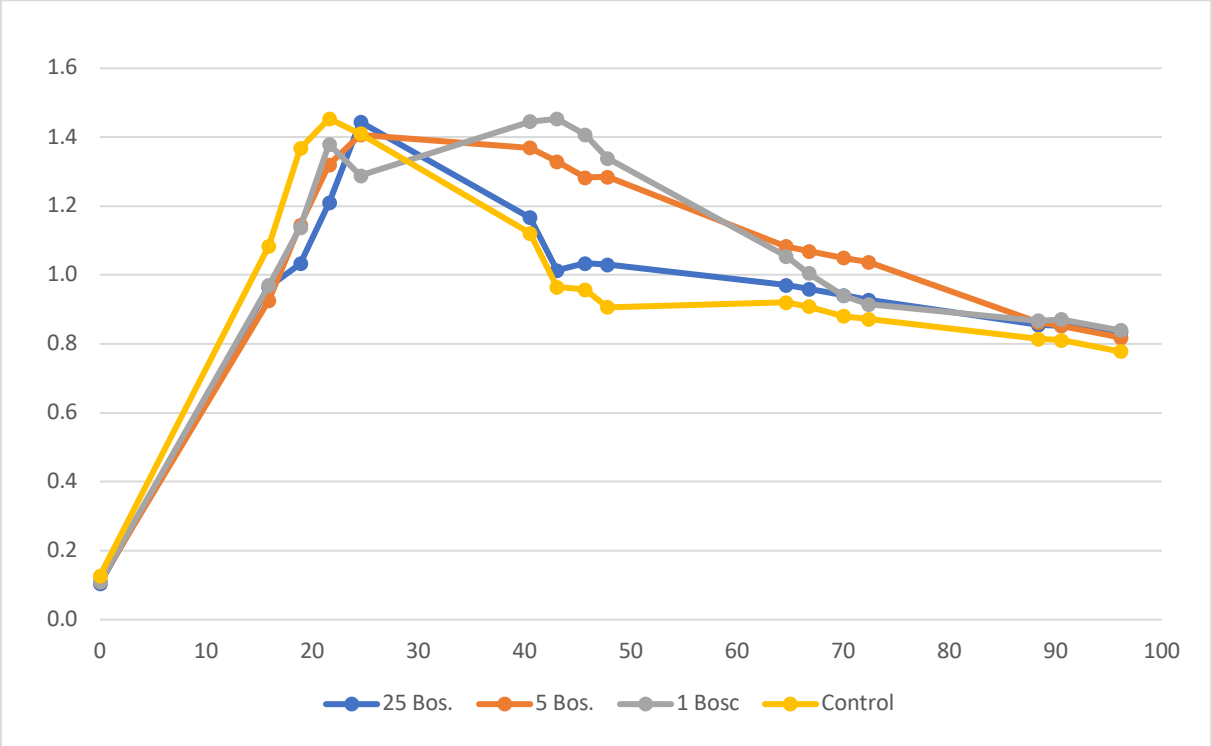


Figure 3.7. The OD curve of isolate 53 over 4 days. 3 doses of boscalid 1,5, and 25 ppm and shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis are illustrated.

In the second run of the experiment, the observed peak values all occurred at the same point (41.4 hours), with the exception of 25 ppm boscalid which reached a peak value sooner after 27.63 hours. In this case significantly higher ODs were observed with the boscalid experiments at some of the assessments leading up to the peak values, especially with 25 ppm boscalid (Fig. 3.8). After reaching the peak values there was then a fall in the ODs but again as in the first experiment, the ODs of the I and 5 ppm treatments were generally numerically if not statistically higher than those of the control. With 25ppm the peak value was reached earlier and so the decline also began earlier with a plateau being reached after 44.7 hours whereas with

1 and 5 ppm a plateau was reached after 49.79 hours and for the control somewhat later at 65.73 hours. (Note no assessment was available between 49.77 and 65.73 hours, so the plateau with the control could have been reached earlier). Some significant differences in the OD were observed in the assessments at 90.6 hours and thereafter between the 1 and 5 ppm treatments and the control (Table 3.24). Looking at the "growth" for these treatments all three treatments with boscalid were higher than the control and bordering (but not quite attaining statistical significance (Table 3.26).

In these two experiments, the boscalid treatments have led to increased OD values compared to the controls and these OD values have been maintained at a higher value compared to the control during the period of decline after the peak values have been reached and it is this difference which has led to increased "growth" of the boscalid treatments over the course of the experiment. With 25 ppm, the initial growth prior to reaching the peak, differed compared to the control in the two experiments. In the first experiment ODs were lower than the control during this period but in the second experiment, higher. Nonetheless, in both cases there was a quicker decline once the peak had been reached and a plateau was reached earlier (after 43-44 hours compared to 50 60 hours for the control and the other boscalid treatments).

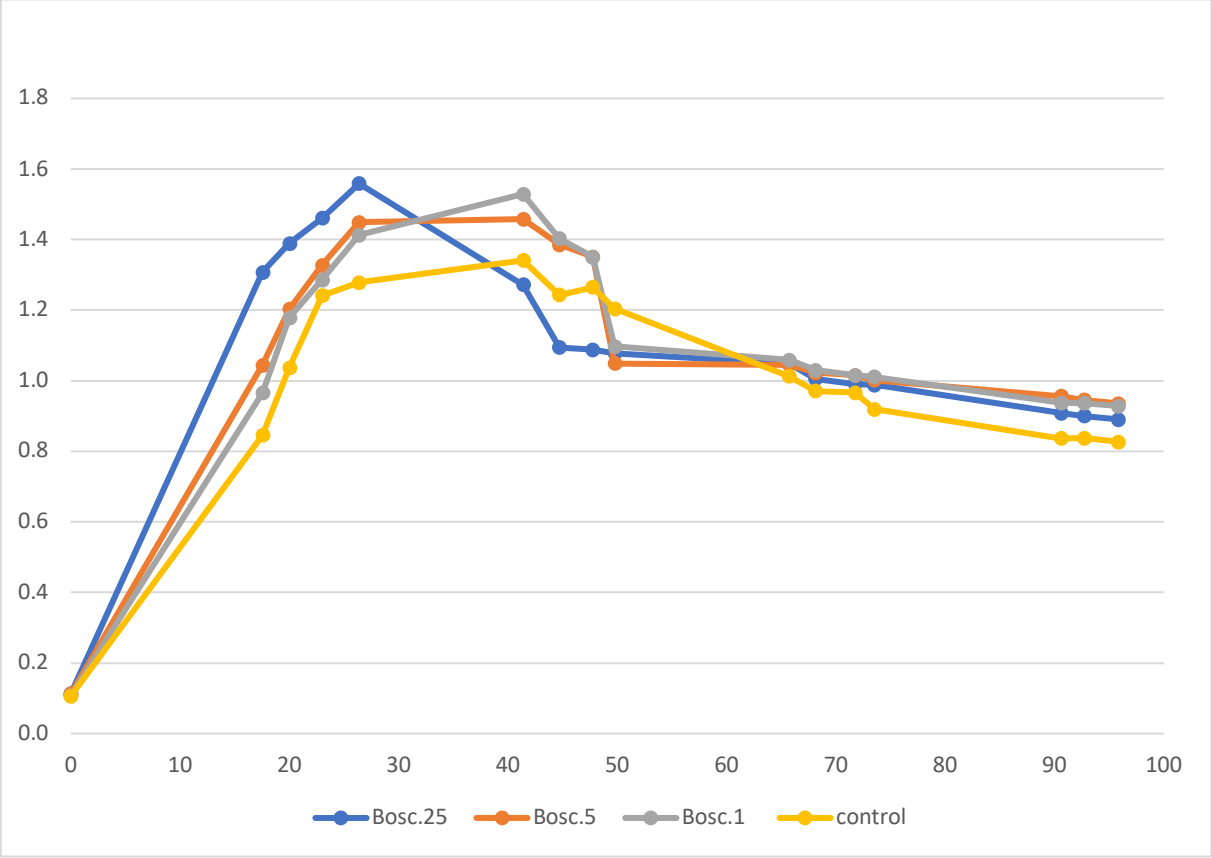


Figure 3.8. The OD curve of isolate 53 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis are illustrated.

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.105	0.110	0.109	0.127	2.0E-10	0.009	0.011	0.015
15.88	0.965	0.926	0.969	1.084	1.5E-04	0.167	0.222	0.288
18.88	1.033	1.145	1.138	1.368	2.9E-08	0.222	0.295	0.383
21.58	1.209	1.320	1.380	1.454	2.1E-08	0.214	0.284	0.369
24.53	1.444	1.407	1.288	1.410	4.9E-03	0.306	0.406	0.526
40.48	1.167	1.369	1.445	1.121	2.8E-03	0.257	0.341	0.442
43.03	1.014	1.329	1.453	0.966	5.3E-05	0.229	0.305	0.395
45.67	1.034	1.283	1.407	0.958	1.1E-04	0.219	0.291	0.377
47.80	1.031	1.284	1.338	0.906	1.4E-05	0.183	0.243	0.316
64.62	0.971	1.083	1.054	0.920	6.4E-03	0.120	0.159	0.207
66.75	0.960	1.069	1.005	0.908	2.3E-02	0.109	0.144	0.187
70.03	0.941	1.050	0.941	0.881	3.0E-02	0.105	0.140	0.181
72.35	0.928	1.037	0.915	0.872	4.6E-02	0.107	0.142	0.185
88.33	0.856	0.863	0.867	0.814	3.9E-01	0.100	0.133	0.172
90.53	0.853	0.853	0.872	0.811	4.9E-01	0.103	0.137	0.177
96.10	0.834	0.818	0.840	0.778	6.3E-01	0.107	0.142	0.184

Table 3.23. The mean ODs at each assessment time for isolate 53 in the presence of three concentrations of boscalid. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance level significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Bosc.25	Bosc.5	Bosc.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.113	0.112	0.108	0.107	7.7E-13	0.006	0.008	0.011
17.55	1.308	1.044	0.965	0.845	3.1E-15	0.155	0.206	0.268
20.02	1.389	1.203	1.179	1.037	1.4E-21	0.130	0.173	0.226
22.98	1.461	1.328	1.286	1.241	1.8E-19	0.161	0.214	0.278
26.37	1.558	1.449	1.413	1.278	1.8E-14	0.217	0.288	0.376
41.40	1.272	1.458	1.529	1.341	7.6E-11	0.270	0.359	0.467
44.70	1.094	1.385	1.403	1.243	9.8E-11	0.266	0.354	0.461
47.75	1.088	1.351	1.351	1.264	1.5E-10	0.264	0.351	0.457
49.77	1.078	1.049	1.097	1.204	7.6E-11	0.217	0.289	0.377
65.73	1.049	1.046	1.059	1.012	2.7E-14	0.160	0.213	0.278
68.13	1.005	1.023	1.030	0.971	6.8E-16	0.135	0.180	0.234
71.73	0.991	1.015	1.016	0.967	9.0E-16	0.132	0.176	0.229
73.53	0.988	1.001	1.010	0.919	4.1E-18	0.113	0.151	0.197
90.60	0.908	0.956	0.937	0.836	6.7E-24	0.082	0.109	0.142
92.72	0.900	0.946	0.936	0.837	4.3E-23	0.078	0.104	0.136
95.85	0.890	0.935	0.928	0.826	1.1E-23	0.082	0.109	0.142

Table. 3.24. The mean ODs at each assessment time for isolate 53 in the presence of three concentrations of boscalid. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Cross screening test with isolate 53 were run on three occasions. In the first test (Fig. 3.9), control and treatments with Revysol® reached a peak value after 21.22 hours. The OD with control started to decline after reaching the peak, while with 5 and 1 ppm there appeared to be a short decline between 24 and 43 hours and then started the death phase until the end of

experiment. However, with treatments OD was always bigger than the control. With 1 and 5 ppm Revysol® the OD value was significantly higher than the control continuously from the beginning of death phase at 43 hours for ca. 21 hours until 64 hours. The isolate with 25 ppm Revysol® as an exception behaved differently. After having a significant lower exponential growth, OD value started to plateau after 24 hours and halted in this phase until the of experiment (Table 3.27).

Treatment	"Growth"
Bosc. 25	81.85
Bosc. 5	89.67
Bosc. 1	89.32
Control	78.49
LSD5%	8.53
LSD1%	11.32
LSD0.1%	14.68

Treatment	"Growth"
Bosc.25	90.72
Bosc.5	90.10
Bosc.1	90.33
Control	82.78
LSD5%	8.020
LSD1%	10.675
LSD0.1%	13.904

Table 3.25. and 3.26. The mean "growth" of isolate 53 in treatments with 25, 5, and 1 ppm of boscalid over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

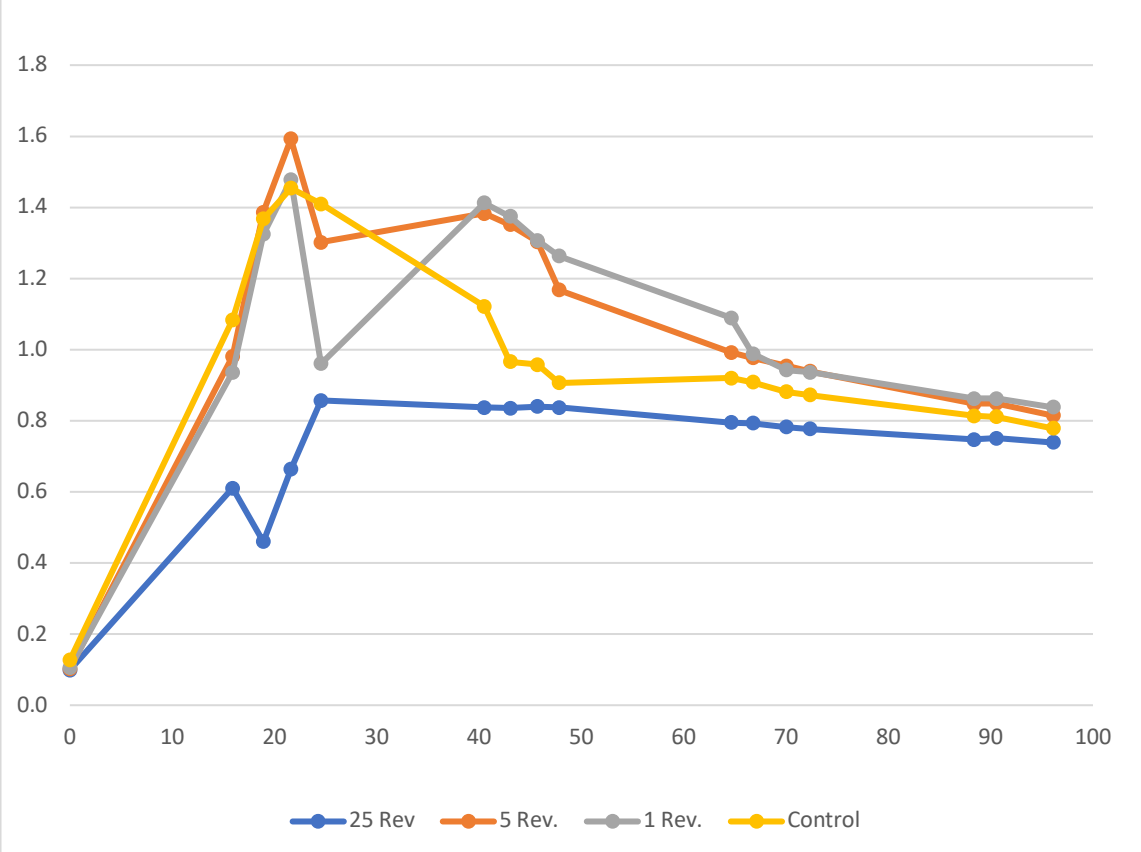


Figure 3.9. The OD curve of isolate 53 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis are illustrated.

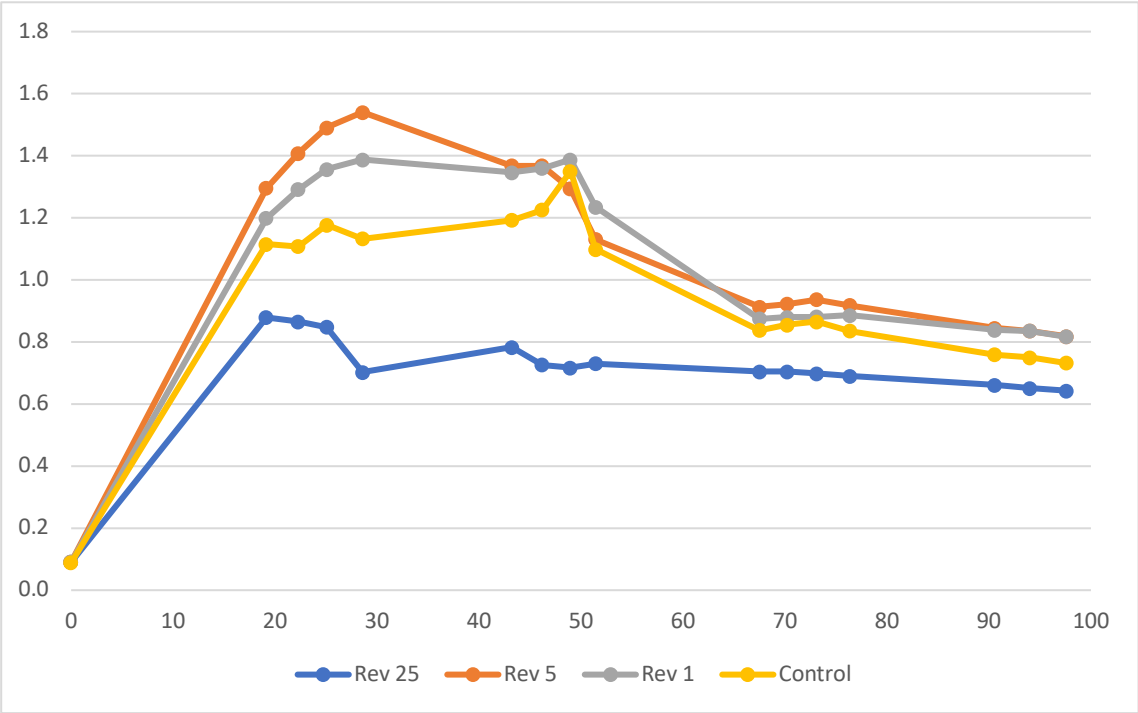


Figure 3.10. The OD curve of isolate 53 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis are illustrated.

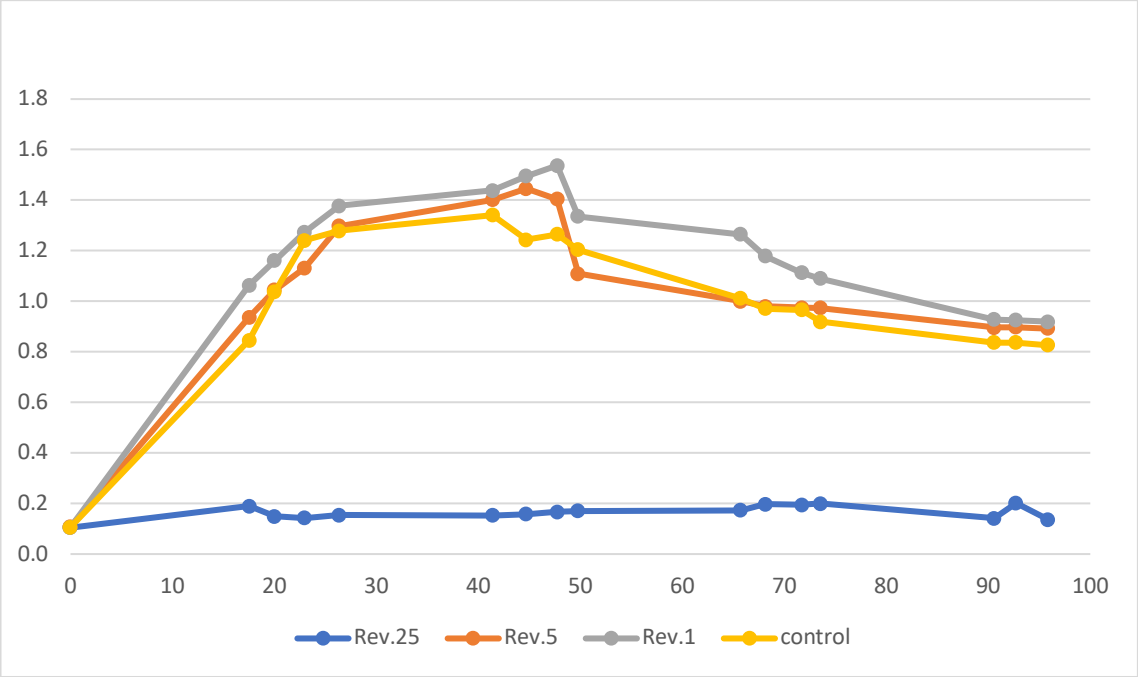


Figure 3.11. The OD curve of isolate 53 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis are illustrated.

In the second trial (Fig. 3.10), the OD with 1 and 5 ppm Revysol® increased at the same rate as with the control until 19 hours, where the first peak was recorded with the control. After reaching the peak, OD with control started to plateau for almost 37 hours, while 1 and 5 ppm continued to increase until 28 hours where 5 ppm reached the highest peak and significantly

different from the control. With both treatments, OD started to fall after the Peak until 49 hours when the control reached its second peak and to the treatments with 1 and 5 ppm. The OD with control and 1, and 5 ppm Revysol® started to drop down simultaneously with plateau reached at 67.50 hours. Between first and second peak of the control the ODs with 1 and 5 ppm were constantly higher than the control but only numerically. With 25 ppm, OD showed significantly lower exponential growth with Plateau started after 19 hours and continued until the trial finished (Table 3.28).

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.099	0.103	0.105	0.127	1.96E-10	0.009	0.011	0.015
15.88	0.610	0.982	0.937	1.084	1.50E-04	0.167	0.222	0.288
18.88	0.460	1.386	1.325	1.368	2.88E-08	0.222	0.295	0.383
21.58	0.664	1.592	1.478	1.454	2.15E-08	0.214	0.284	0.369
24.53	0.857	1.301	0.961	1.410	4.85E-03	0.306	0.406	0.526
40.48	0.838	1.383	1.412	1.121	2.82E-03	0.257	0.341	0.442
43.03	0.836	1.351	1.375	0.966	5.31E-05	0.229	0.305	0.395
45.67	0.840	1.303	1.308	0.958	1.14E-04	0.219	0.291	0.377
47.80	0.838	1.168	1.263	0.906	1.37E-05	0.183	0.243	0.316
64.62	0.795	0.992	1.089	0.920	6.41E-03	0.120	0.159	0.207
66.75	0.793	0.977	0.989	0.908	2.28E-02	0.109	0.144	0.187
70.03	0.783	0.954	0.943	0.881	2.96E-02	0.105	0.140	0.181
72.35	0.777	0.939	0.936	0.872	4.60E-02	0.107	0.142	0.185
88.33	0.748	0.848	0.863	0.814	3.90E-01	0.100	0.133	0.172
90.53	0.751	0.849	0.863	0.811	4.90E-01	0.103	0.137	0.177
96.10	0.739	0.815	0.838	0.778	6.28E-01	0.107	0.142	0.184

Table 3.27. The mean ODs at each assessment time for isolate 53 in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In the third run of experiment (Fig. 3.11), control and 1 and 5 ppm Revysol® showed the similar pattern of the growth curve. The initial OD peak was recorded at 26.37 hours. Up to this point the OD value with 1 and 5 ppm was not significantly but numerically higher than the control. After initial peak, the ODs continued to grow in the control, 1, and 5 ppm Revysol®. With control the second peak was recorded earlier at 41.40 hours and so the decline also began earlier. Later the higher peak was reached with Revysol® 1 ppm at 47.75 hours (the highest peak recorded) and 5 ppm at 44.70 hours. The OD with both treatments started to fall at the same point. The highest peak with 1 ppm is reflected in the significant differences observed from 47.75 hours until the end of experiment but with 5 ppm the increased OD value tend to be at particular assessment timings and just numerically. Despite of 1 and 5 ppm, with 25 ppm the OD was remarkedly less than the control and almost didn't growth at all (Table 3.29).

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.090	0.091	0.090	0.089	7.8E-02	0.002	0.003	0.004
19.25	0.879	1.296	1.199	1.115	5.0E-03	0.250	0.332	0.433
22.37	0.865	1.408	1.292	1.107	4.6E-02	0.323	0.430	0.559
25.17	0.848	1.490	1.357	1.177	2.0E-01	0.352	0.469	0.611
28.68	0.702	1.540	1.387	1.133	1.2E-04	0.330	0.439	0.572
43.32	0.783	1.368	1.347	1.192	9.0E-03	0.266	0.355	0.462
46.27	0.726	1.367	1.359	1.226	6.6E-04	0.271	0.361	0.470
49.03	0.717	1.293	1.388	1.350	3.3E-04	0.261	0.348	0.453
67.62	0.730	1.130	1.234	1.099	3.5E-03	0.219	0.291	0.379
70.32	0.705	0.913	0.875	0.838	4.6E-03	0.154	0.204	0.266
73.20	0.705	0.922	0.880	0.855	1.8E-02	0.142	0.189	0.246
76.52	0.698	0.937	0.881	0.865	1.5E-02	0.140	0.187	0.243
90.78	0.690	0.918	0.886	0.836	7.6E-03	0.140	0.186	0.243
94.18	0.662	0.845	0.839	0.760	7.8E-03	0.133	0.177	0.230
97.77	0.651	0.836	0.836	0.750	5.1E-03	0.129	0.172	0.224
97.60	0.643	0.818	0.817	0.732	6.5E-03	0.130	0.173	0.225

Figure 3.28. The mean ODs at each assessment time for isolate 53 in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Rev.25	Rev.5	Rev.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.104	0.108	0.106	0.107	7.7E-13	0.006	0.008	0.011
17.55	0.189	0.937	1.063	0.845	3.1E-15	0.155	0.206	0.268
20.02	0.149	1.044	1.161	1.037	1.4E-21	0.130	0.173	0.226
22.98	0.143	1.131	1.273	1.241	1.8E-19	0.161	0.214	0.278
26.37	0.154	1.298	1.378	1.278	1.8E-14	0.217	0.288	0.376
41.40	0.153	1.402	1.438	1.341	7.6E-11	0.270	0.359	0.467
44.70	0.157	1.446	1.495	1.243	9.8E-11	0.266	0.354	0.461
47.75	0.166	1.405	1.537	1.264	1.5E-10	0.264	0.351	0.457
49.77	0.170	1.110	1.336	1.204	7.6E-11	0.217	0.289	0.377
65.73	0.173	1.001	1.264	1.012	2.7E-14	0.160	0.213	0.278
68.13	0.197	0.980	1.179	0.971	6.8E-16	0.135	0.180	0.234
71.73	0.195	0.975	1.113	0.967	9.0E-16	0.132	0.176	0.229
73.53	0.200	0.974	1.090	0.919	4.1E-18	0.113	0.151	0.197
90.60	0.142	0.896	0.928	0.836	6.7E-24	0.082	0.109	0.142
92.72	0.202	0.897	0.926	0.837	4.3E-23	0.078	0.104	0.136
95.85	0.136	0.893	0.919	0.826	1.1E-23	0.082	0.109	0.142

Table 3.29. The mean ODs at each assessment time for isolate 53 in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In these three experiments, the Revysol® treatments have led to increased OD values compared to the controls and these OD values have been maintained at a higher value compared to the control during the period of decline after the peak values have been reached and it is this difference which has led to increased "growth" of the Revysol® treatments over the course of

the experiment. With 25 ppm, the growth was either greatly lower than the control with a very short exponential growth or there was no growth at all (Table 30-32).

Treatment	"Growth"
Rev. 25	58.91
Rev. 5	87.98
Rev. 1	86.03
Control	78.49
LSD 5%	8.53
LSD 1%	11.32
LSD 0.1%	14.68

Treatment	"Growth"
Rev. 25	57.13
Rev. 5	93.02
Rev. 1	89.85
Control	75.82
LSD 5%	13.88
LSD 1%	18.47
LSD 0.1%	24.05

Treatment	"Growth"
Rev.25	5.62
Rev.5	85.62
Rev.1	96.33
Control	82.78
LSD5%	8.020
LSD1%	10.675
LSD0.1%	13.904

Table 3.30 and 3.31 and 3.32, The "growth" of isolate 53 in treatments with 25, 5, and 1 ppm of Revysol®, compared to the control over the four days of the experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

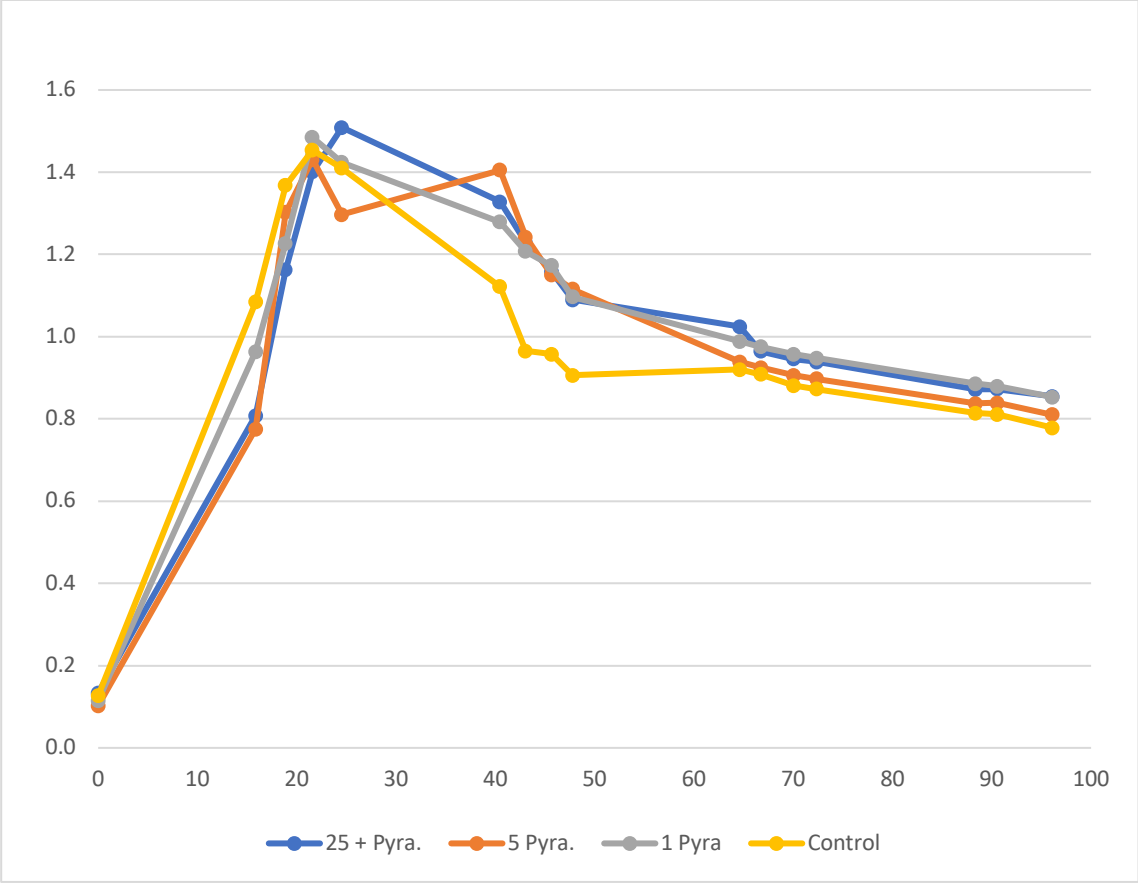


Figure 3.12. The OD curve of isolate 53 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis are illustrated.

Cross screening test with Isolate 53 were run on two occasions. In the first trial (Fig. 3.12) the isolate in treatments with pyraclostrobin started to grow slower than in the control, which tend to be significant in the beginning of first day. The observed peak OD value with the control and treatment 1 and 5 ppm pyraclostrobin occurred at the same point (21.58 hours) and with 25 ppm

in the next assessment (24.53 hours). After reaching the peak OD values, there was then a fall in the ODs until the end of experiment. Comparing the reduction in the control and treatments, it's taken place with the control faster led to indicate remarkable higher OD values in treatments until the end of second 24 hours. However, the higher growth with treatments were occasionally significant (Table 3.33).

In the second trial (Fig. 3.13) the initial peak with the control and all treatments with pyraclostrobin was recorded at 23 hours. Within the first 23 hours the treatment with pyraclostrobin 25 ppm had the remarkable growth higher than the control consistently and then pyraclostrobin 1 and 5 ppm were in the second and third place respectively, they tend not to be constant over multiple timings though (Table 3.34).

After reaching the peak, OD value with treatment 25 ppm started to decline distinctly, while with the control and treatment 1 and 5 ppm high ODs around the peak values were maintained for a longer period before they started to decline after 41.40 hours. At this point despite the control, all treatments indicated a great reduction, which is reflected in occasionally statistically significant lower OD values with 5 and 25 ppm pyraclostrobin until the test stopped.

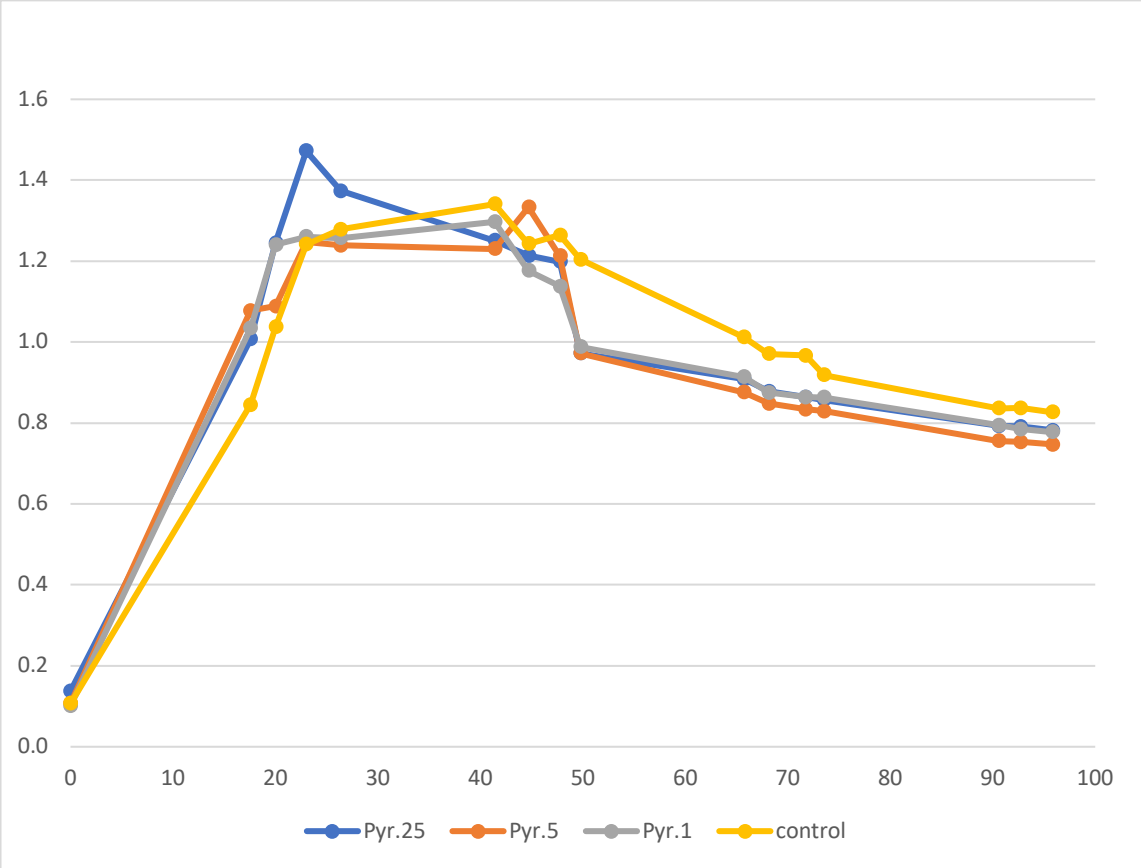


Figure 3.13. The OD curve of isolate 53 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis are illustrated.

Ass. (h)	Pyra.25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1% LSD	0.1% LSD
0.00	0.134	0.103	0.115	0.127	1.962E-10	0.009	0.011	0.015
15.88	0.808	0.775	0.964	1.084	1.501E-04	0.167	0.222	0.288
18.88	1.162	1.303	1.227	1.368	2.884E-08	0.222	0.295	0.383
21.58	1.401	1.431	1.485	1.454	2.147E-08	0.214	0.284	0.369
24.53	1.509	1.297	1.424	1.410	4.852E-03	0.306	0.406	0.526
40.48	1.328	1.405	1.279	1.121	2.820E-03	0.257	0.341	0.442
43.03	1.229	1.242	1.208	0.966	5.305E-05	0.229	0.305	0.395
45.67	1.159	1.150	1.173	0.958	1.142E-04	0.219	0.291	0.377
47.80	1.090	1.116	1.097	0.906	1.365E-05	0.183	0.243	0.316
64.62	1.025	0.939	0.989	0.920	6.411E-03	0.120	0.159	0.207
66.75	0.965	0.925	0.975	0.908	2.279E-02	0.109	0.144	0.187
70.03	0.946	0.906	0.958	0.881	2.964E-02	0.105	0.140	0.181
72.35	0.939	0.898	0.948	0.872	4.603E-02	0.107	0.142	0.185
88.33	0.872	0.838	0.886	0.814	3.898E-01	0.100	0.133	0.172
90.53	0.872	0.839	0.880	0.811	0.490054	0.103	0.137	0.177
96.10	0.855	0.810	0.853	0.778	0.627457	0.107	0.142	0.184

Table 3.33. The mean ODs at each assessment time for isolate 53 in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.138	0.107	0.101	0.107	7.668E-13	0.006	0.008	0.011
17.55	1.007	1.078	1.034	0.845	3.135E-15	0.155	0.206	0.268
20.02	1.246	1.089	1.241	1.037	1.372E-21	0.130	0.173	0.226
22.98	1.472	1.248	1.260	1.241	1.781E-19	0.161	0.214	0.278
26.37	1.374	1.239	1.257	1.278	1.845E-14	0.217	0.288	0.376
41.40	1.251	1.231	1.297	1.341	7.618E-11	0.270	0.359	0.467
44.70	1.214	1.333	1.177	1.243	9.775E-11	0.266	0.354	0.461
47.75	1.199	1.213	1.137	1.264	1.534E-10	0.264	0.351	0.457
49.77	0.973	0.973	0.988	1.204	7.617E-11	0.217	0.289	0.377
65.73	0.909	0.876	0.914	1.012	2.748E-14	0.160	0.213	0.278
68.13	0.878	0.848	0.875	0.971	6.799E-16	0.135	0.180	0.234
71.73	0.864	0.834	0.864	0.967	9.008E-16	0.132	0.176	0.229
73.53	0.857	0.829	0.863	0.919	4.150E-18	0.113	0.151	0.197
90.60	0.793	0.756	0.794	0.836	6.677E-24	0.082	0.109	0.142
92.72	0.791	0.753	0.785	0.837	4.335E-23	0.078	0.104	0.136
95.85	0.782	0.747	0.778	0.826	1.112E-23	0.082	0.109	0.142

Table 3.34. The mean ODs at each assessment time for isolate 53 in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In both trials, the highest peak OD value was recorded with pyraclostrobin 25 ppm (Fig. 3.12-13). Also, the isolate indicated the higher growth with treatments but occasionally (within first 24 hours and second 24 hours in the first and second trial respectively). Therefore, through the average of growth evaluation with none of treatments with pyraclostrobin the isolate 53 had

higher growth than the control (Table 3.35) and in the second run, the growth with treatments were only numerically higher (Table 3.36).

Treatment	"Growth"
Pyra. 25	83.16
Pyra. 5	83.07
Pyra. 1	85.46
Control	78.49
LSD5%	8.529
LSD1%	11.322
LSD0.1%	14.685

Treatment	"Growth"
Pyra. 25	78.20
Pyra. 5	78.19
Pyra. 1	80.25
Control	82.78
LSD5%	8.020
LSD1%	10.675
LSD0.1%	13.904

Table 3.35 and 3.36. The mean "growth" of isolate 53 in treatments with 25, 5, and 1 ppm of pyraclostrobin, over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

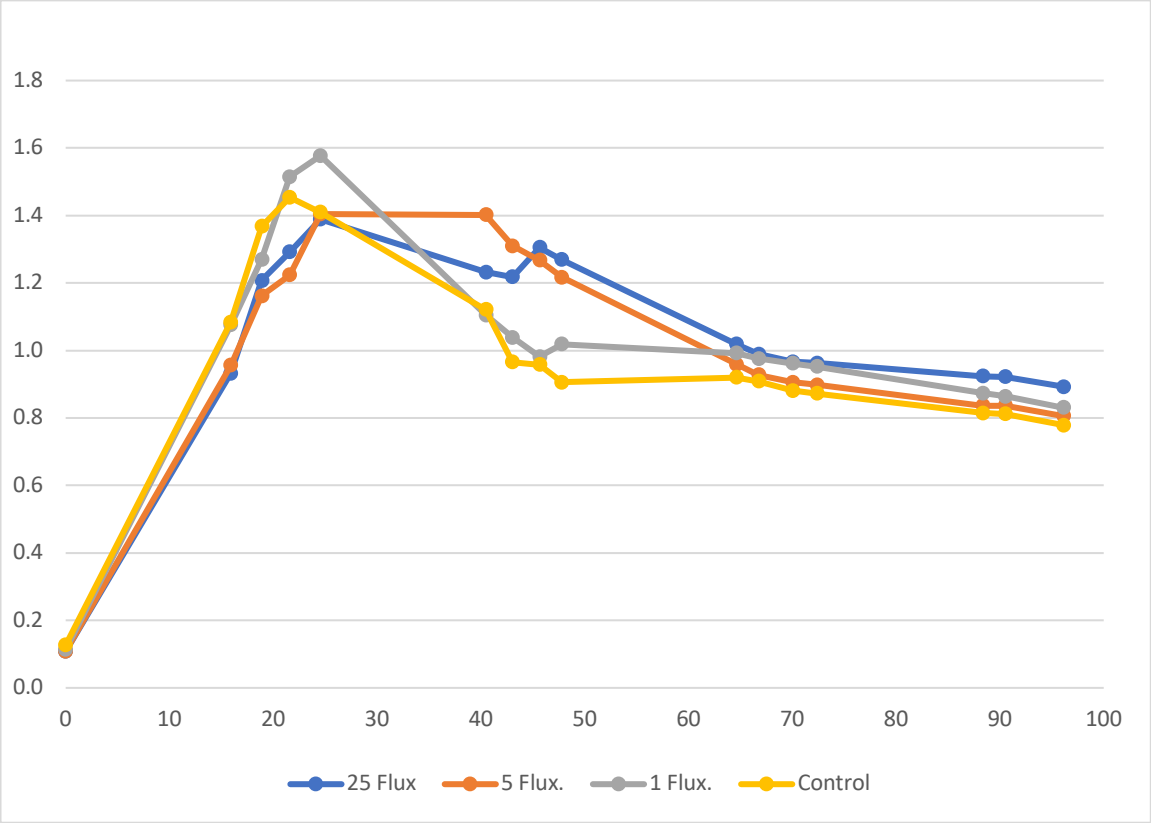


Figure 3.14. The OD curve of isolate 53 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis are illustrated.

Cross screening test with isolate 53 contained treatments with fluxapyroxad were run twice. In the first test all treatments showed exponential growth during the first 24 hours. The first peak was reached with the control at 21.53 hours and the highest peak, 1 ppm fluxapyroxad at 24.53 hours. Later, both control and with 1 ppm fluxapyroxad showed a quick drop in the OD values which then started to level off from 48 hours onwards (Fig. 3.14).

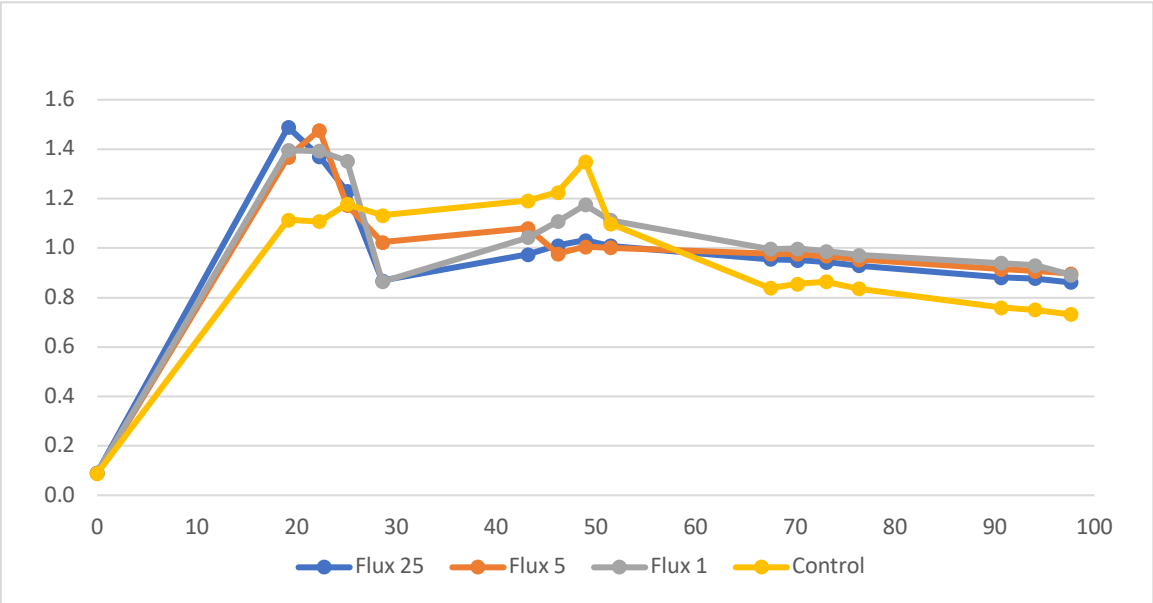


Figure 3.15. The OD curve of isolate 53 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis are illustrated.

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.108	0.109	0.113	0.127	1.962E-10	0.009	0.011	0.015
15.88	0.932	0.956	1.076	1.084	1.501E-04	0.167	0.222	0.288
18.88	1.208	1.162	1.270	1.368	2.884E-08	0.222	0.295	0.383
21.58	1.292	1.224	1.515	1.454	2.147E-08	0.214	0.284	0.369
24.53	1.389	1.404	1.577	1.410	4.852E-03	0.306	0.406	0.526
40.48	1.232	1.402	1.105	1.121	2.820E-03	0.257	0.341	0.442
43.03	1.218	1.310	1.039	0.966	5.305E-05	0.229	0.305	0.395
45.67	1.305	1.267	0.981	0.958	1.142E-04	0.219	0.291	0.377
47.80	1.269	1.217	1.019	0.906	1.365E-05	0.183	0.243	0.316
64.62	1.019	0.959	0.992	0.920	6.411E-03	0.120	0.159	0.207
66.75	0.990	0.928	0.976	0.908	2.279E-02	0.109	0.144	0.187
70.03	0.966	0.906	0.961	0.881	2.964E-02	0.105	0.140	0.181
72.35	0.963	0.899	0.952	0.872	4.603E-02	0.107	0.142	0.185
88.33	0.924	0.837	0.873	0.814	3.898E-01	0.100	0.133	0.172
90.53	0.922	0.837	0.864	0.811	4.901E-01	0.103	0.137	0.177
96.10	0.892	0.806	0.831	0.778	6.275E-01	0.107	0.142	0.184

Table 3.37. The mean ODs at each assessment time for isolate 53 in the presence of three concentrations of fluxapyroxad. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Peak OD values of the 5 and 25 ppm fluxapyroxad treatments were also recorded at the same point as the one with 1 ppm (at 24.53 hours) although in both cases the peak OD reached was lower than for 1ppm. Declines in ODs followed but more gradually than observed for the control and the 1ppm treatment. This is reflected in the significant differences observed between these two treatments and the control between 40.48 and 47.80 hours. From this point, all

treatments and the control began to level out in parallel. The OD values with 25 ppm became significantly higher again within last 8 hours (Table 3.37).

Ass. (h)	Flux.25	Flux.5	Flux.1	Control	P-value	5% LSD	1% LSD	0.1% LSD
0.00	0.091	0.089	0.092	0.089	7.775E-02	0.002	0.003	0.004
19.25	1.489	1.368	1.396	1.115	5.041E-03	0.250	0.332	0.433
22.37	1.371	1.477	1.392	1.107	4.607E-02	0.323	0.430	0.559
25.17	1.229	1.174	1.353	1.177	1.972E-01	0.352	0.469	0.611
28.68	0.868	1.023	0.864	1.133	1.176E-04	0.330	0.439	0.572
43.32	0.975	1.080	1.043	1.192	9.008E-03	0.266	0.355	0.462
46.27	1.010	0.978	1.107	1.226	6.583E-04	0.271	0.361	0.470
49.03	1.032	1.005	1.175	1.350	3.257E-04	0.261	0.348	0.453
67.62	1.009	1.002	1.113	1.099	3.477E-03	0.219	0.291	0.379
70.32	0.955	0.977	0.997	0.838	4.579E-03	0.154	0.204	0.266
73.20	0.951	0.976	0.998	0.855	1.768E-02	0.142	0.189	0.246
76.52	0.944	0.968	0.987	0.865	1.526E-02	0.140	0.187	0.243
90.78	0.929	0.954	0.972	0.836	7.605E-03	0.140	0.186	0.243
94.18	0.881	0.915	0.939	0.760	7.806E-03	0.133	0.177	0.230
97.77	0.878	0.906	0.931	0.750	5.096E-03	0.129	0.172	0.224
97.60	0.862	0.896	0.892	0.732	6.507E-03	0.130	0.173	0.225

Table 3.38. The mean ODs at each assessment time for isolate 53 in the presence of three concentrations of fluxapyroxad. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
25 Flux.	87.47
5 Flux.	85.87
1 Flux.	84.92
Control	78.49
LSD5%	8.53
LSD1%	11.32
LSD0.1%	14.68

Treatment	"Growth"
Flux. 25	82.94
Flux. 5	85.06
Flux. 1	86.30
Control	75.82
LSD5%	13.88
LSD1%	18.47
LSD0.1%	24.05

Table 3.39 and 3.40. The mean "growth" of isolate 53 in treatments with 25, 5, and 1 ppm of fluxapyroxad over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

In the second run of the experiment, the highest peak OD values were recorded with 25 and 5 ppm at 19.15 and 22.27 hours respectively. The 1 ppm reached a lower peak at the same point at 19.15 hours. The lowest peak value was recorded with the control later at 25.07 hours. In all treatments after the peak values were reached there was a rapid initial decline which then became more moderate for the remainder of the experiment. Significant differences between the control and the fluxapyroxad treatments were observed during the first 24 hours as a result of the initial rapid growth and then from 70 hours onwards, statistically significant differences were observed reflecting that the fluxapyroxad treatments maintained a higher OD than the

control (Table 3.38). In both runs of the experiment similar trends in the OD values were observed. Looking at the total "growth" throughout the experiment, in the first experiment all of the fluxapyroxad treatments showed a higher "growth" than the control although this was only statistically significant at the 5% level for the 25ppm dose (Table 3.39). In the second experiment again all of the fluxapyroxad treatments showed more "growth" than the control although here the differences were not statistically significant (Table 3.40). Nonetheless the two experiments suggest that the "growth" of strain 53 is enhanced in the presence of fluxapyroxad.

3.2.4.2. Isolate 61.a

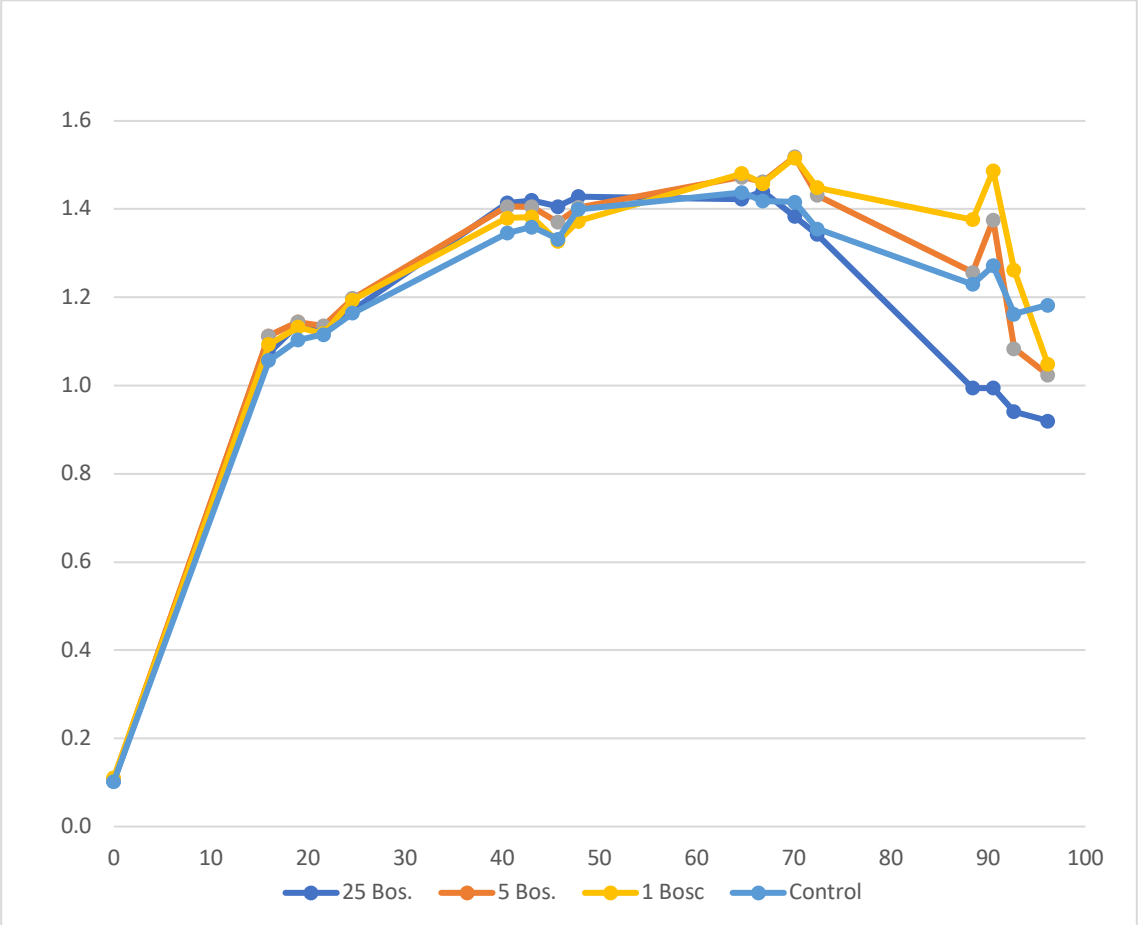


Figure 3.16. The OD curve of isolate 61.a over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Cross screening with the isolate 61.a in treatments with boscalid 1,5, and 25 ppm were run on three occasions. In the first run (Fig. 3.16), all treatments and the control demonstrated a similar growth pattern within first 48 hours. At this point, the treatment with 25 ppm and the control reached a peak OD value and from 48 hours onwards OD values with them started to plateau.

Despite that, the treatment with 5 and 1 ppm continued their exponential growth with a peak OD value at 70.08 hours simultaneously. OD values with the control and 25 ppm at 66.80 hours and treatment 1 and 5 ppm in the next assessment point at 70.08 hours after reaching peak OD values started to decline until the end of experiment. The decrease with boscalid 25 ppm was more rapidly than the control reflected in occasionally statistically significant lower OD values until the test was stopped. Meanwhile, with 1 and 5 ppm the OD values were maintained numerically higher than the control until 92.67 hours where the growth curve of treatments crossed the one with the control (Table 3.41)

In the second run (Fig. 3.17), all treatments and the control had the same level of growth until 42.02 hours. At this point, treatments with 1 and 5 ppm continued their exponential growth while the control started to plateau. With the 25 ppm treatment there was a rapid decline similar to the one in the first trial, after reaching the peak OD value at 42.02 hours until the end of experiment. After another 24 hours, from 65.58 hours, the ODs with the control also started to decline leading to occasionally significant higher OD values with 5 ppm and to a lesser extent, 1 ppm until the test was stopped (Table 3.42).

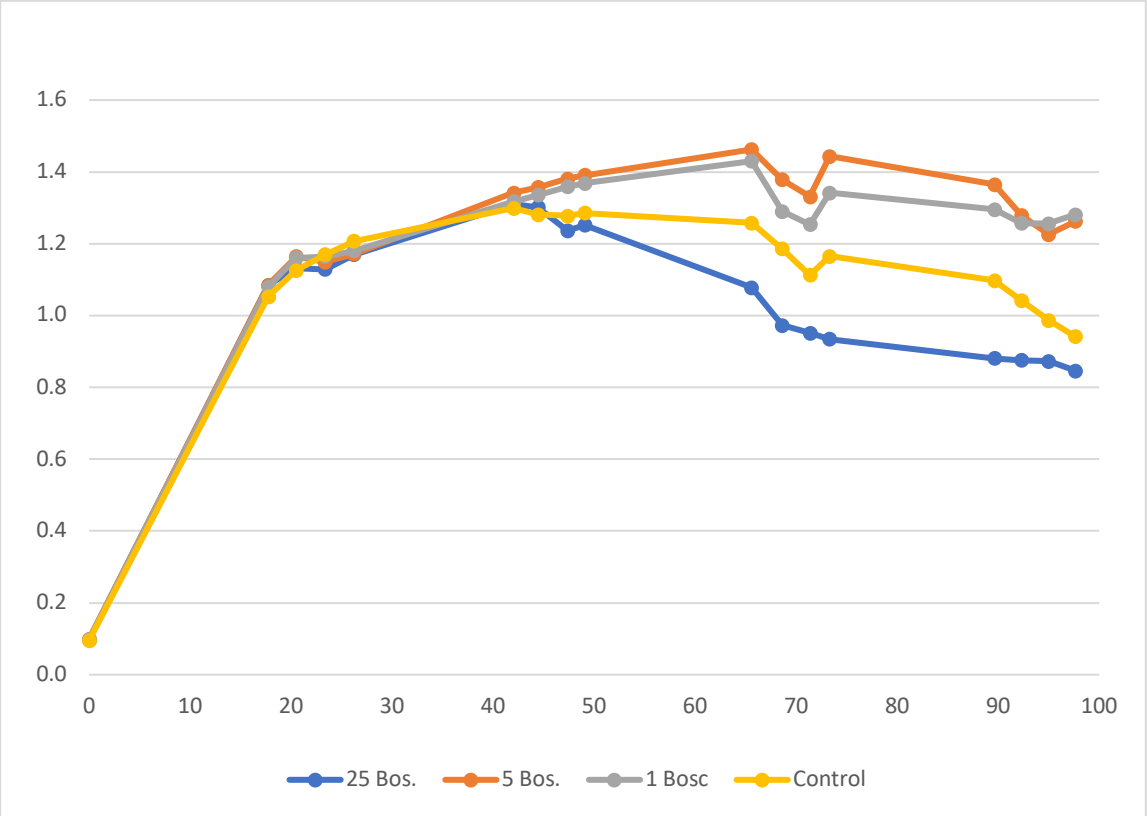


Figure 3.17. The OD curve of isolate 61.a over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

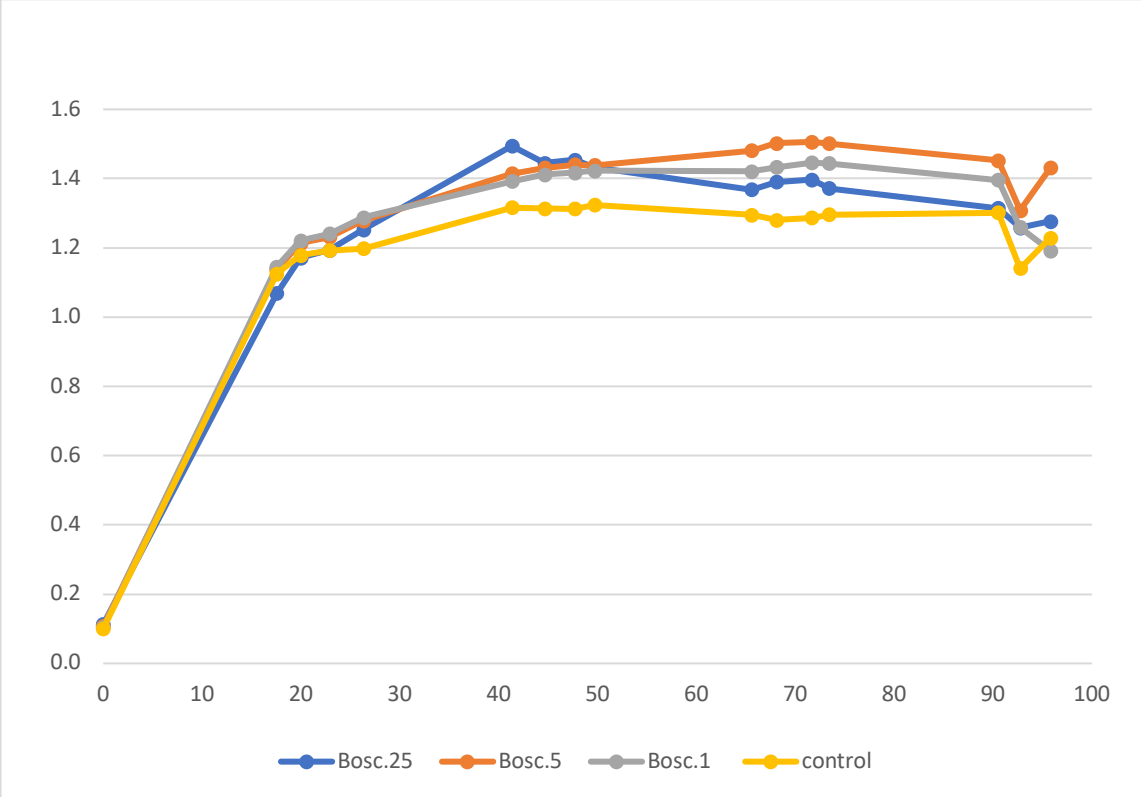


Figure 3.18. The OD curve of isolate 61.a over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Bosc.25	Bosc.5	Bosc.1	Control	P-value	5% LSD	1%LSD	0.1 %LSD
0.00	0.104	0.107	0.111	0.103	3.013E-12	0.007	0.009	0.012
15.95	1.075	1.113	1.093	1.057	7.042E-06	0.146	0.194	0.252
18.97	1.138	1.144	1.133	1.104	1.830E-04	0.150	0.199	0.258
21.60	1.127	1.135	1.118	1.116	7.574E-04	0.150	0.199	0.258
24.60	1.171	1.198	1.194	1.164	8.494E-04	0.156	0.207	0.269
40.50	1.414	1.406	1.380	1.346	1.736E-02	0.184	0.245	0.318
43.05	1.420	1.405	1.382	1.360	1.406E-02	0.184	0.245	0.317
45.72	1.406	1.371	1.327	1.332	1.168E-03	0.169	0.224	0.291
47.82	1.429	1.404	1.373	1.400	1.536E-03	0.176	0.234	0.303
64.67	1.423	1.473	1.481	1.438	2.267E-14	0.139	0.185	0.239
66.80	1.441	1.462	1.459	1.419	4.486E-12	0.150	0.200	0.259
70.08	1.384	1.519	1.517	1.416	2.179E-10	0.177	0.235	0.304
72.38	1.343	1.431	1.449	1.355	8.168E-09	0.176	0.234	0.304
88.40	0.995	1.257	1.377	1.230	7.594E-09	0.185	0.246	0.319
90.53	0.995	1.376	1.487	1.272	6.692E-08	0.244	0.324	0.421
92.67	0.941	1.084	1.262	1.162	3.367E-04	0.244	0.324	0.420
96.13	0.920	1.024	1.048	1.183	5.917E-05	0.195	0.259	0.336

Table 3.41. The mean ODs at each assessment time for isolate 61.a in the presence of three concentrations of boscalid. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In the last run (Fig. 3.18), the highest peak was recorded with 25 ppm at 41.33 hours. However, after reaching the peak it started to decline slowly until the end of experiment. Meanwhile, the highest OD with the control occurred at 49.68 hours and with the 1 and 5 ppm boscalid treatments the highest ODs recorded did not occur until 65.65 hours. However, since the initial growth of the control was lower than the one with treatments, the growth of treatment 5 ppm and to a lesser extent, 1 and 25 ppm was significantly higher than the control from 41.33 hours onwards (Table 3.43). The growth over the course of the experiment was numerically greater for all of the boscalid treatments compared to the control although statistical significance at the 5% level was only reached in the case of the 5ppm treatment (Table 3.46).

In all three experiments, the isolate 61.a showed numerically greater growth than the control at the doses 1 and 5 ppm and in the second experiments to a lesser extent with 25 ppm. Statistical significance was only seen in the third experiment with the 5ppm boscalid treatment. Also, growth in treatments with 1 and 5 ppm and in the last trial with 25 ppm too (Table 3.44, 3.45, 3.46). The general pattern of growth was similar in the three experiments with initial rapid increases in the OD which plateaued or slightly decreased after peak values were observed. The only exception to this was the treatment with 25 ppm falling more rapidly after reaching the peak value and generally remaining lower than the control in the first two runs of this experiment.

Ass. (h)	Bosc.25	Bosc.5	Bosc. 1	Control	P-value	5% LSD	1% LSD	0.1% LSD
0.00	0.098	0.099	0.098	0.095	1.042E-40	0.003	0.004	0.005
17.73	1.085	1.085	1.080	1.052	1.258E-05	0.125	0.166	0.216
20.52	1.132	1.164	1.161	1.126	3.217E-04	0.143	0.189	0.246
23.35	1.129	1.149	1.165	1.170	4.153E-04	0.140	0.186	0.241
26.22	1.169	1.172	1.181	1.207	3.437E-03	0.156	0.207	0.269
42.02	1.310	1.342	1.318	1.299	2.882E-11	0.134	0.178	0.231
44.43	1.301	1.357	1.336	1.281	7.057E-08	0.176	0.233	0.302
47.35	1.236	1.381	1.359	1.277	9.508E-09	0.169	0.225	0.291
49.10	1.251	1.390	1.368	1.286	3.238E-08	0.172	0.229	0.297
65.58	1.078	1.462	1.430	1.258	6.884E-06	0.236	0.313	0.406
68.62	0.973	1.378	1.290	1.186	2.521E-06	0.212	0.281	0.364
71.37	0.952	1.331	1.254	1.112	2.000E-06	0.182	0.241	0.313
73.32	0.934	1.444	1.341	1.166	1.889E-07	0.213	0.283	0.367
89.63	0.881	1.365	1.296	1.098	1.722E-06	0.230	0.305	0.396
92.32	0.876	1.279	1.257	1.041	4.6885E-06	0.206	0.274	0.356
94.98	0.873	1.225	1.256	0.987	7.2993E-06	0.197	0.261	0.339
97.65	0.846	1.263	1.281	0.941	3.845E-06	0.217	0.288	0.374

Table 3.42. The mean ODs at each assessment time for isolate 61.a in the presence of three concentrations of boscalid. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Bosc.25	Bosc.5	Bosc.1	Control	P-value	5%LSD	1%LSD	0.1%LSD
0.00	0.113	0.108	0.105	0.100	6.898E-06	0.012	0.016	0.021
17.55	1.068	1.138	1.146	1.125	4.397E-05	0.128	0.170	0.222
19.98	1.171	1.214	1.221	1.179	1.169E-03	0.119	0.159	0.207
22.88	1.194	1.231	1.241	1.192	7.046E-02	0.090	0.120	0.157
26.33	1.253	1.279	1.288	1.198	6.368E-02	0.093	0.123	0.160
41.33	1.494	1.415	1.392	1.316	1.491E-04	0.079	0.105	0.137
44.65	1.445	1.431	1.412	1.314	1.865E-03	0.092	0.123	0.160
47.68	1.455	1.440	1.417	1.313	5.631E-04	0.094	0.125	0.163
49.68	1.432	1.438	1.423	1.324	3.473E-03	0.094	0.125	0.162
65.60	1.368	1.481	1.421	1.295	1.804E-01	0.161	0.214	0.279
68.08	1.391	1.502	1.433	1.281	1.618E-01	0.185	0.247	0.321
71.67	1.397	1.506	1.446	1.287	1.318E-01	0.198	0.264	0.344
73.43	1.372	1.502	1.444	1.296	1.013E-01	0.201	0.267	0.348
90.52	1.314	1.452	1.396	1.302	5.685E-01	0.293	0.391	0.509
92.75	1.258	1.308	1.259	1.140	2.566E-01	0.159	0.212	0.276
95.80	1.277	1.431	1.192	1.229	1.535E-01	0.287	0.382	0.498

Table 3.43. The mean ODs at each assessment time for isolate 61.a in the presence of three concentrations of boscalid. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Bosc.25	101.10
Bosc.5	106.54
Bosc.1	107.26
Control	103.67
LSD 5%	12.36
LSD 1%	16.40
LSD 0.1%	21.27

Treatment	"Growth"
Bosc. 25	87.75
Bosc. 5	107.46
Bosc.1	104.76
Control	96.13
LSD5%	12.46
LSD1%	16.54
LSD 0.1%	21.45

Treatment	"Growth"
Bosc.25	105.56
Bosc.5	110.99
Bosc.1	108.48
Control	100.48
LSD5%	8.022
LSD1%	10.678
LSD 0.1%	13.907

Table 3.44. and 3.45. and 3.46. The mean "growth" of isolate 61.a in treatments with 25, 5, and 1 ppm of boscalid over the four day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

Cross screening test with the isolate 61.a containing treatments with Revysol® were run three times. In the first run, the control and treatments with 1 and 5 ppm demonstrated a similar trend in the OD values. They reached peak OD values at 66.80 hours and then started to decline gradually until the test was stopped (Fig. 3.19). In general, the 1 and 5 ppm Revysol® treatments showed numerically higher OD values than the ones with the control over the 4 experimental days. The exception with this was 25 ppm, which had a lower peak OD value at 40.50 and then started to decrease. The low OD values with 25 ppm were always statistically significantly lower over the whole experimental period (Table 3.47, 3.50).

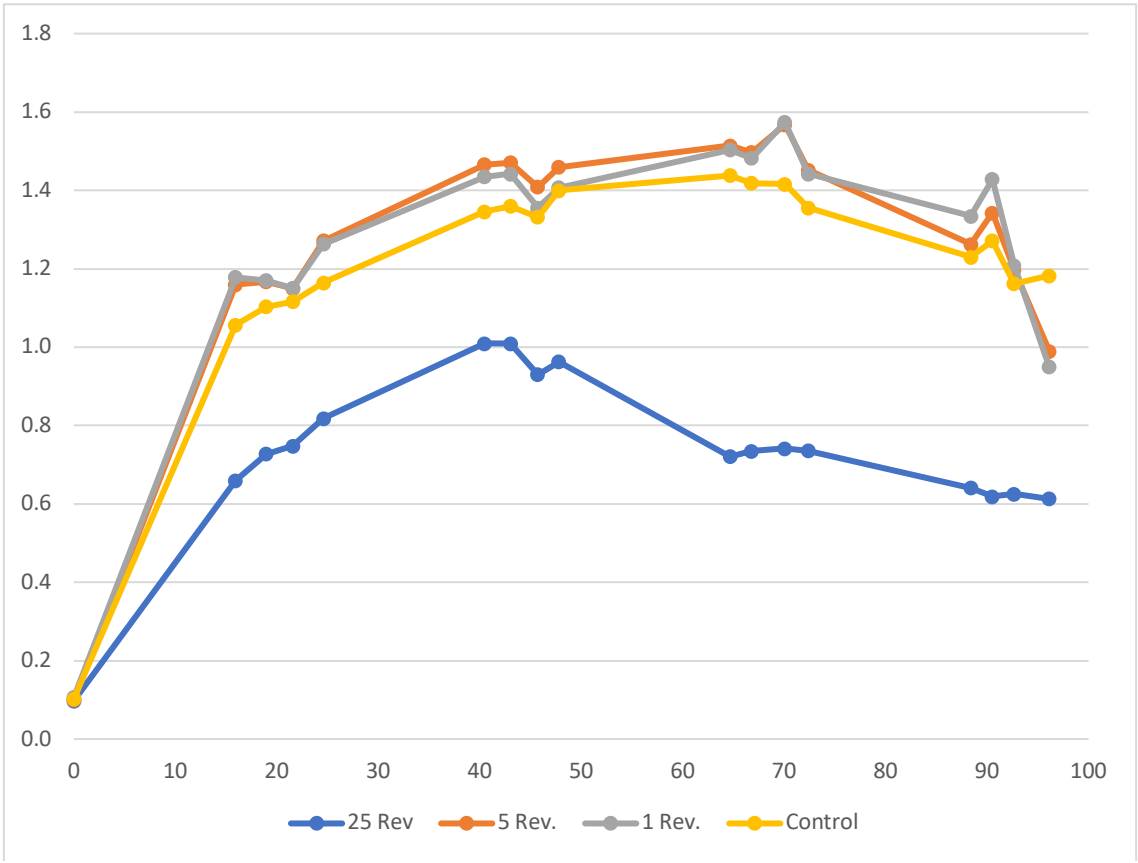


Figure 3.19. The OD curve of isolate 61.a over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis

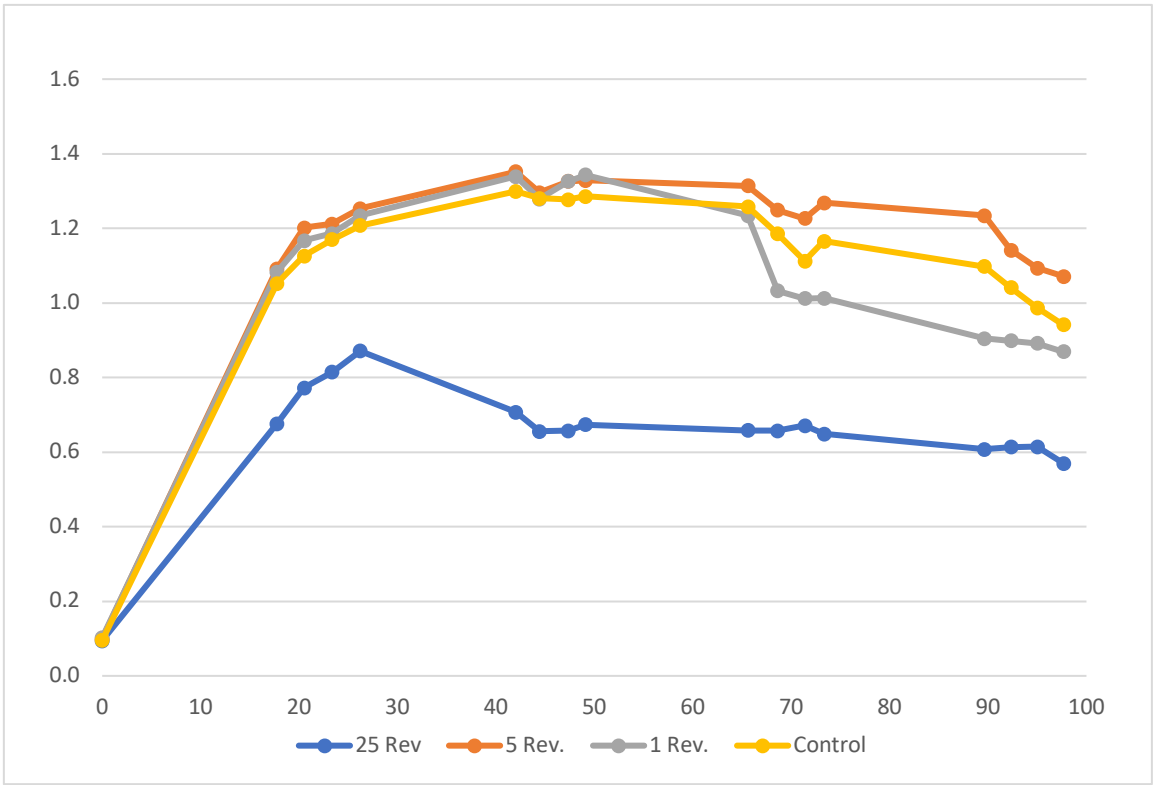


Figure 3.20. The OD curve of isolate 61.a over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

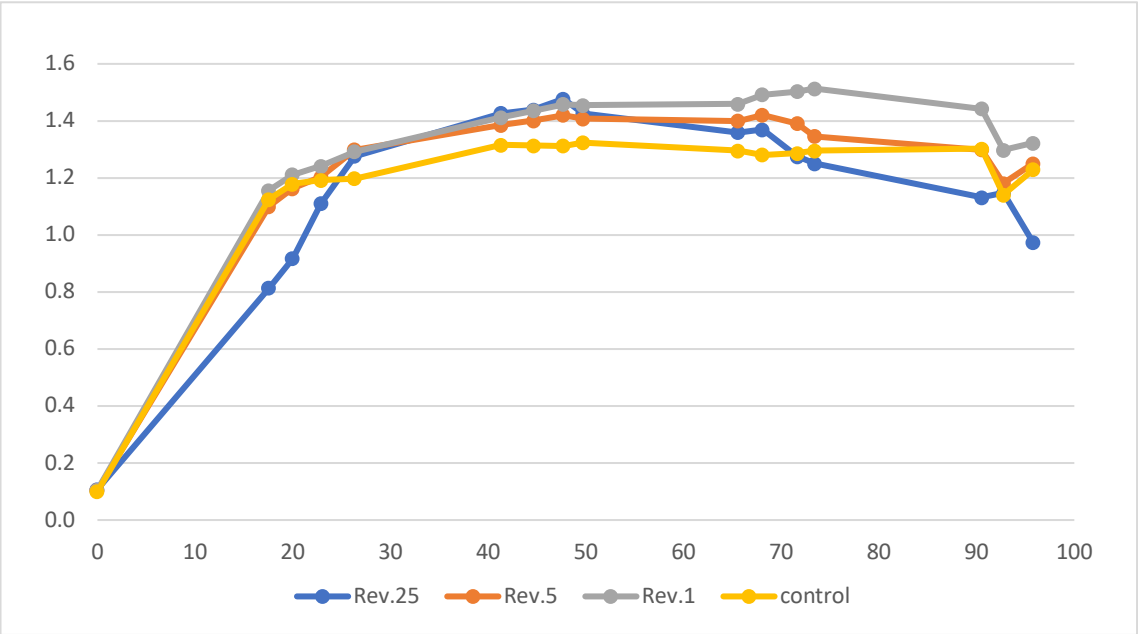


Figure 3.21. The OD curve of isolate 61.a over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.098	0.100	0.106	0.103	3.013E-12	0.007	0.009	0.012
15.95	0.660	1.160	1.179	1.057	7.042E-06	0.146	0.194	0.252
18.97	0.728	1.167	1.170	1.104	1.830E-04	0.150	0.199	0.258
21.60	0.748	1.150	1.151	1.116	7.574E-04	0.150	0.199	0.258
24.60	0.819	1.272	1.263	1.164	8.494E-04	0.156	0.207	0.269
40.50	1.009	1.466	1.435	1.346	1.736E-02	0.184	0.245	0.318
43.05	1.009	1.471	1.442	1.360	1.406E-02	0.184	0.245	0.317
45.72	0.930	1.408	1.356	1.332	1.168E-03	0.169	0.224	0.291
47.82	0.963	1.460	1.407	1.400	1.536E-03	0.176	0.234	0.303
64.67	0.721	1.514	1.503	1.438	2.267E-14	0.139	0.185	0.239
66.80	0.735	1.497	1.482	1.419	4.486E-12	0.150	0.200	0.259
70.08	0.742	1.568	1.574	1.416	2.179E-10	0.177	0.235	0.304
72.38	0.736	1.452	1.442	1.355	8.168E-09	0.176	0.234	0.304
88.40	0.641	1.263	1.334	1.230	7.594E-09	0.185	0.246	0.319
90.53	0.619	1.343	1.428	1.272	6.692E-08	0.244	0.324	0.421
92.67	0.625	1.197	1.209	1.162	3.367E-04	0.244	0.324	0.420
96.13	0.613	0.989	0.950	1.183	5.917E-05	0.195	0.259	0.336

Table 3.47 The mean ODs at each assessment time for isolate 61.a in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In the second trial like the first run, treatments with 1 and 5 ppm and the control indicated the analogous growth pattern with earlier peak OD values at 42.02 hours. However, after reaching the peak the ODs with the 1 ppm treatment were lower than those of the control and the 5ppm treatment until the end of experiment (Fig. 3.20). Meanwhile, the ODs with the control and 5 ppm started to level off after reaching the peak value until 68.62 hours. From this point on, the

ODs with control and 5 ppm also started to fall. However, since the reduction with 1 ppm was faster it caused the numerically lower average growth with 1ppm. Moreover, like in the first run, the treatment 25 ppm showed the lowest growth which was significantly lower at the 0.5% level compared to the control over the 4 days (Table 3.48, 3.51).

In the third trial OD values with 25 ppm were only significantly lower than the control during the first 23 hours (Fig. 3.21). The control and treatments 1 and 5 ppm showed a similar trend during the first 24 hours. The control reached a peak value at 49:68 hours and the treatments at 47:68 hours after which the ODs started to level off. The generally slower OD increase with the control resulted in statistical differences between 41 and 50 hours. With the 1 ppm treatment the ODs tended to slightly increase and the ODs with the 25 ppm treatment started to decline after 49,68 until the test was finished although there were no statistically significant differences between this treatment and the control (Table 3.49). Again the "growth" with 25 ppm was slightly less than the control, whereas the 1 and 5 ppm treatments showed greater "growth" than the control which was statistically significant in the former case (Table 3.52).

Over the three trials conducted, 3 trials on isolate 61.a with Revysol® suggest that there was a trend towards an increase in the "growth" of isolate with 1 and 5 ppm of Revysol®, although statistical significance at the 5% level was only observed in the third trial The treatment with 25 ppm Revysol® led to a reduction in "growth" which was statistically significant in the first two trials where the effect was most pronounced.

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.095	0.100	0.102	0.095	1.042E-40	0.003	0.004	0.005
17.73	0.676	1.091	1.083	1.052	1.258E-05	0.125	0.166	0.216
20.52	0.773	1.202	1.167	1.126	3.217E-04	0.143	0.189	0.246
23.35	0.814	1.212	1.186	1.170	4.153E-04	0.140	0.186	0.241
26.22	0.871	1.253	1.234	1.207	3.437E-03	0.156	0.207	0.269
42.02	0.707	1.352	1.339	1.299	2.882E-11	0.134	0.178	0.231
44.43	0.656	1.296	1.279	1.281	7.057E-08	0.176	0.233	0.302
47.35	0.658	1.326	1.326	1.277	9.508E-09	0.169	0.225	0.291
49.10	0.674	1.329	1.343	1.286	3.238E-08	0.172	0.229	0.297
65.58	0.659	1.314	1.234	1.258	6.884E-06	0.236	0.313	0.406
68.62	0.657	1.249	1.033	1.186	2.521E-06	0.212	0.281	0.364
71.37	0.671	1.227	1.012	1.112	2.000E-06	0.182	0.241	0.313
73.32	0.649	1.269	1.012	1.166	1.889E-07	0.213	0.283	0.367
89.63	0.608	1.234	0.905	1.098	1.722E-06	0.230	0.305	0.396
92.32	0.614	1.142	0.899	1.041	4.6885E-06	0.206	0.274	0.356
94.98	0.614	1.093	0.892	0.987	7.2993E-06	0.197	0.261	0.339
97.65	0.570	1.071	0.870	0.941	3.845E-06	0.217	0.288	0.374

Table 3.48. The mean ODs at each assessment time for isolate 61.a in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Rev.25	Rev. 5	Rev.1	Control	P-value	5%LSD	1%LSD	0.1%LSD
0.00	0.108	0.104	0.106	0.100	6.898E-06	0.012	0.016	0.021
17.55	0.813	1.100	1.156	1.125	4.397E-05	0.128	0.170	0.222
19.98	0.918	1.163	1.212	1.179	1.169E-03	0.119	0.159	0.207
22.88	1.110	1.203	1.242	1.192	7.046E-02	0.090	0.120	0.157
26.33	1.277	1.299	1.292	1.198	6.368E-02	0.093	0.123	0.160
41.33	1.427	1.385	1.412	1.316	1.491E-04	0.079	0.105	0.137
44.65	1.439	1.401	1.436	1.314	1.865E-03	0.092	0.123	0.160
47.68	1.477	1.420	1.458	1.313	5.631E-04	0.094	0.125	0.163
49.68	1.426	1.407	1.455	1.324	3.473E-03	0.094	0.125	0.162
65.60	1.359	1.400	1.460	1.295	1.804E-01	0.161	0.214	0.279
68.08	1.370	1.421	1.492	1.281	1.618E-01	0.185	0.247	0.321
71.67	1.276	1.391	1.503	1.287	1.318E-01	0.198	0.264	0.344
73.43	1.250	1.346	1.514	1.296	1.013E-01	0.201	0.267	0.348
90.52	1.132	1.299	1.443	1.302	5.685E-01	0.293	0.391	0.509
92.75	1.147	1.181	1.298	1.140	2.566E-01	0.159	0.212	0.276
95.80	0.973	1.250	1.323	1.229	1.535E-01	0.287	0.382	0.498

Table 3.49. The mean ODs at each assessment time for isolate 61.a in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Rev. 25	60.41
Rev. 5	110.74
Rev. 1	109.92
Control	103.67
LSD5%	12.36
LSD1%	16.40
LSD0.1%	21.27

Treatment	"Growth"
Rev. 25	52.68
Rev. 5	101.88
Rev. 1	92.33
Control	96.13
LSD5%	12.46
LSD1%	16.54
LSD0.1%	21.45

Treatment	"Growth"
Rev. 25	97.93
Rev. 5	105.29
Rev. 1	111.25
Control	100.48
LSD5%	8.022
LSD1%	10.678
LSD0.1%	13.907

Table 3.50. and 3.51. and 3.52. and 3.53. The mean "growth" of isolate 61.a in treatments with 25, 5, and 1 ppm of Revysol® over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

Cross screening test with isolate 61.a with pyraclostrobin were run three times. In first two experiments, the isolate started to grow in the control and treatments in parallel. In the first trial (Fig. 3.22) all 3 treatments and the control reached maximum observed OD values after 66.80 hours but in the second trial (Fig. 3.23) earlier at 44.49 hours. In the first trial, after reaching peak values there was a fall with OD values in the treatments and the control until the test was stopped. In the second run, after reaching the peak, the control started slowly to decline. With the pyraclostrobin treatments OD values declined until 68.62 hours and from then onwards they started to level off until the test finished resulting in numerical differences between the pyraclostrobin in treatments and the control.

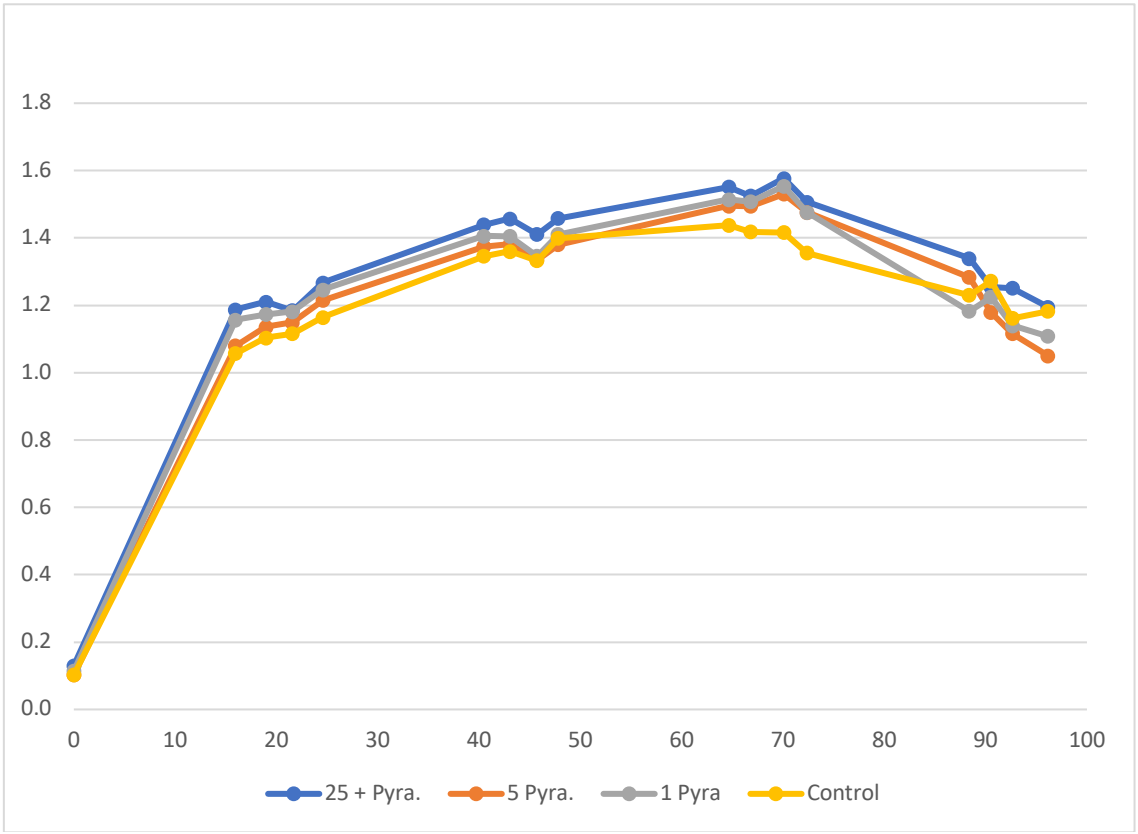


Figure 3.22. The OD curve of isolate 61.a over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis

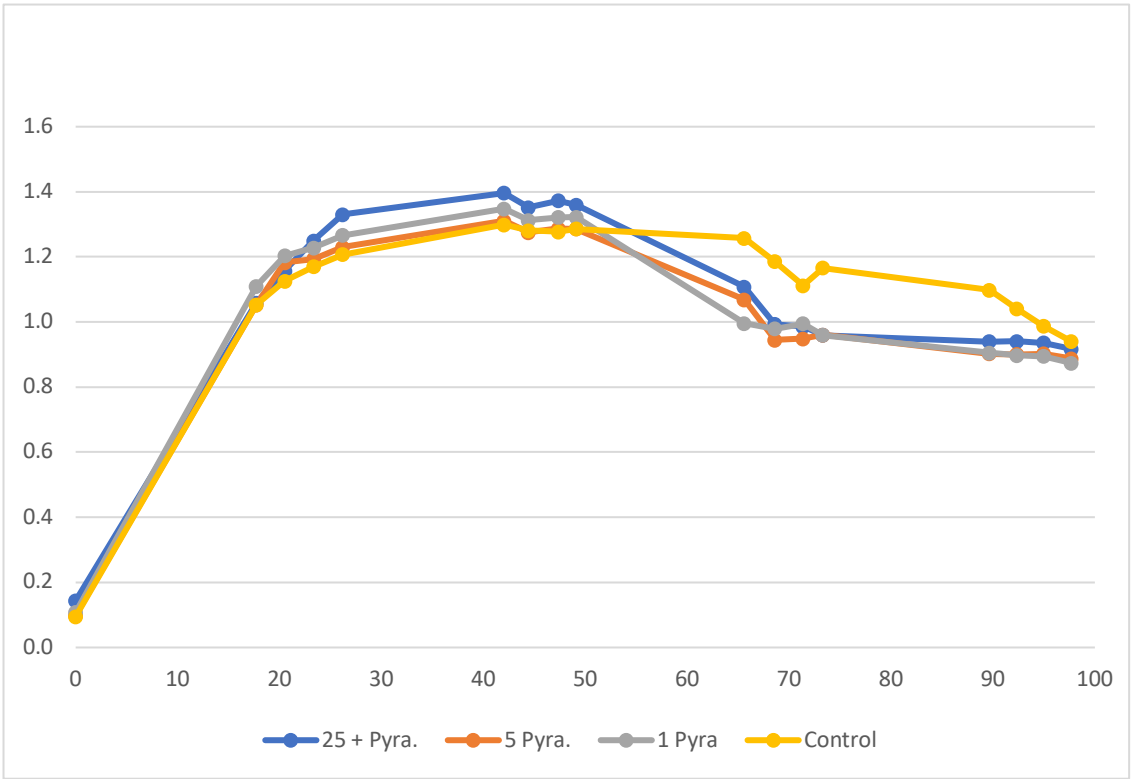


Figure 3.23. The OD curve of isolate 61.a over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

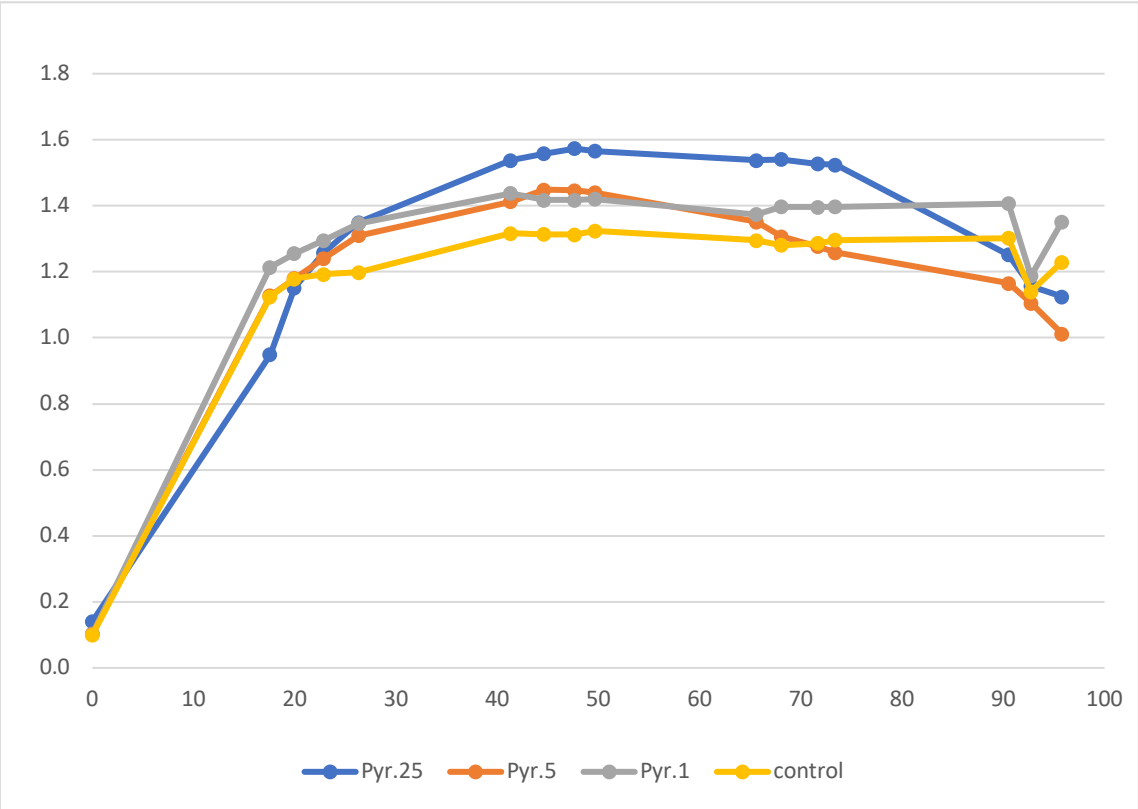


Figure 3.24. The OD curve of isolate 61.a over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.130	0.103	0.114	0.103	3.0E-12	0.007	0.009	0.012
15.95	1.187	1.080	1.157	1.057	7.0E-06	0.146	0.194	0.252
18.97	1.210	1.136	1.173	1.104	1.8E-04	0.150	0.199	0.258
21.60	1.184	1.149	1.182	1.116	7.6E-04	0.150	0.199	0.258
24.60	1.267	1.215	1.247	1.164	8.5E-04	0.156	0.207	0.269
40.50	1.439	1.374	1.406	1.346	1.7E-02	0.184	0.245	0.318
43.05	1.458	1.382	1.405	1.360	1.4E-02	0.184	0.245	0.317
45.72	1.410	1.335	1.346	1.332	1.2E-03	0.169	0.224	0.291
47.82	1.458	1.381	1.411	1.400	1.5E-03	0.176	0.234	0.303
64.67	1.552	1.496	1.515	1.438	2.3E-14	0.139	0.185	0.239
66.80	1.524	1.494	1.508	1.419	4.5E-12	0.150	0.200	0.259
70.08	1.577	1.530	1.554	1.416	2.2E-10	0.177	0.235	0.304
72.38	1.507	1.476	1.477	1.355	8.2E-09	0.176	0.234	0.304
88.40	1.340	1.284	1.182	1.230	7.6E-09	0.185	0.246	0.319
90.53	1.255	1.179	1.224	1.272	6.7E-08	0.244	0.324	0.421
92.67	1.251	1.116	1.140	1.162	3.4E-04	0.244	0.324	0.420
96.13	1.195	1.050	1.108	1.183	5.9E-05	0.195	0.259	0.336

Table 3.53. The mean ODs at each assessment time for isolate 61.a in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.144	0.099	0.108	0.095	1.0E-40	0.003	0.004	0.005
17.73	1.059	1.053	1.110	1.052	1.3E-05	0.125	0.166	0.216
20.52	1.157	1.182	1.204	1.126	3.2E-04	0.143	0.189	0.246
23.35	1.249	1.193	1.228	1.170	4.2E-04	0.140	0.186	0.241
26.22	1.331	1.230	1.267	1.207	3.4E-03	0.156	0.207	0.269
42.02	1.396	1.311	1.348	1.299	2.9E-11	0.134	0.178	0.231
44.43	1.352	1.275	1.313	1.281	7.1E-08	0.176	0.233	0.302
47.35	1.372	1.287	1.321	1.277	9.5E-09	0.169	0.225	0.291
49.10	1.360	1.286	1.322	1.286	3.2E-08	0.172	0.229	0.297
65.58	1.108	1.069	0.996	1.258	6.9E-06	0.236	0.313	0.406
68.62	0.993	0.945	0.980	1.186	2.5E-06	0.212	0.281	0.364
71.37	0.986	0.949	0.995	1.112	2.0E-06	0.182	0.241	0.313
73.32	0.959	0.961	0.959	1.166	1.9E-07	0.213	0.283	0.367
89.63	0.940	0.902	0.905	1.098	1.7E-06	0.230	0.305	0.396
92.32	0.940	0.900	0.898	1.041	4.7E-06	0.206	0.274	0.356
94.98	0.936	0.903	0.895	0.987	7.3E-06	0.197	0.261	0.339
97.65	0.917	0.887	0.874	0.941	3.8E-06	0.217	0.288	0.374

Table 3.54. The mean ODs at each assessment time for isolate 61.a in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.141	0.105	0.102	0.100	6.9E-06	0.012	0.016	0.021
17.55	0.949	1.127	1.214	1.125	4.4E-05	0.128	0.170	0.222
19.98	1.152	1.180	1.256	1.179	1.2E-03	0.119	0.159	0.207
22.88	1.259	1.241	1.295	1.192	7.0E-02	0.090	0.120	0.157
26.33	1.350	1.310	1.347	1.198	6.4E-02	0.093	0.123	0.160
41.33	1.538	1.413	1.437	1.316	1.5E-04	0.079	0.105	0.137
44.65	1.558	1.449	1.418	1.314	1.9E-03	0.092	0.123	0.160
47.68	1.573	1.446	1.417	1.313	5.6E-04	0.094	0.125	0.163
49.68	1.566	1.440	1.421	1.324	3.5E-03	0.094	0.125	0.162
65.60	1.538	1.351	1.373	1.295	1.8E-01	0.161	0.214	0.279
68.08	1.541	1.307	1.397	1.281	1.6E-01	0.185	0.247	0.321
71.67	1.527	1.276	1.396	1.287	1.3E-01	0.198	0.264	0.344
73.43	1.524	1.259	1.397	1.296	1.0E-01	0.201	0.267	0.348
90.52	1.252	1.166	1.406	1.302	5.7E-01	0.293	0.391	0.509
92.75	1.157	1.105	1.188	1.140	2.6E-01	0.159	0.212	0.276
95.80	1.124	1.012	1.352	1.229	1.5E-01	0.287	0.382	0.498

Table 3.55. The mean ODs at each assessment time for isolate 61.a in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In the third trial (Fig. 3.24), all treatments and the control reached their maximum observed OD values at 41.33 hours. However, from 19.98 hours the OD values with the pyraclostrobin treatments were higher than the ones with the control resulting in statistically significant

differences between the pyraclostrobin treatments and the control, especially with 25 ppm where the significant differences persisted for longer (Table 3.55). In contrast to the first two experiments where no significant differences were observed. Nonetheless in the first two runs, the pyraclostrobin treatments showed numerically higher ODs than the control during this period. This is reflected in significant differences between control and treatments 1 and 5 ppm over second 24 hours at 5 and 1 % level and for a longer time and more significant with 25 ppm from the second 24 hours for 2 days (Table 3.53, 3.54).

OD values with control and treatments with 1 and 5 ppm started to level off after reaching the peak value until the test was stopped but with 25 ppm reached the highest peak but started to decline after level off for 24 hours.

Treatment	"Growth"
Pyra. 25	110.23
Pyra. 5	106.78
Pyra. 1	107.30
Control	103.67
LSD5%	12.36
LSD1%	16.40
LSD0.1%	21.27

Treatment	"Growth"
Pyra. 25	89.18
Pyra. 5	89.03
Pyra. 1	89.62
Control	96.13
LSD5%	12.46
LSD1%	16.54
LSD0.1%	21.45

Treatment	"Growth"
Pyra. 25	107.90
Pyra. 5	102.80
Pyra. 1	109.69
Control	100.48
LSD5%	8.022
LSD1%	10.678
LSD0.1%	13.907

Table 3.56, 3.57 and 3.58. The mean "growth" of isolate 61.a in treatments with 25, 5, and 1 ppm of pyraclostrobin over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

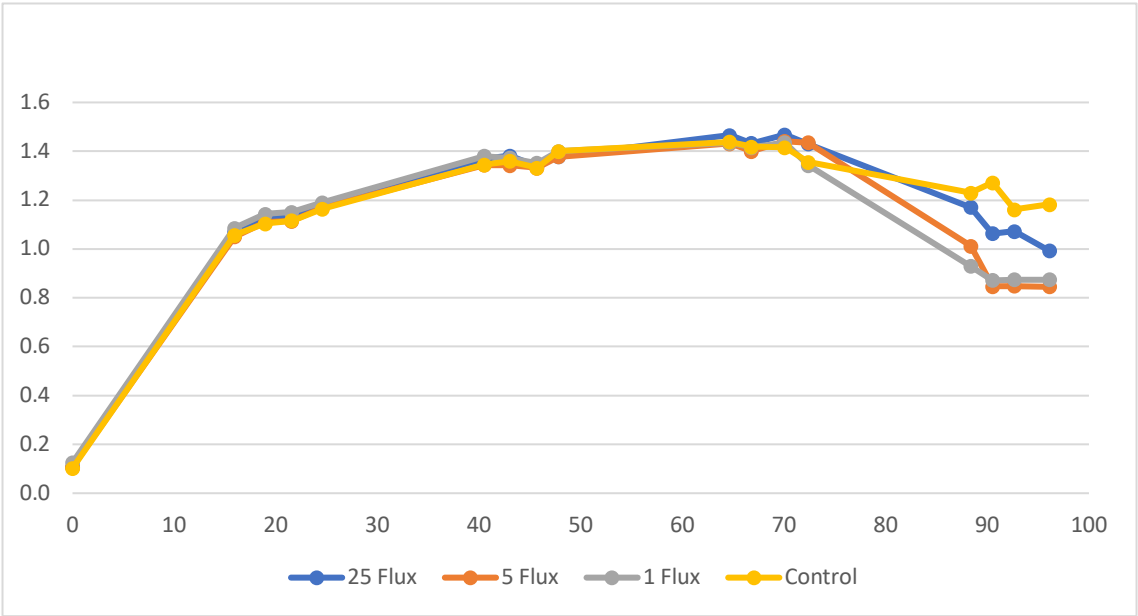


Figure 3.25. The OD curve of isolate 61.a over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis

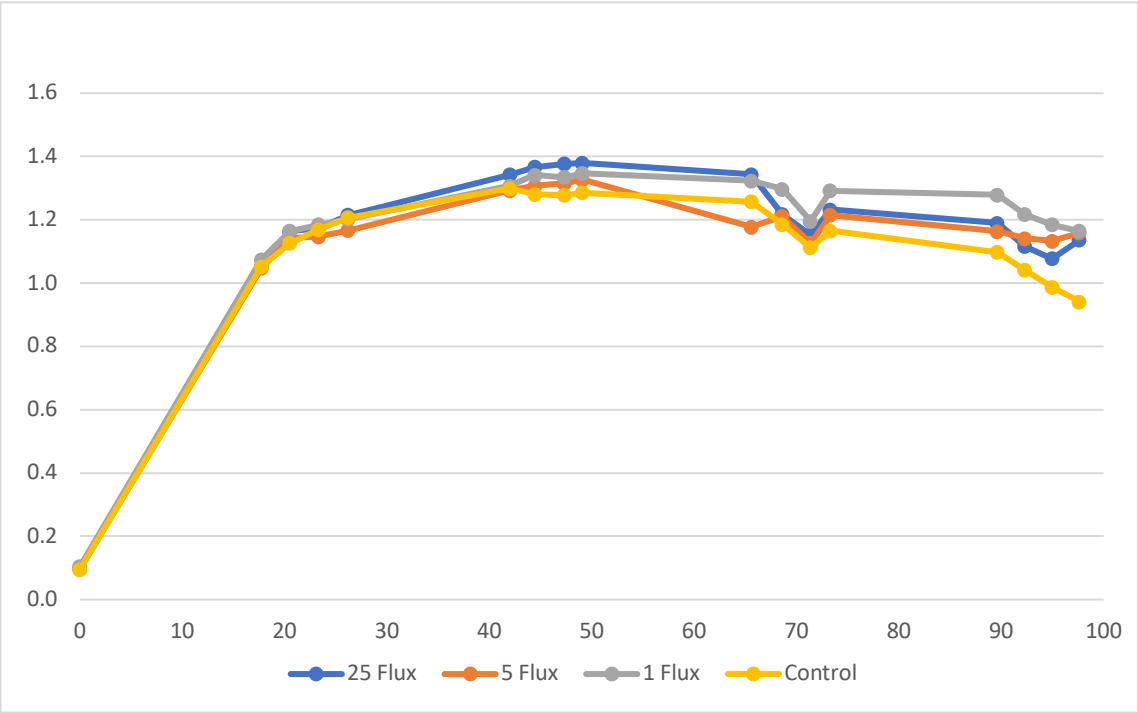


Figure 3.26. The OD curve of isolate 61.a over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

In general, treatments with different doses of pyraclostrobin indicated an improved "growth" of isolate 61.a in the first and third trials (Table 3.56, 3.58). This increase was statistically significant (5% level) in the third trial with the 1ppm pyraclostrobin treatment. However, in the second trial none of treatments showed higher "growth" than the control (Table 3.57).

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.108	0.108	0.125	0.103	3.0E-12	0.007	0.009	0.012
15.95	1.069	1.050	1.085	1.057	7.0E-06	0.146	0.194	0.252
18.97	1.128	1.109	1.143	1.104	1.8E-04	0.150	0.199	0.258
21.60	1.133	1.115	1.152	1.116	7.6E-04	0.150	0.199	0.258
24.60	1.184	1.170	1.191	1.164	8.5E-04	0.156	0.207	0.269
40.50	1.369	1.344	1.381	1.346	1.7E-02	0.184	0.245	0.318
43.05	1.381	1.343	1.373	1.360	1.4E-02	0.184	0.245	0.317
45.72	1.338	1.332	1.352	1.332	1.2E-03	0.169	0.224	0.291
47.82	1.379	1.378	1.401	1.400	1.5E-03	0.176	0.234	0.303
64.67	1.466	1.432	1.435	1.438	2.3E-14	0.139	0.185	0.239
66.80	1.433	1.400	1.416	1.419	4.5E-12	0.150	0.200	0.259
70.08	1.468	1.441	1.435	1.416	2.2E-10	0.177	0.235	0.304
72.38	1.430	1.435	1.342	1.355	8.2E-09	0.176	0.234	0.304
88.40	1.170	1.012	0.930	1.230	7.6E-09	0.185	0.246	0.319
90.53	1.063	0.847	0.872	1.272	6.7E-08	0.244	0.324	0.421
92.67	1.073	0.848	0.875	1.162	3.4E-04	0.244	0.324	0.420
96.13	0.993	0.845	0.874	1.183	5.9E-05	0.195	0.259	0.336

Table 3.59. The mean ODs at each assessment time for isolate 61.a in the presence of three concentrations of fluxapyroxad. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.099	0.098	0.104	0.095	1.0E-40	0.003	0.004	0.005
17.73	1.054	1.047	1.074	1.052	1.3E-05	0.125	0.166	0.216
20.52	1.164	1.141	1.165	1.126	3.2E-04	0.143	0.189	0.246
23.35	1.173	1.148	1.185	1.170	4.2E-04	0.140	0.186	0.241
26.22	1.216	1.166	1.202	1.207	3.4E-03	0.156	0.207	0.269
42.02	1.342	1.293	1.307	1.299	2.9E-11	0.134	0.178	0.231
44.43	1.367	1.308	1.342	1.281	7.1E-08	0.176	0.233	0.302
47.35	1.377	1.315	1.335	1.277	9.5E-09	0.169	0.225	0.291
49.10	1.380	1.327	1.347	1.286	3.2E-08	0.172	0.229	0.297
65.58	1.344	1.178	1.324	1.258	6.9E-06	0.236	0.313	0.406
68.62	1.217	1.212	1.297	1.186	2.5E-06	0.212	0.281	0.364
71.37	1.152	1.126	1.196	1.112	2.0E-06	0.182	0.241	0.313
73.32	1.232	1.215	1.292	1.166	1.9E-07	0.213	0.283	0.367
89.63	1.190	1.163	1.279	1.098	1.7E-06	0.230	0.305	0.396
92.32	1.116	1.141	1.217	1.041	4.7E-06	0.206	0.274	0.356
94.98	1.077	1.133	1.185	0.987	7.3E-06	0.197	0.261	0.339
97.65	1.136	1.159	1.164	0.941	3.8E-06	0.217	0.288	0.374

Table 3.60. The mean ODs at each assessment time for isolate 61.a in the presence of three concentrations of fluxapyroxad. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Flux. 25	103.29
Flux. 5	99.31
Flux. 1	97.78
Control	103.67
LSD5%	12.36
LSD1%	16.40
LSD0.1%	21.27

Treatment	"Growth"
Flux. 25	100.91
Flux. 5	97.25
Flux. 1	101.90
Control	96.13
LSD5%	12.46
LSD1%	16.54
LSD0.1%	21.45

Table 3.61, 3.62. The mean "growth" of isolate 61.a in treatments with 25, 5, and 1 ppm of fluxapyroxad over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

Cross screening with isolate 61.a with fluxapyroxad treatments were run on two occasions. In both runs (Fig. 3.25-26). Both the fluxapyroxad treatments and the control simultaneously reached the maximum observed OD values at 64.67 and 49.10 hours in the first and second trials respectively, the maximum value reached were comparable in both trials. (c. 1.4). After the maximum values had been reached, there was a decline in the ODs until the test was stopped. In the first trial the decline was more rapid than in the second. In the first test, the control consistently showed OD values which were numerically (but not statistically) greater than the fluxapyroxad treatments after the maximum ODs had been reached but in the second trial the reverse was true, in fact the order of the treatments in terms of OD values following the maxima

was reversed compared to the first trial. (Table 3.59, 3.60). In general, the fluxapyroxad treatments showed a numerically improved "growth" only in the second trial (Table 3.61, 3.62).

3.2.4.3. Isolate 61.b

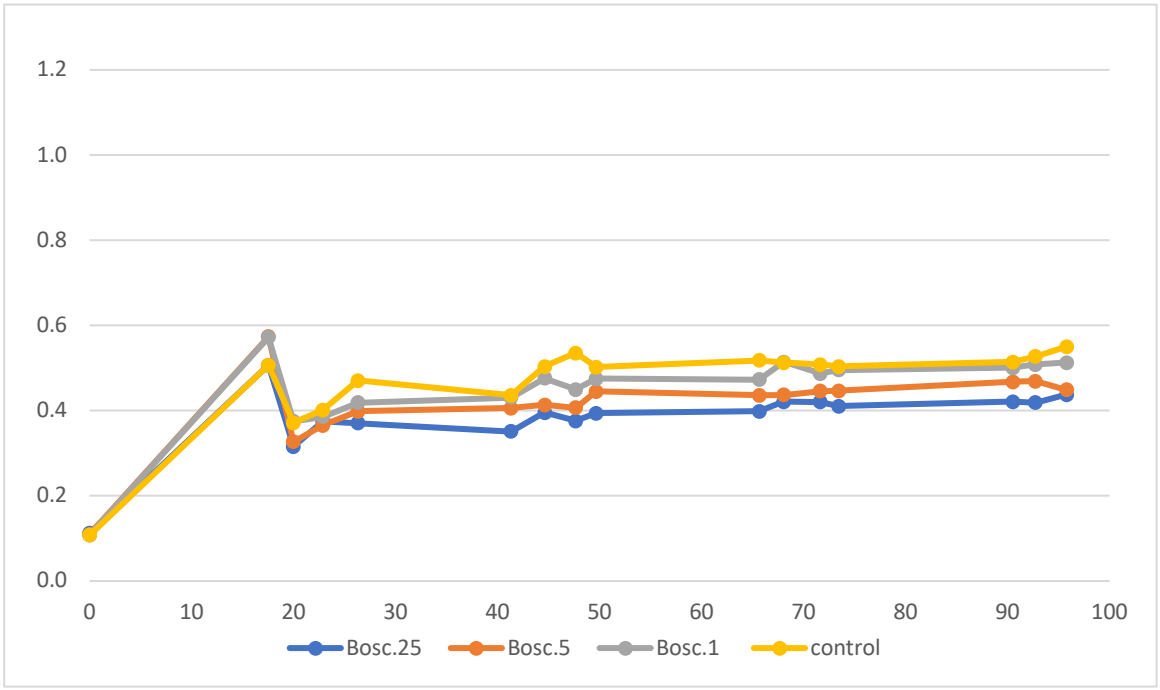


Figure 3.27. The OD curve of isolate 61.b over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

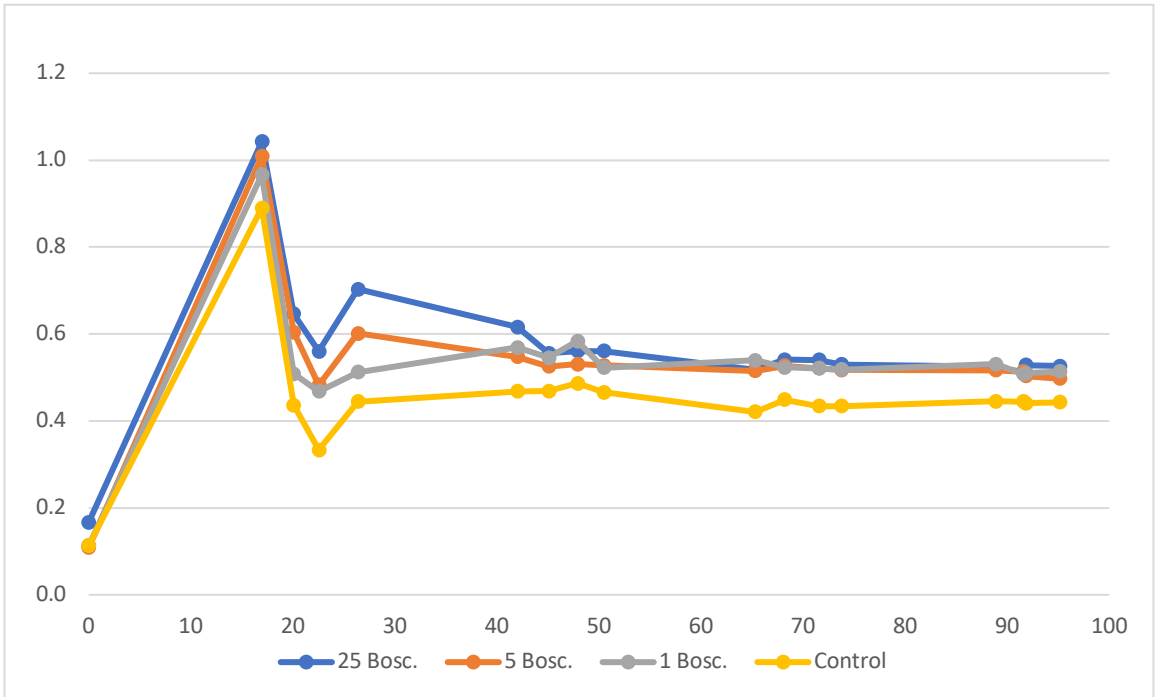


Figure 3.28. The OD curve of isolate 61.b over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Bosc.25	Bosc.5	Bosc.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.112	0.110	0.108	0.107	3.0E-04	0.015	0.020	0.026
17.53	0.507	0.573	0.572	0.507	1.5E-02	0.080	0.107	0.139
19.97	0.316	0.327	0.375	0.371	1.1E-01	0.134	0.179	0.233
22.87	0.375	0.366	0.386	0.401	1.8E-01	0.090	0.120	0.156
26.32	0.371	0.399	0.419	0.470	3.9E-04	0.055	0.073	0.095
41.32	0.351	0.406	0.431	0.436	4.6E-02	0.068	0.091	0.119
44.62	0.396	0.414	0.476	0.504	3.9E-03	0.082	0.109	0.142
47.65	0.376	0.407	0.450	0.535	2.4E-02	0.086	0.114	0.148
49.67	0.394	0.445	0.475	0.502	1.2E-01	0.090	0.120	0.156
65.63	0.399	0.436	0.473	0.518	1.1E-01	0.092	0.123	0.160
68.05	0.421	0.437	0.514	0.513	1.2E-02	0.086	0.115	0.150
71.63	0.421	0.446	0.487	0.509	1.4E-01	0.088	0.118	0.153
73.42	0.411	0.446	0.496	0.504	4.3E-02	0.087	0.116	0.151
90.50	0.421	0.468	0.501	0.514	2.8E-02	0.081	0.108	0.141
92.73	0.419	0.469	0.508	0.527	3.3E-02	0.086	0.114	0.149
95.78	0.438	0.449	0.513	0.551	3.3E-02	0.097	0.129	0.167

Table 3.63. The mean ODs at each assessment time for isolate 61.b in the presence of three concentrations of boscalid. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1% LSD
0.00	0.167	0.108	0.113	0.113	1.9E-22	0.014	0.018	0.023
16.98	1.042	1.009	0.966	0.890	1.8E-25	0.096	0.127	0.164
20.07	0.646	0.604	0.509	0.437	1.6E-18	0.072	0.095	0.124
22.57	0.560	0.483	0.468	0.333	2.7E-17	0.062	0.082	0.106
26.38	0.703	0.601	0.513	0.444	1.8E-16	0.085	0.113	0.146
42.00	0.616	0.548	0.569	0.468	5.9E-29	0.046	0.061	0.079
45.07	0.555	0.525	0.545	0.469	1.3E-26	0.046	0.060	0.078
47.90	0.561	0.531	0.584	0.487	9.8E-29	0.046	0.061	0.079
50.48	0.561	0.528	0.522	0.466	1.8E-26	0.047	0.063	0.082
65.30	0.517	0.515	0.539	0.421	1.4E-26	0.045	0.060	0.078
68.20	0.541	0.527	0.523	0.449	2.9E-14	0.082	0.108	0.141
71.53	0.540	0.521	0.521	0.434	2.5E-13	0.082	0.109	0.141
73.78	0.530	0.517	0.518	0.434	5.3E-13	0.081	0.108	0.140
88.90	0.524	0.517	0.531	0.445	7.0E-11	0.091	0.120	0.156
91.57	0.512	0.514	0.508	0.444	3.4E-10	0.094	0.124	0.161
91.80	0.528	0.503	0.510	0.441	3.0E-11	0.089	0.119	0.154
95.15	0.526	0.498	0.514	0.443	1.4E-11	0.089	0.118	0.153

Table 3.64. The mean ODs at each assessment time for isolate 61.b in the presence of three concentrations of boscalid. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The cross-screening tests with isolate 61.b and boscalid were run twice. Similar to 61.b with Revysol®, the isolate showed the same growth curve as the one in 30 minutes test; a rapid initial increase in the OD in first 15 hours or so to reach a maximum value followed by a decline.

Treatment	"Growth"
Bosc.25	25.49
Bosc.5	29.11
Bosc.1	32.07
Control	33.29
LSD5%	5.796
LSD1%	7.715
LSD0.1%	10.048

Treatment	"Growth"
Bosc. 25	39.82
Bosc. 5	41.70
Bosc. 1	40.43
Control	33.08
LSD5%	3.86
LSD1%	5.12
LSD0.1%	6.64

Table 3.65. and 3.66. The mean "growth" of isolate 61.b in treatments with 25, 5, and 1 ppm of boscalid over the four day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

In the first trial (Fig. 3.27), the decrease with the control was less than with the boscalid treatments leading to significantly lower OD values with 25 ppm from 26 hours onwards and 5 ppm at 2 specific timings (Table 3.63). Unsurprisingly then, significant differences compared to the control at the 5 % level were observed. Furthermore, in the "growth" assessment, all treatments were numerically lower than that of the control with the highest dose, 25ppm being significantly lower at the 5% level (Table 3.65). The peak values were observed at the first assessment on the day after setting up the experiment. It could be that the peak has already occurred here, indeed the first assessment in the first trial was 30 minutes or so later.

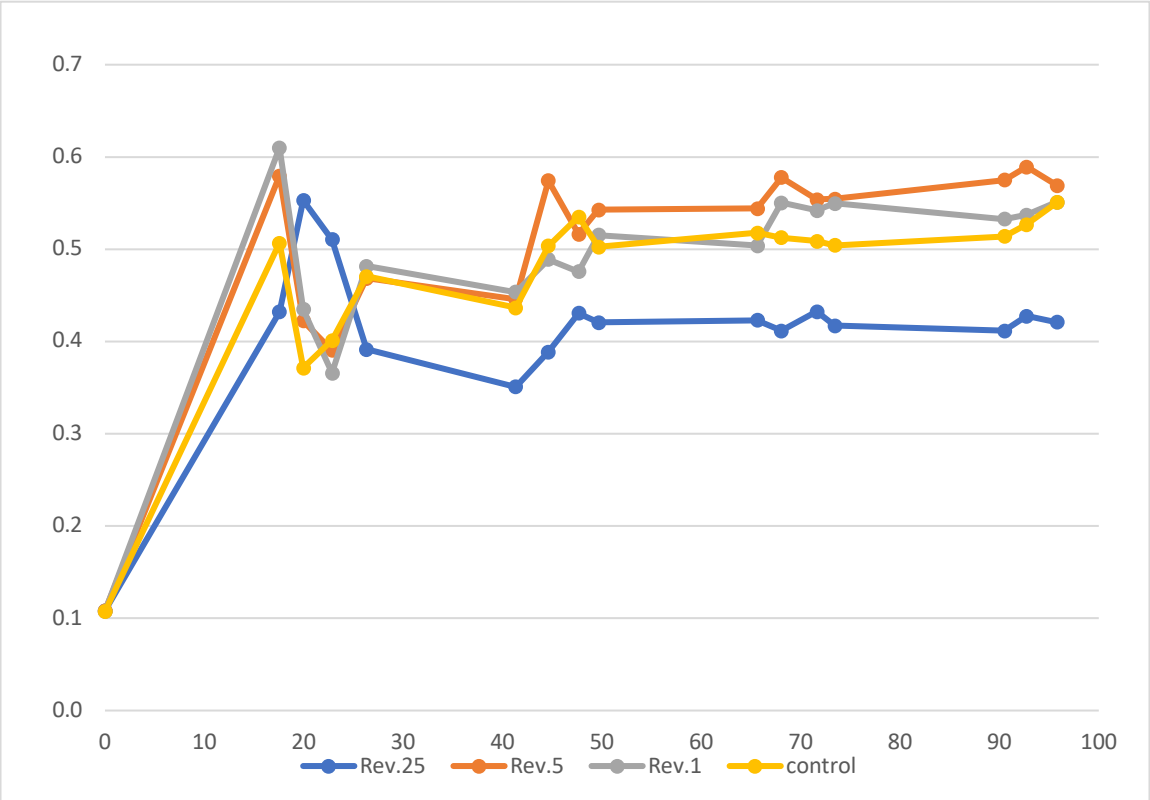


Figure 3.29. The OD curve of isolate 61.b over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

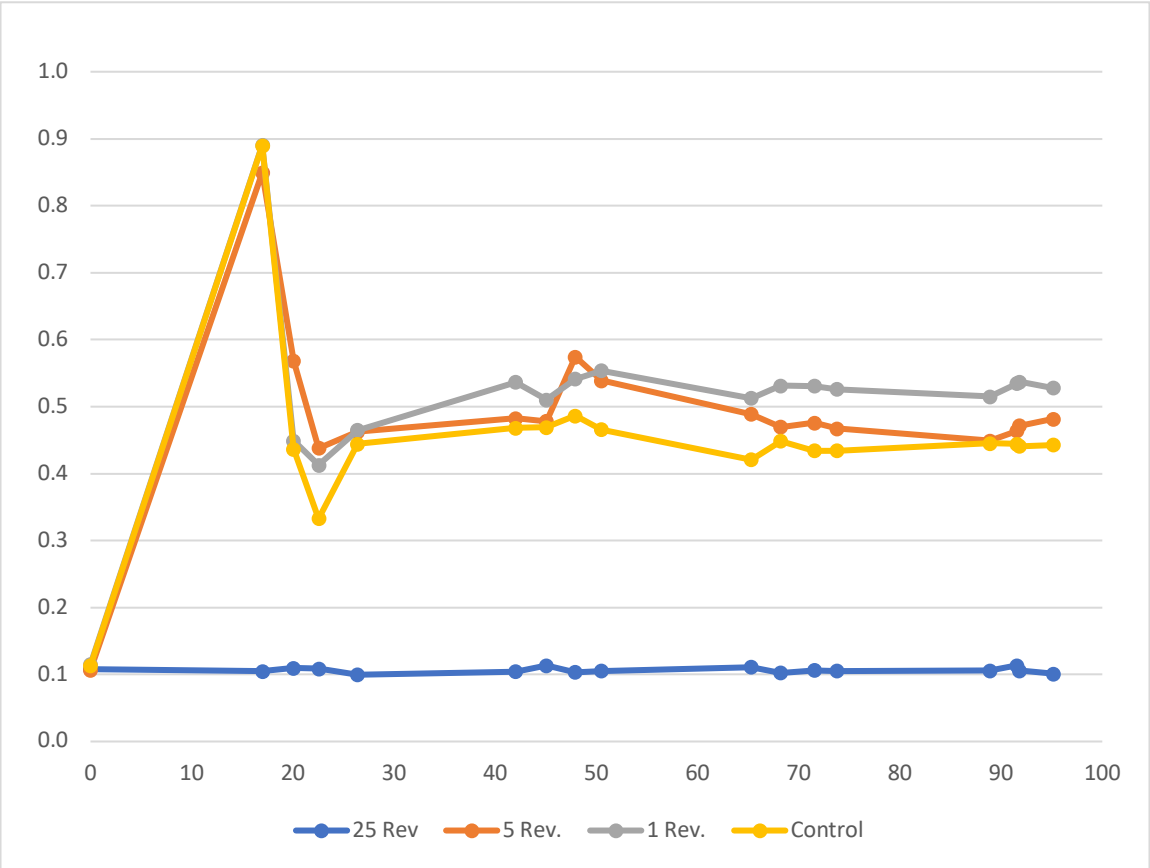


Figure 3.30 The OD curve of isolate 61.b over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Rev.25	Rev.5	Rev.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.108	0.108	0.108	0.107	3.0E-04	0.015	0.020	0.026
17.53	0.432	0.579	0.610	0.507	1.5E-02	0.080	0.107	0.139
19.97	0.553	0.423	0.435	0.371	1.1E-01	0.134	0.179	0.233
22.87	0.511	0.391	0.366	0.401	1.8E-01	0.090	0.120	0.156
26.32	0.392	0.469	0.482	0.470	3.9E-04	0.055	0.073	0.095
41.32	0.351	0.446	0.454	0.436	4.6E-02	0.068	0.091	0.119
44.62	0.389	0.574	0.489	0.504	3.9E-03	0.082	0.109	0.142
47.65	0.431	0.516	0.476	0.535	2.4E-02	0.086	0.114	0.148
49.67	0.421	0.543	0.515	0.502	1.2E-01	0.090	0.120	0.156
65.63	0.423	0.544	0.504	0.518	1.1E-01	0.092	0.123	0.160
68.05	0.412	0.578	0.550	0.513	1.2E-02	0.086	0.115	0.150
71.63	0.433	0.554	0.542	0.509	1.4E-01	0.088	0.118	0.153
73.42	0.417	0.555	0.550	0.504	4.3E-02	0.087	0.116	0.151
90.50	0.412	0.575	0.533	0.514	2.8E-02	0.081	0.108	0.141
92.73	0.428	0.589	0.537	0.527	3.3E-02	0.086	0.114	0.149
95.78	0.421	0.569	0.551	0.551	3.3E-02	0.097	0.129	0.167

Table 3.67. The mean ODs at each assessment time for isolate 61.b in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.108	0.107	0.115	0.113	1.877E-22	0.014	0.018	0.023
16.98	0.105	0.849	0.891	0.890	1.805E-25	0.096	0.127	0.164
20.07	0.110	0.569	0.449	0.437	1.640E-18	0.072	0.095	0.124
22.57	0.109	0.438	0.413	0.333	2.668E-17	0.062	0.082	0.106
26.38	0.100	0.462	0.465	0.444	1.796E-16	0.085	0.113	0.146
42.00	0.105	0.483	0.537	0.468	5.892E-29	0.046	0.061	0.079
45.07	0.114	0.478	0.510	0.469	1.332E-26	0.046	0.060	0.078
47.90	0.104	0.574	0.541	0.487	9.780E-29	0.046	0.061	0.079
50.48	0.105	0.539	0.554	0.466	1.799E-26	0.047	0.063	0.082
65.30	0.111	0.489	0.513	0.421	1.412E-26	0.045	0.060	0.078
68.20	0.103	0.470	0.531	0.449	2.891E-14	0.082	0.108	0.141
71.53	0.106	0.476	0.531	0.434	2.506E-13	0.082	0.109	0.141
73.78	0.106	0.467	0.526	0.434	5.346E-13	0.081	0.108	0.140
88.90	0.106	0.449	0.515	0.445	6.970E-11	0.091	0.120	0.156
91.57	0.113	0.464	0.535	0.444	3.437E-10	0.094	0.124	0.161
91.80	0.106	0.471	0.537	0.441	2.980E-11	0.089	0.119	0.154
95.15	0.101	0.482	0.528	0.443	1.391E-11	0.089	0.118	0.153

Table 3.68. The mean ODs at each assessment time for isolate 61.b in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Rev.25	26.88
Rev.5	36.57
Rev.1	35.34
Control	33.29
LSD5%	5.796
LSD1%	7.715
LSD0.1%	10.048

Treatment	"Growth"
Rev. 25	-0.23
Rev. 5	36.39
Rev. 1	38.28
Control	33.08
LSD5%	3.86
LSD1%	5.12
LSD0.1%	6.64

Table 3.69. and 3.70. The mean "growth" of isolate 61.b in treatments with 25, 5, and 1 ppm of Revysol® over the four day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

In the second trial (Fig. 3.28), the control indicated a lower initial increase, the lowest peak OD value, and the greatest decline reflected in significant positive differences with all treatments frequently at 0.1% level (Table 3.64) resulting in significantly increased average "growth" of all treatments at 0.1% level (Table 3.66).

In the first of these two trials looking at "growth" there was only a significant effect at the highest dose where the "growth" was reduced (significant at the 1% level). In the first run there was a tendency for boscalid to have an inhibitory effect whilst in the second, there was a clear trend to a positive effect. significant at the 0.1% level at all doses.

The two cross-screening tests with isolate 61.b with Revysol® treatments showed similar growth curves, similar to the growth curve seen for strain 61.b in the 30 minutes test. In the second trial the Revysol® treatments and the control achieved the higher peak values except with 25 ppm which in this case didn't grow at all (Fig. 3.30). Unsurprisingly then very significant differences compared to the control at the 0.1 % level were observed (Table 3.68, 3.70).

After reaching the maximum values, there was a steep fall with all treatments and the control in both trials. Although in the first trial (Fig. 3.29) the decrease of ODs with 25 ppm was greater than with the other Revysol® treatments more until 41.32, while with other treatments and the control the ODs appeared to increase between 20 and 22 hours followed by a plateauing out of all the treatments and control (including 25 ppm Revysol®) around 50 hours (Table 3.67).

In the second trial (Fig. 3.30), the 1 and 5 ppm treatments and the control started to level off directly after the steep fall at 26:38 hours However, the decrease with the control was greater than with the Revysol® treatments resulting in significant differences between 5 ppm and for a longer period of time 1 ppm and the control (Table 3.68).

According to the results, it seemed that 25 ppm Revysol® had an inhibitory effect on the "growth" while with 1 and 5 ppm an improved "growth" was seen, and statistical significance was observed in the second run with the 1 ppm treatment (Table 3.69, 3.70).

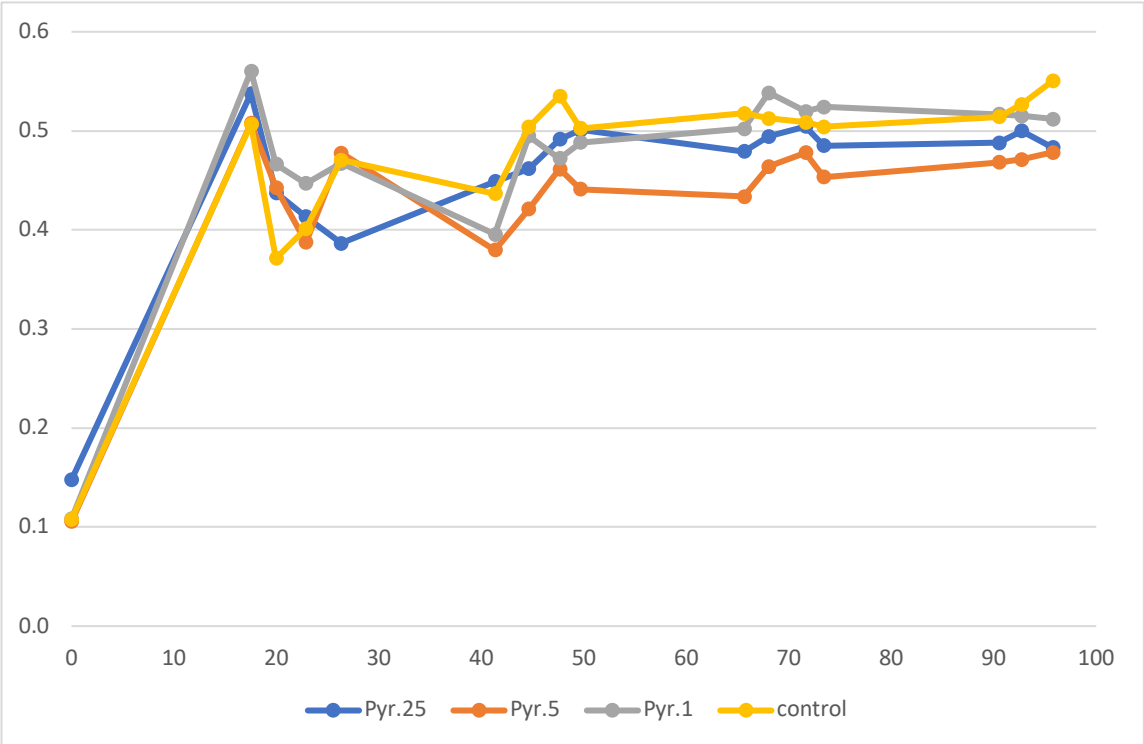


Figure 3.31. The OD curve of isolate 61.b over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

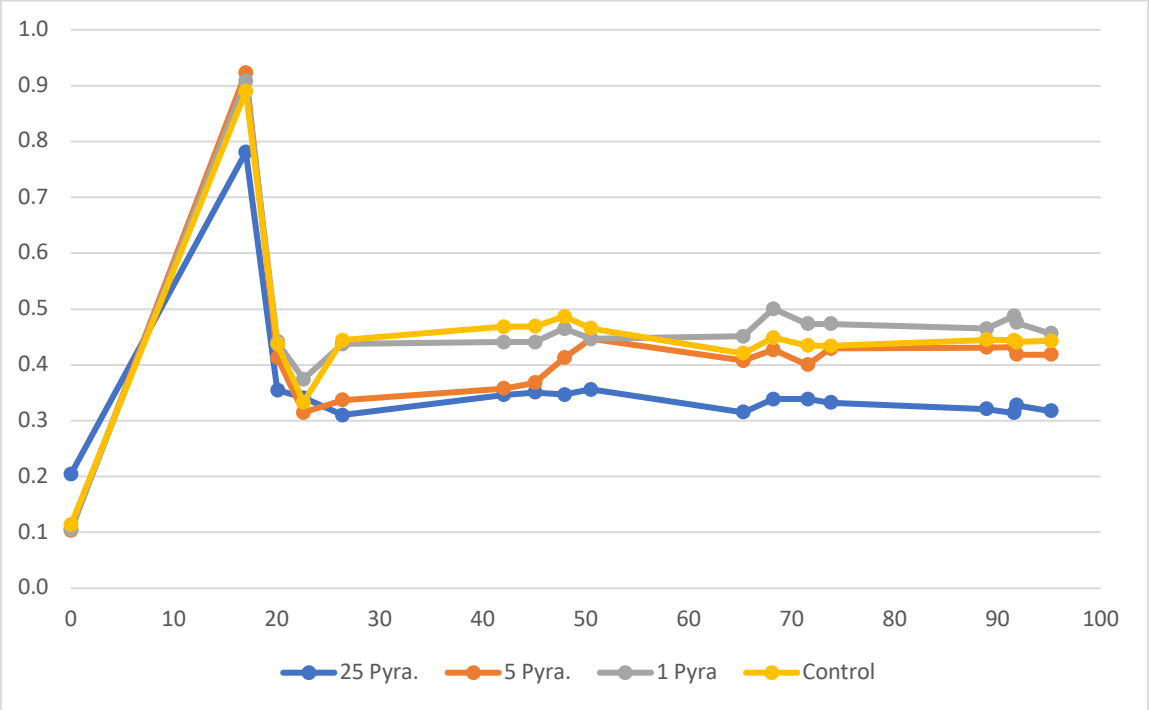


Figure 3.32. The OD curve of isolate 61.b over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Pyra.25	Pyra.5	Pyra.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.148	0.106	0.108	0.107	3.0E-04	0.015	0.020	0.026
17.53	0.538	0.508	0.560	0.507	1.5E-02	0.080	0.107	0.139
19.97	0.438	0.443	0.466	0.371	1.1E-01	0.134	0.179	0.233
22.87	0.413	0.387	0.447	0.401	1.8E-01	0.090	0.120	0.156
26.32	0.387	0.478	0.467	0.470	3.9E-04	0.055	0.073	0.095
41.32	0.449	0.380	0.395	0.436	4.6E-02	0.068	0.091	0.119
44.62	0.462	0.421	0.495	0.504	3.9E-03	0.082	0.109	0.142
47.65	0.492	0.462	0.472	0.535	2.4E-02	0.086	0.114	0.148
49.67	0.501	0.441	0.488	0.502	1.2E-01	0.090	0.120	0.156
65.63	0.479	0.434	0.502	0.518	1.1E-01	0.092	0.123	0.160
68.05	0.494	0.464	0.538	0.513	1.2E-02	0.086	0.115	0.150
71.63	0.504	0.478	0.520	0.509	1.4E-01	0.088	0.118	0.153
73.42	0.485	0.454	0.524	0.504	4.3E-02	0.087	0.116	0.151
90.50	0.488	0.468	0.517	0.514	2.8E-02	0.081	0.108	0.141
92.73	0.500	0.471	0.515	0.527	3.3E-02	0.086	0.114	0.149
95.78	0.483	0.478	0.512	0.551	3.3E-02	0.097	0.129	0.167

Table 3.71. The mean ODs at each assessment time for isolate 61.b in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The cross-screening test with isolate 61.b and the fungicide pyraclostrobin was run on 2 occasions. In both cases a similar growth curve to the one observed with this isolate in the "30 minutes test" resulted.

In the first trial (Fig. 3.31), the control consistently showed OD values which were numerically (but not statistically) lower than the treatments until 23 hours but thereafter during the plateau period, the pyraclostrobin treatments generally had lower ODs than the control. As the result, the average "growth" of the 5 and 25ppm treatments were numerically lower than the control (Table 3.73).

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.204	0.104	0.106	0.113	1.9E-22	0.014	0.018	0.023
16.98	0.781	0.923	0.909	0.890	1.8E-25	0.096	0.127	0.164
20.07	0.355	0.414	0.441	0.437	1.6E-18	0.072	0.095	0.124
22.57	0.341	0.315	0.374	0.333	2.7E-17	0.062	0.082	0.106
26.38	0.310	0.337	0.437	0.444	1.8E-16	0.085	0.113	0.146
42.00	0.346	0.358	0.441	0.468	5.9E-29	0.046	0.061	0.079
45.07	0.351	0.368	0.441	0.469	1.3E-26	0.046	0.060	0.078
47.90	0.347	0.413	0.465	0.487	9.8E-29	0.046	0.061	0.079
50.48	0.356	0.447	0.447	0.466	1.8E-26	0.047	0.063	0.082
65.30	0.315	0.408	0.451	0.421	1.4E-26	0.045	0.060	0.078
68.20	0.339	0.427	0.500	0.449	2.9E-14	0.082	0.108	0.141
71.53	0.339	0.400	0.474	0.434	2.5E-13	0.082	0.109	0.141
73.78	0.332	0.429	0.474	0.434	5.3E-13	0.081	0.108	0.140
88.90	0.321	0.431	0.465	0.445	7.0E-11	0.091	0.120	0.156
91.57	0.314	0.432	0.487	0.444	3.4E-10	0.094	0.124	0.161
91.80	0.328	0.418	0.476	0.441	3.0E-11	0.089	0.119	0.154
95.15	0.318	0.418	0.456	0.443	1.4E-11	0.089	0.118	0.153

Table 3.72. The mean ODs at each assessment time for isolate 61.b in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Pyra.25	28.46
Pyra.5	30.08
Pyra.1	33.62
Control	33.29
LSD5%	5.796
LSD1%	7.715
LSD0.1%	10.048

Treatment	"Growth"
Pyra.25	15.64
Pyra.5	30.83
Pyra.1	34.65
Control	33.08
LSD5%	3.86
LSD1%	5.12
LSD0.1%	6.64

Table 3.73. and 3.74. The mean "growth" of isolate 61.b in treatments with 25, 5, and 1 ppm of pyraclostrobin over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

In first 17 hours there was a rapid rise to a peak value followed by a steep decline until 23 hours or so. From the second 24 hours onwards, there was an eventual level plateauing out until the test was finished (Table 3.71). The treatment with 25 ppm showed the lowest OD value over the experiment, statistically lower than the control and resulting in a very significant (0.1%)

lower “growth” with 25 ppm. With other two doses no significant difference in “growth” with isolate 61.b was observed.

The two experiments suggest that the “growth” of isolate 61.b in the presence of 5 and 25 ppm pyraclostrobin have an inhibitory effect on the growth of the isolate 61.b. Although statistical significance was only observed in the first run of the experiment at the higher dose, the same dose in the second run and the 5ppm dose in the first run both bordered on statistical significance (Table 3.71, 3.72, 3.73, 3.74).

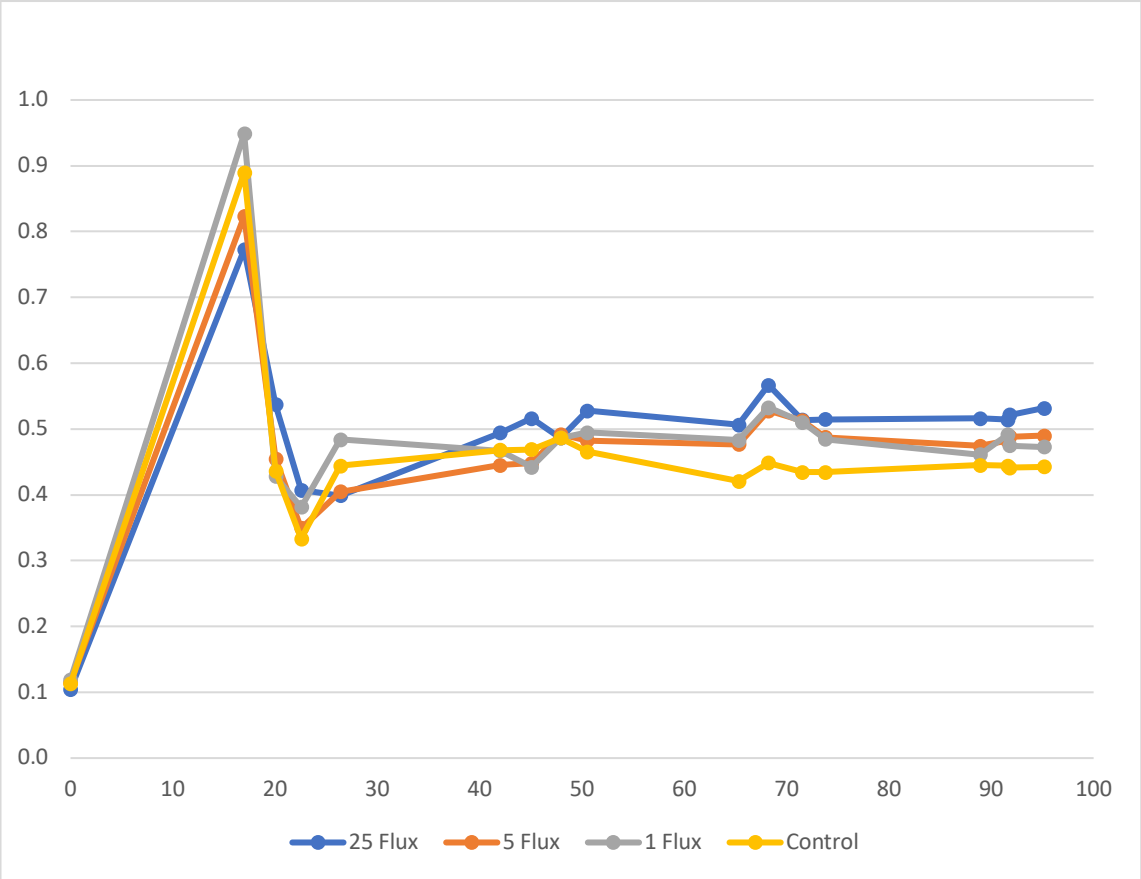


Figure 3.33. The OD curve of isolate 61.b over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

The cross-screening test with isolate 61.b and the fungicide fluxapyroxad showed a similar growth curve to the one observed with this isolate in the “30 minutes test” (Fig. 3.33). Moreover, the isolate indicated higher OD values with the fluxapyroxad treatments compared to the control which led to significant differences especially with 25 ppm. OD values with other treatments tend to be numerically greater than the control but the differences were occasionally statistically significant (Table 3.75). In terms of “growth”, none of treatments were statistically different from the control (Table 3.76).

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.104	0.115	0.119	0.113	1.9E-22	0.014	0.018	0.023
16.98	0.773	0.823	0.949	0.890	1.8E-25	0.096	0.127	0.164
20.07	0.537	0.455	0.428	0.437	1.6E-18	0.072	0.095	0.124
22.57	0.407	0.349	0.381	0.333	2.7E-17	0.062	0.082	0.106
26.38	0.399	0.405	0.484	0.444	1.8E-16	0.085	0.113	0.146
42.00	0.494	0.445	0.467	0.468	5.9E-29	0.046	0.061	0.079
45.07	0.516	0.448	0.442	0.469	1.3E-26	0.046	0.060	0.078
47.90	0.486	0.492	0.486	0.487	9.8E-29	0.046	0.061	0.079
50.48	0.528	0.483	0.495	0.466	1.8E-26	0.047	0.063	0.082
65.30	0.506	0.477	0.483	0.421	1.4E-26	0.045	0.060	0.078
68.20	0.567	0.528	0.533	0.449	2.9E-14	0.082	0.108	0.141
71.53	0.514	0.513	0.511	0.434	2.5E-13	0.082	0.109	0.141
73.78	0.515	0.488	0.485	0.434	5.3E-13	0.081	0.108	0.140
88.90	0.516	0.474	0.461	0.445	7.0E-11	0.091	0.120	0.156
91.57	0.514	0.482	0.492	0.444	3.4E-10	0.094	0.124	0.161
91.80	0.522	0.488	0.475	0.441	3.0E-11	0.089	0.119	0.154
95.15	0.532	0.490	0.473	0.443	1.4E-11	0.089	0.118	0.153

Table 3.75. The mean ODs at each assessment time for isolate 61.b in the presence of three concentrations of fluxapyroxad. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Flux. 25	36.73
Flux. 5	33.77
Flux. 1	35.59
Control	33.08
LSD5%	3.86
LSD1%	5.12
LSD0.1%	6.64

Table 3.76. The mean "growth" of isolate 61.b in treatments with 25, 5, and 1 ppm of fluxapyroxad over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

3.2.4.4. Isolate 80

The cross-screening test with isolate 80 and the fungicide boscalid were run three times. In all 3 tests, 25 ppm indicated the lowest OD values reflected in significant differences compared to the control at 0.1% level, suggesting that this highest rate of boscalid was inhibitory to the growth of isolate 80 (Table 3.77, 3.78, 3.79).

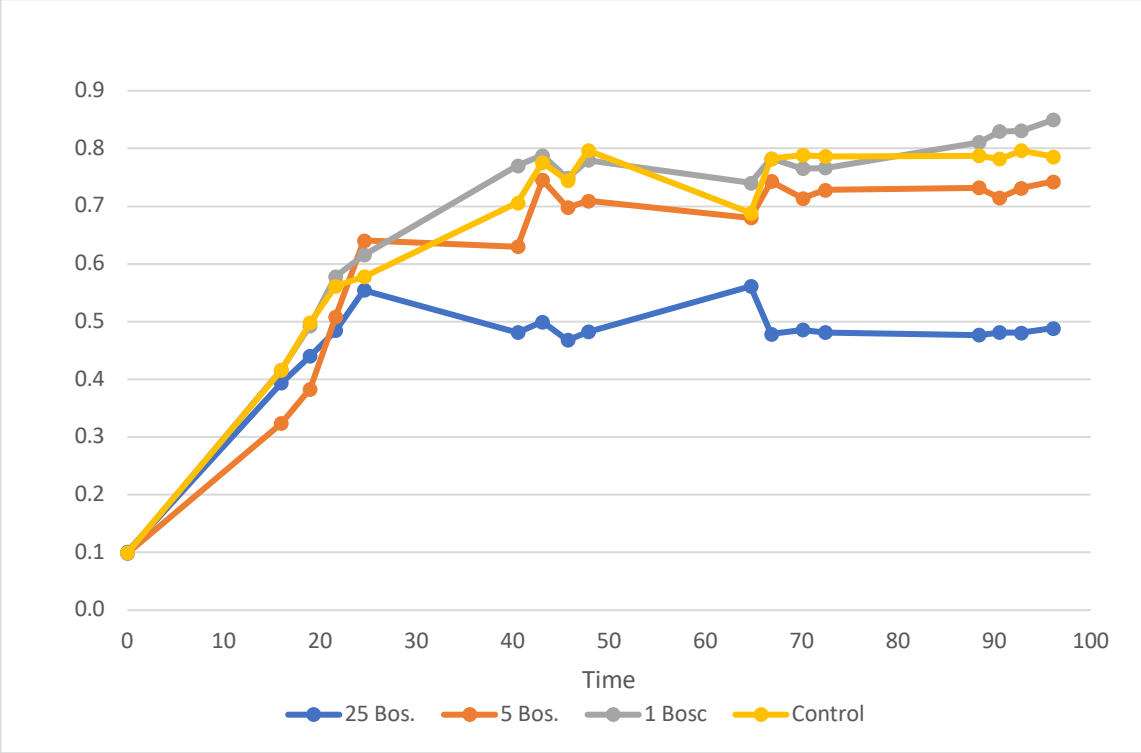


Figure 3.34. The OD curve of isolate 80 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

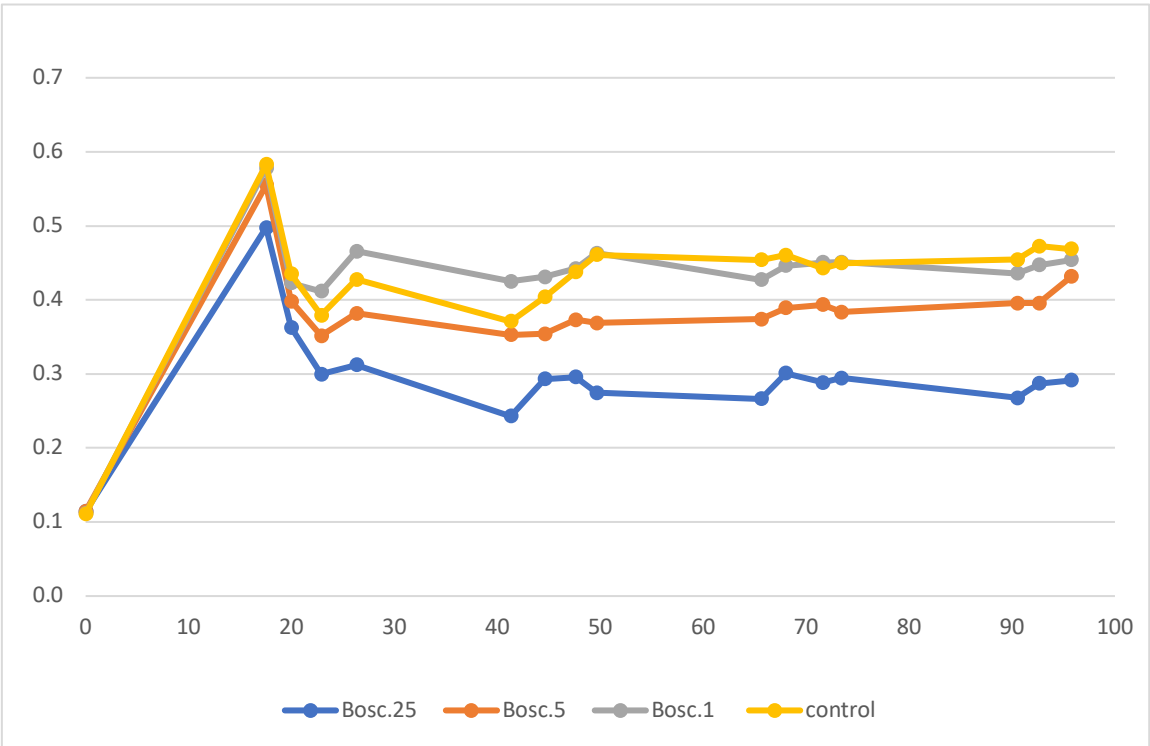


Figure 3.35. The OD curve of isolate 80 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

In the second and third trials, treatments and the control achieved peak values around 17 hours or so followed by a steep decline. Then they started to level off until the test was finished

(Fig. 35-36). Moreover, in the second trial not only with 25 ppm but also with 5 ppm OD values were significantly lower than with the control (Table 78).

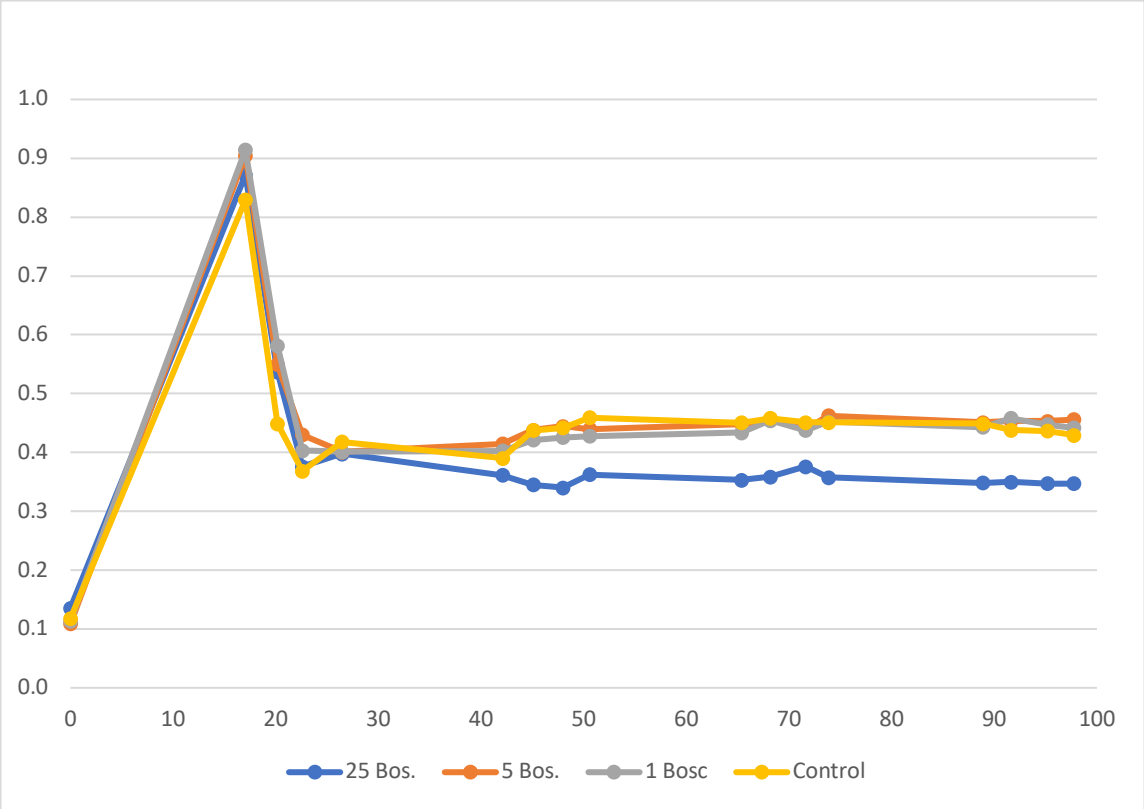


Figure 3.36. The OD curve of isolate 80 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.102	0.099	0.099	0.100	3.6E-43	0.003	0.004	0.005
15.95	0.394	0.324	0.416	0.416	4.3E-03	0.088	0.117	0.152
18.95	0.440	0.383	0.493	0.498	3.4E-02	0.109	0.144	0.187
21.62	0.485	0.509	0.578	0.562	1.1E-02	0.083	0.110	0.143
24.55	0.555	0.641	0.615	0.578	2.2E-07	0.075	0.099	0.129
40.50	0.481	0.630	0.770	0.706	6.0E-14	0.060	0.079	0.102
43.07	0.500	0.746	0.788	0.776	3.9E-08	0.099	0.131	0.170
45.70	0.468	0.698	0.749	0.744	2.6E-11	0.091	0.120	0.156
47.83	0.482	0.709	0.780	0.797	2.9E-09	0.096	0.127	0.165
64.70	0.561	0.680	0.740	0.688	5.0E-08	0.089	0.119	0.154
66.82	0.479	0.743	0.783	0.783	3.8E-07	0.117	0.156	0.202
70.10	0.486	0.714	0.765	0.788	3.4E-08	0.106	0.140	0.182
72.43	0.481	0.728	0.766	0.786	9.6E-10	0.101	0.135	0.175
88.40	0.477	0.732	0.811	0.788	1.2E-08	0.104	0.138	0.180
90.53	0.481	0.714	0.830	0.782	2.2E-08	0.116	0.154	0.199
92.78	0.481	0.732	0.831	0.796	2.8E-08	0.112	0.149	0.193
96.10	0.489	0.743	0.850	0.786	3.2E-07	0.119	0.157	0.204

Table 3.77. The mean ODs at each assessment time for isolate 80 in the presence of three concentrations of boscalid. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Bosc.25	Bosc.5	Bosc.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.114	0.115	0.112	0.111	1.2E-33	0.004	0.006	0.008
17.53	0.498	0.555	0.578	0.583	2.1E-27	0.048	0.063	0.082
19.97	0.363	0.398	0.423	0.435	6.1E-39	0.019	0.025	0.033
22.90	0.300	0.352	0.412	0.379	4.6E-20	0.031	0.041	0.054
26.32	0.312	0.382	0.466	0.428	5.9E-10	0.065	0.087	0.113
41.30	0.243	0.353	0.425	0.371	2.0E-09	0.056	0.074	0.097
44.62	0.293	0.354	0.431	0.404	2.1E-10	0.049	0.066	0.086
47.63	0.296	0.373	0.442	0.438	1.5E-10	0.052	0.069	0.090
49.65	0.296	0.373	0.442	0.438	2.2E-11	0.052	0.069	0.090
65.62	0.275	0.369	0.463	0.461	1.5E-14	0.047	0.063	0.082
68.03	0.266	0.374	0.427	0.454	2.8E-13	0.050	0.067	0.087
71.63	0.301	0.389	0.446	0.460	4.2E-14	0.054	0.072	0.093
73.43	0.289	0.394	0.451	0.443	7.3E-16	0.048	0.064	0.083
90.50	0.294	0.384	0.451	0.450	1.2E-14	0.053	0.070	0.092
92.63	0.268	0.396	0.436	0.454	7.5E-15	0.053	0.071	0.092
95.75	0.291	0.431	0.454	0.469	7.5E-15	0.072	0.096	0.125

Table 3.78. The mean ODs at each assessment time for isolate 80 in the presence of three concentrations of boscalid. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.136	0.109	0.114	0.118	1.6E-23	0.007	0.010	0.013
17.03	0.872	0.905	0.914	0.829	4.6E-03	0.077	0.102	0.132
20.13	0.537	0.550	0.582	0.449	7.0E-04	0.086	0.114	0.148
22.58	0.376	0.430	0.403	0.368	3.9E-06	0.042	0.055	0.072
26.43	0.398	0.401	0.401	0.418	6.3E-01	0.044	0.059	0.077
42.08	0.361	0.415	0.403	0.390	7.5E-04	0.032	0.043	0.056
45.07	0.345	0.438	0.422	0.438	1.4E-05	0.043	0.057	0.074
47.97	0.340	0.445	0.426	0.442	1.1E-05	0.039	0.052	0.067
50.55	0.363	0.440	0.428	0.459	1.7E-03	0.040	0.054	0.069
65.37	0.353	0.448	0.434	0.451	3.1E-07	0.036	0.048	0.062
68.18	0.359	0.455	0.454	0.458	5.5E-06	0.040	0.053	0.069
71.60	0.376	0.440	0.438	0.451	2.4E-05	0.041	0.054	0.070
73.82	0.358	0.463	0.452	0.451	6.1E-07	0.042	0.056	0.072
88.87	0.349	0.451	0.443	0.449	3.5E-07	0.041	0.054	0.070
91.60	0.350	0.454	0.458	0.438	2.5E-07	0.046	0.061	0.079
95.18	0.347	0.454	0.447	0.437	6.4E-08	0.042	0.055	0.072
97.73	0.348	0.456	0.442	0.430	2.6E-08	0.042	0.055	0.072

Table 3.79. The mean ODs at each assessment time for isolate 80 in the presence of three concentrations of boscalid. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In the first trial (Fig. 3.34), with the control and 1 and 5 ppm boscalid treatments reached the maximum observed OD values at 45 hours or so values and then started to level off.

The “growth” assessments for the 1ppm boscalid treatment exceeded that of the control in all three runs of the experiment although in no case reached statistical significance. Also, it is clear that 25 ppm had an inhibitory effect on the “growth” in all trials with the differences to the control reaching statistical significance at the 0.1 % level in all three trials (Table 3.80-82).

Treatment	“Growth”
Bosc.25	33.99
Bosc.5	47.70
Bosc.1	53.60
Control	51.92
LSD5%	5.18
LSD1%	6.87
LSD0.1%	8.91

Treatment	“Growth”
Bosc.25	16.89
Bosc.5	24.67
Bosc.1	30.10
Control	29.63
LSD5%	3.267
LSD1%	4.348
LSD0.1%	5.663

Treatment	“Growth”
Bosc.25	25.77
Bosc.5	34.40
Bosc.1	33.41
Control	32.27
LSD5%	2.48
LSD1%	3.29
LSD0.1%	4.27

Table 3.80. and 3.81. and 3.82 The mean “growth” of isolate 80 in treatments with 25, 5, and 1 ppm of boscalid over the four-day experimental period. Significant increases in “growth” compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

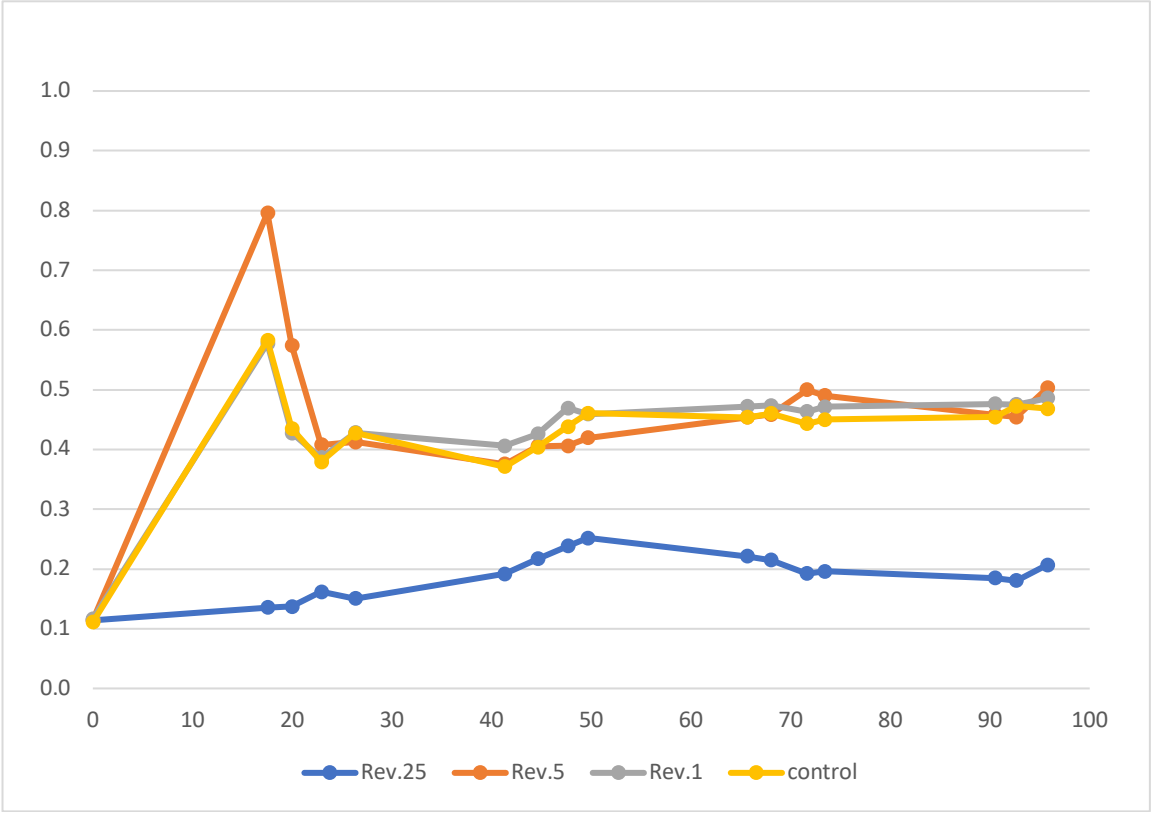


Figure 3.37. The OD curve of isolate 80 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

The cross-screening tests with isolate 80 and the fungicide Revysol® were run three times. In all 3 trials, the isolate in the Revysol® treatments and the control reached the maximum OD

value around 17 hours except with 25 ppm in the first and third runs where there was lower growth and the point at which the maximum value was observed was delayed.

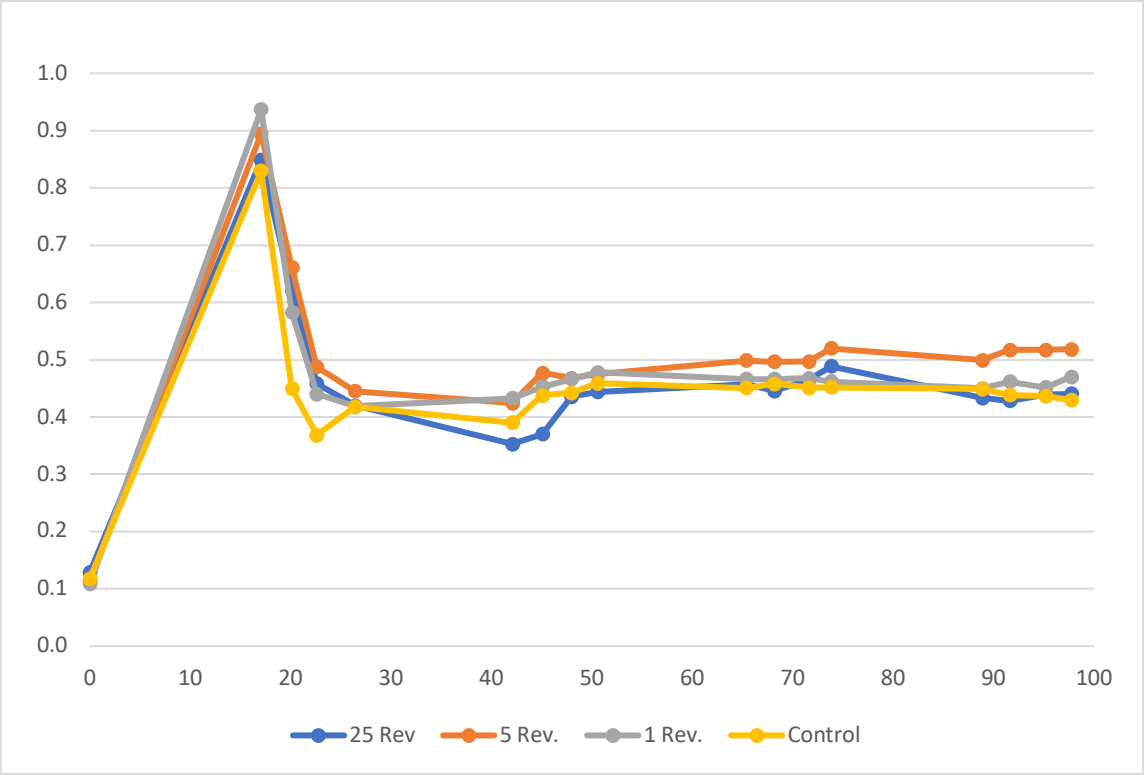


Figure 3.38. The OD curve of isolate 80 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

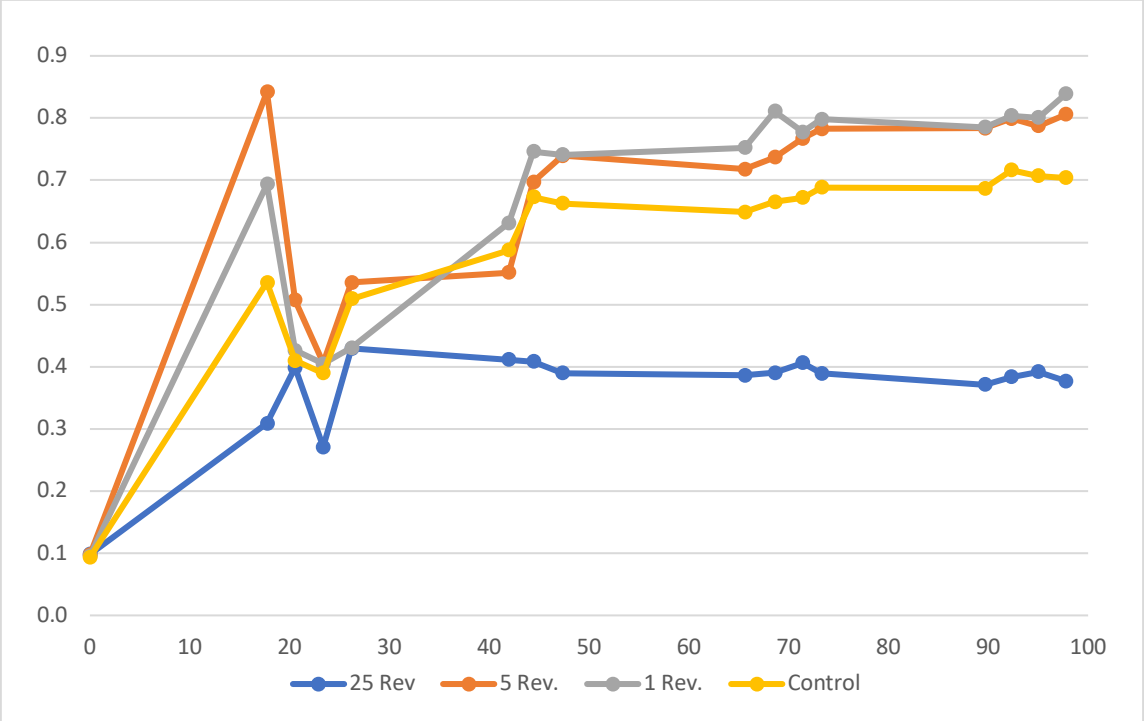


Figure 3.39. The OD curve of isolate 80 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Rev.25	Rev.5	Rev.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.114	0.114	0.117	0.111	1.2E-33	0.004	0.006	0.008
17.53	0.136	0.796	0.578	0.583	2.1E-27	0.048	0.063	0.082
19.97	0.137	0.574	0.428	0.435	6.1E-39	0.019	0.025	0.033
22.90	0.162	0.408	0.386	0.379	4.6E-20	0.031	0.041	0.054
26.32	0.151	0.412	0.428	0.428	5.9E-10	0.065	0.087	0.113
41.30	0.192	0.376	0.406	0.371	2.0E-09	0.056	0.074	0.097
44.62	0.217	0.406	0.426	0.404	2.1E-10	0.049	0.066	0.086
47.63	0.239	0.406	0.470	0.438	1.5E-10	0.052	0.069	0.090
49.65	0.239	0.406	0.470	0.438	2.2E-11	0.052	0.069	0.090
65.62	0.252	0.420	0.459	0.461	1.5E-14	0.047	0.063	0.082
68.03	0.221	0.454	0.472	0.454	2.8E-13	0.050	0.067	0.087
71.63	0.215	0.459	0.474	0.460	4.2E-14	0.054	0.072	0.093
73.43	0.192	0.500	0.464	0.443	7.3E-16	0.048	0.064	0.083
90.50	0.196	0.490	0.472	0.450	1.2E-14	0.053	0.070	0.092
92.63	0.185	0.458	0.477	0.454	7.5E-15	0.053	0.071	0.092
95.75	0.207	0.503	0.486	0.469	7.5E-15	0.072	0.096	0.125

Table 3.83. The mean ODs at each assessment time for isolate 80 in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.129	0.115	0.108	0.118	1.6E-23	0.007	0.010	0.013
17.03	0.849	0.895	0.937	0.829	4.6E-03	0.077	0.102	0.132
20.13	0.621	0.661	0.583	0.449	7.0E-04	0.086	0.114	0.148
22.58	0.458	0.487	0.440	0.368	3.9E-06	0.042	0.055	0.072
26.43	0.420	0.445	0.419	0.418	6.3E-01	0.044	0.059	0.077
42.08	0.353	0.425	0.433	0.390	7.5E-04	0.032	0.043	0.056
45.07	0.370	0.477	0.453	0.438	1.4E-05	0.043	0.057	0.074
47.97	0.436	0.468	0.466	0.442	1.1E-05	0.039	0.052	0.067
50.55	0.444	0.475	0.478	0.459	1.7E-03	0.040	0.054	0.069
65.37	0.457	0.499	0.466	0.451	3.1E-07	0.036	0.048	0.062
68.18	0.445	0.496	0.466	0.458	5.5E-06	0.040	0.053	0.069
71.60	0.465	0.497	0.468	0.451	2.4E-05	0.041	0.054	0.070
73.82	0.489	0.520	0.462	0.451	6.1E-07	0.042	0.056	0.072
88.87	0.434	0.499	0.450	0.449	3.5E-07	0.041	0.054	0.070
91.60	0.428	0.517	0.462	0.438	2.5E-07	0.046	0.061	0.079
95.18	0.439	0.517	0.452	0.437	6.4E-08	0.042	0.055	0.072
97.73	0.441	0.518	0.470	0.430	2.6E-08	0.042	0.055	0.072

Table 3.84. The mean ODs at each assessment time for isolate 80 in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In the first (Fig. 3.37) and third trial (Fig. 3.39), 5 ppm and in the second trial (Fig. 3.38) 1 ppm achieved the maximum observed OD values around 17 hours or so followed by a steep decline until about 22 hours reflected in significant differences with 5 ppm in the first run and with all

treatments in second run compared to the control and then they started to level off. In the second trial OD values with 5 ppm were statistically higher than the control until the test was finished (Table 3.84) and in the last trial 1 and 5 ppm were at most of timings numerically higher (Table 3.83).

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.098	0.097	0.096	0.093	4.5E-09	0.015	0.020	0.026
15.95	0.309	0.842	0.694	0.536	6.0E-06	0.164	0.217	0.282
18.95	0.398	0.507	0.426	0.410	2.0E-01	0.119	0.158	0.204
21.62	0.271	0.406	0.405	0.390	1.8E-02	0.109	0.145	0.188
24.55	0.430	0.536	0.431	0.509	1.1E-01	0.134	0.178	0.230
40.50	0.411	0.551	0.631	0.587	2.1E-01	0.133	0.176	0.228
43.07	0.408	0.697	0.746	0.673	1.7E-02	0.148	0.197	0.256
45.70	0.390	0.740	0.741	0.663	1.6E-02	0.154	0.204	0.264
47.83	0.386	0.718	0.752	0.649	5.3E-04	0.121	0.161	0.209
64.70	0.390	0.737	0.811	0.665	2.3E-04	0.128	0.170	0.221
66.82	0.406	0.767	0.778	0.672	1.3E-03	0.131	0.174	0.226
70.10	0.389	0.782	0.798	0.688	6.6E-05	0.128	0.169	0.220
72.43	0.371	0.784	0.785	0.687	5.7E-06	0.119	0.158	0.204
88.40	0.384	0.799	0.804	0.716	3.2E-05	0.127	0.169	0.219
90.53	0.392	0.787	0.801	0.707	9.6E-05	0.129	0.172	0.223
92.78	0.377	0.806	0.839	0.704	3.6E-05	0.134	0.178	0.231

Table 3.85. The mean ODs at each assessment time for isolate 80 in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Rev.25	6.63
Rev.5	32.04
Rev.1	30.35
Control	29.63
LSD5%	3.267
LSD1%	4.348
LSD0.1%	5.663

Treatment	"Growth"
Rev. 25	31.82
Rev. 5	37.41
Rev. 1	35.96
Control	32.27
LSD5%	2.48
LSD1%	3.29
LSD0.1%	4.27

Treatment	"Growth"
Rev. 25	25.17
Rev. 5	53.82
Rev. 1	52.97
Control	46.40
LSD5%	8.652
LSD1%	11.485
LSD0.1%	14.895

Table 3.86. 3.87. and 3.88. The mean "growth" of isolate 80 in treatments with 25, 5, and 1 ppm of Revysol® over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

In all trials the treatments with 1 and 5 ppm indicated consistent increases in "growth" which were statistically significant at 1 and 5 ppm in the second trial (Table 3.86, 3.87, 3.88). The 25 ppm treatment which showed marked significant reductions in the OD values throughout the first and third trials (Table 3.83 and 3.85) tended to be inhibitory to the growth of this strain.

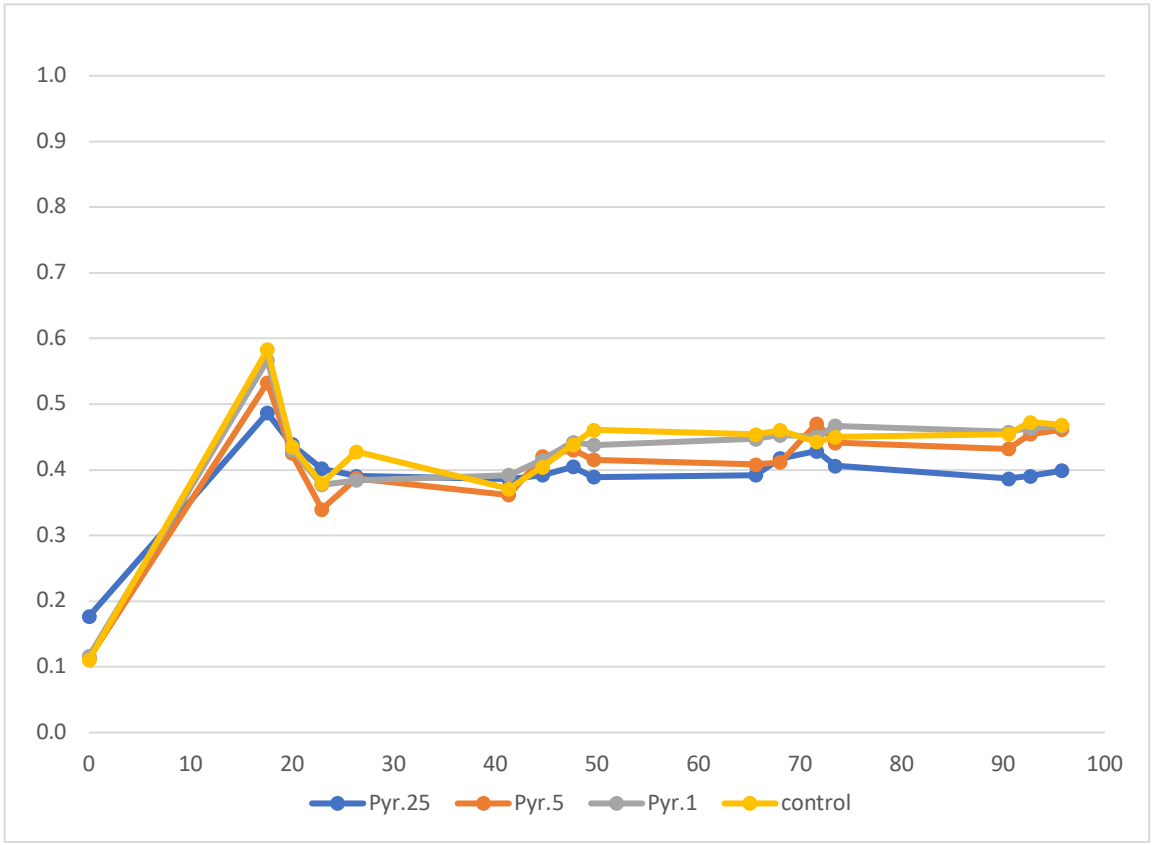


Figure 3.40. The OD curve of isolate 80 over 4 days. 3 doses of pyraclostrobin1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

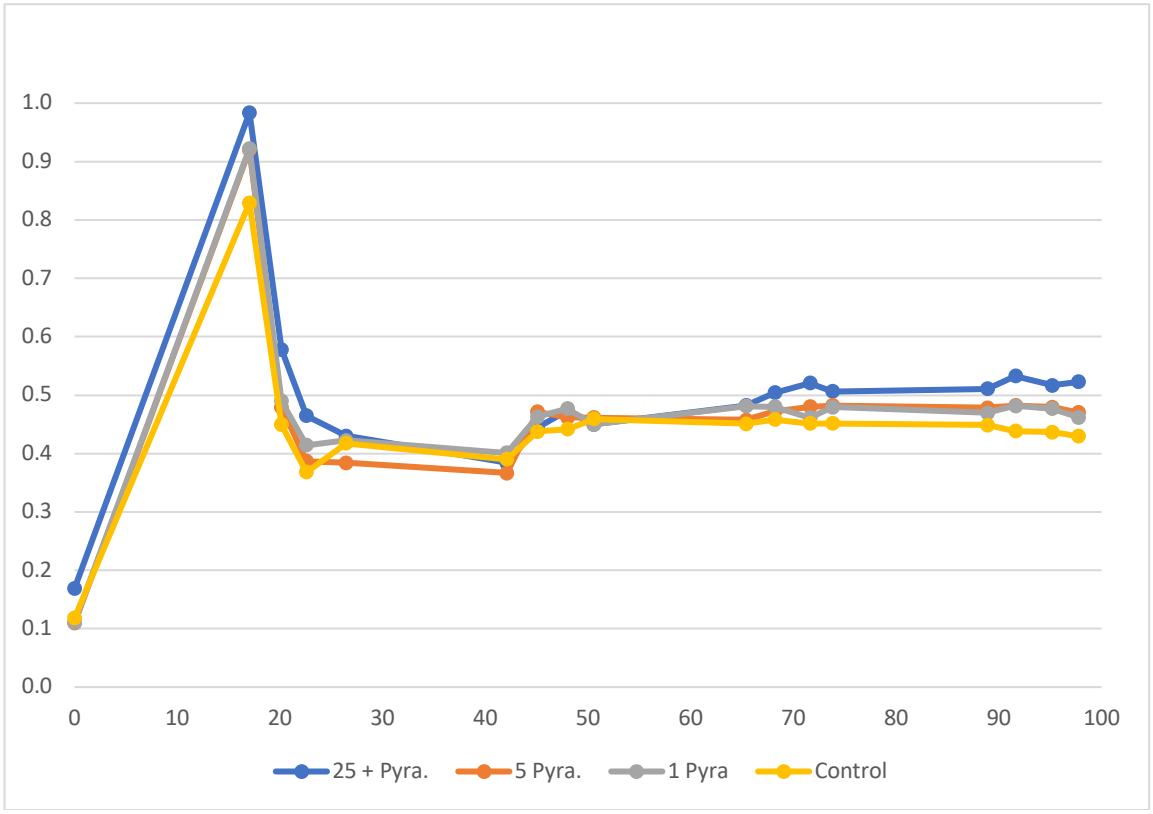


Figure 3.41. The OD curve of isolate 80 over 4 days. 3 doses of pyraclostrobin1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

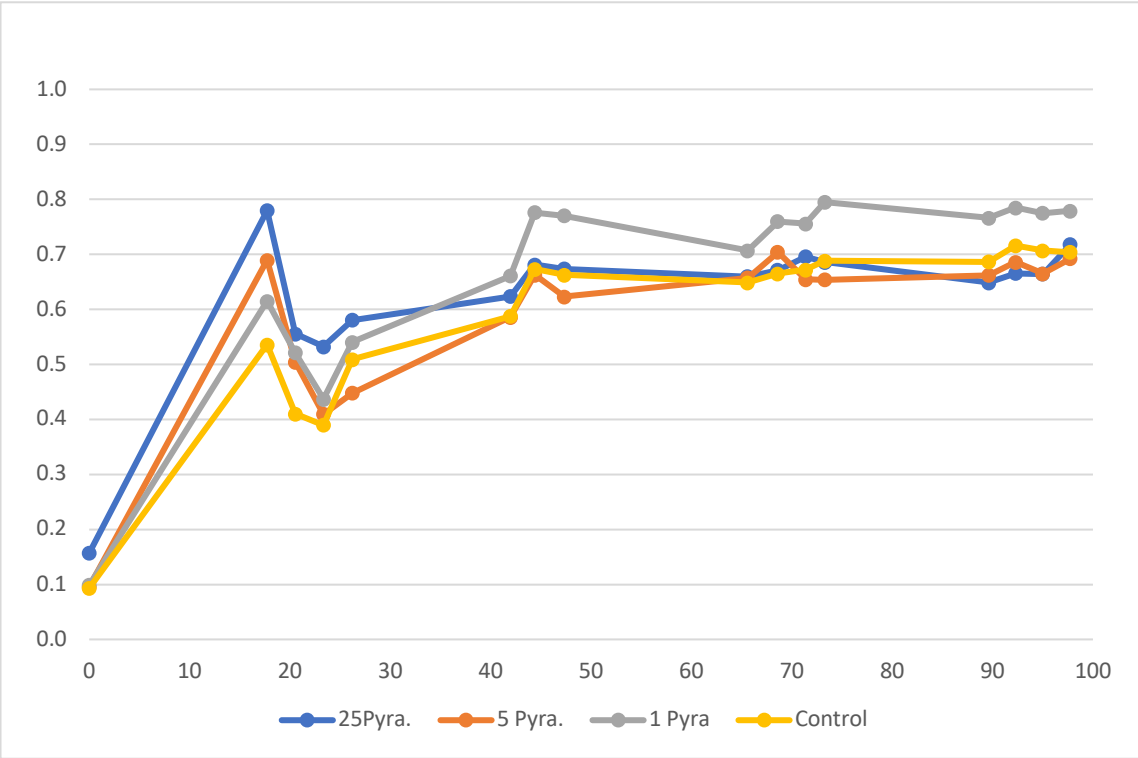


Figure 3.42. The OD curve of isolate 80 over 4 days. 3 doses of pyraclostrobin1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.177	0.113	0.116	0.111	1.2E-33	0.004	0.006	0.008
17.53	0.487	0.533	0.567	0.583	2.1E-27	0.048	0.063	0.082
19.97	0.439	0.425	0.431	0.435	6.1E-39	0.019	0.025	0.033
22.90	0.402	0.340	0.377	0.379	4.6E-20	0.031	0.041	0.054
26.32	0.390	0.387	0.384	0.428	5.9E-10	0.065	0.087	0.113
41.30	0.386	0.362	0.392	0.371	2.0E-09	0.056	0.074	0.097
44.62	0.392	0.420	0.415	0.404	2.1E-10	0.049	0.066	0.086
47.63	0.405	0.430	0.442	0.438	1.5E-10	0.052	0.069	0.090
49.65	0.405	0.430	0.442	0.438	2.2E-11	0.052	0.069	0.090
65.62	0.389	0.415	0.438	0.461	1.5E-14	0.047	0.063	0.082
68.03	0.392	0.408	0.447	0.454	2.8E-13	0.050	0.067	0.087
71.63	0.417	0.412	0.453	0.460	4.2E-14	0.054	0.072	0.093
73.43	0.428	0.471	0.451	0.443	7.3E-16	0.048	0.064	0.083
90.50	0.406	0.441	0.467	0.450	1.2E-14	0.053	0.070	0.092
92.63	0.387	0.432	0.458	0.454	7.5E-15	0.053	0.071	0.092
95.75	0.399	0.461	0.466	0.469	7.5E-15	0.072	0.096	0.125

Table 3.89. The mean ODs at each assessment time for isolate 80 in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The cross-screening test with the isolate 80 and treatments with pyraclostrobin was run 3 times. In all trials, the isolated showed a similar trend to the one recorded in the 30 minutes test with recorded peak OD values around 17 hours. Then in first 2 trials it started to decline and then

plateaued until the end of the test (Fig. 3.40,41). The third trial also showed the same trend but there was a gradual increase following the decline until 47.33 hours when the control and treatments started to level off until the test was stopped. (Fig. 3.42).

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.169	0.109	0.111	0.118	1.6E-23	0.007	0.010	0.013
17.03	0.984	0.921	0.923	0.829	4.6E-03	0.077	0.102	0.132
20.13	0.578	0.479	0.490	0.449	7.0E-04	0.086	0.114	0.148
22.58	0.465	0.387	0.414	0.368	3.9E-06	0.042	0.055	0.072
26.43	0.430	0.384	0.423	0.418	6.3E-01	0.044	0.059	0.077
42.08	0.384	0.367	0.401	0.390	7.5E-04	0.032	0.043	0.056
45.07	0.445	0.471	0.463	0.438	1.4E-05	0.043	0.057	0.074
47.97	0.473	0.462	0.477	0.442	1.1E-05	0.039	0.052	0.067
50.55	0.450	0.461	0.450	0.459	1.7E-03	0.040	0.054	0.069
65.37	0.482	0.458	0.481	0.451	3.1E-07	0.036	0.048	0.062
68.18	0.504	0.472	0.480	0.458	5.5E-06	0.040	0.053	0.069
71.60	0.520	0.480	0.462	0.451	2.4E-05	0.041	0.054	0.070
73.82	0.507	0.482	0.480	0.451	6.1E-07	0.042	0.056	0.072
88.87	0.511	0.478	0.470	0.449	3.5E-07	0.041	0.054	0.070
91.60	0.533	0.482	0.482	0.438	2.5E-07	0.046	0.061	0.079
95.18	0.517	0.479	0.477	0.437	6.4E-08	0.042	0.055	0.072
97.73	0.523	0.470	0.462	0.430	2.6E-08	0.042	0.055	0.072

Table 3.90. The mean ODs at each assessment time for isolate 80 in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.157	0.096	0.099	0.093	4.5E-09	0.015	0.020	0.026
15.95	0.780	0.690	0.615	0.536	6.0E-06	0.164	0.217	0.282
18.95	0.555	0.504	0.521	0.410	2.0E-01	0.119	0.158	0.204
21.62	0.532	0.410	0.437	0.390	1.8E-02	0.109	0.145	0.188
24.55	0.580	0.448	0.540	0.509	1.1E-01	0.134	0.178	0.230
40.50	0.624	0.586	0.661	0.587	2.1E-01	0.133	0.176	0.228
43.07	0.681	0.663	0.776	0.673	1.7E-02	0.148	0.197	0.256
45.70	0.674	0.623	0.770	0.663	1.6E-02	0.154	0.204	0.264
47.83	0.660	0.657	0.707	0.649	5.3E-04	0.121	0.161	0.209
64.70	0.672	0.704	0.760	0.665	2.3E-04	0.128	0.170	0.221
66.82	0.696	0.655	0.756	0.672	1.3E-03	0.131	0.174	0.226
70.10	0.686	0.654	0.795	0.688	6.6E-05	0.128	0.169	0.220
72.43	0.649	0.662	0.766	0.687	5.7E-06	0.119	0.158	0.204
88.40	0.666	0.686	0.785	0.716	3.2E-05	0.127	0.169	0.219
90.53	0.664	0.665	0.775	0.707	9.6E-05	0.129	0.172	0.223
92.78	0.718	0.692	0.779	0.704	3.6E-05	0.134	0.178	0.231

Table 3.91. The mean ODs at each assessment time for isolate 80 in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Pyra.25	20.00
Pyra.5	27.19
Pyra.1	28.81
Control	29.63
LSD5%	3.267
LSD1%	4.348
LSD0.1%	5.663

Treatment	"Growth"
Pyra.25	32.34
Pyra.5	34.75
Pyra.1	35.43
Control	32.27
LSD5%	2.48
LSD1%	3.29
LSD0.1%	4.27

Treatment	"Growth"
Pyra.25	44.76
Pyra.5	46.46
Pyra.1	52.86
Control	46.40
LSD5%	8.652
LSD1%	11.485
LSD0.1%	14.895

Table 3.92 and 3.93 and 3.94. The mean "growth" of isolate 80 in treatments with 25, 5, and 1 ppm of pyraclostrobin over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

Considering the individual assessments in the first trial (Table 3.89), although there were only some assessments with 25 ppm when the OD value was statistically lower than the control nonetheless the "growth" assessment showed that great significantly lower growth was recorded with 25 ppm. In the other two runs of the experiment there were no significant differences between the "growth" of the control and the 25ppm treatment although in the third trial (Table 3.91) there was a numerically lower "growth" value for this treatment. In all three trials (Table. 3.92, 3.93, and 3.94 respectively) the 1ppm pyraclostrobin treatment showed the highest "growth" value of all the treatments including the control and both the 1 and 5 ppm treatments in the second trial (Table.3.93) were significantly greater than the control suggesting at these concentrations a tendency for increased "growth".

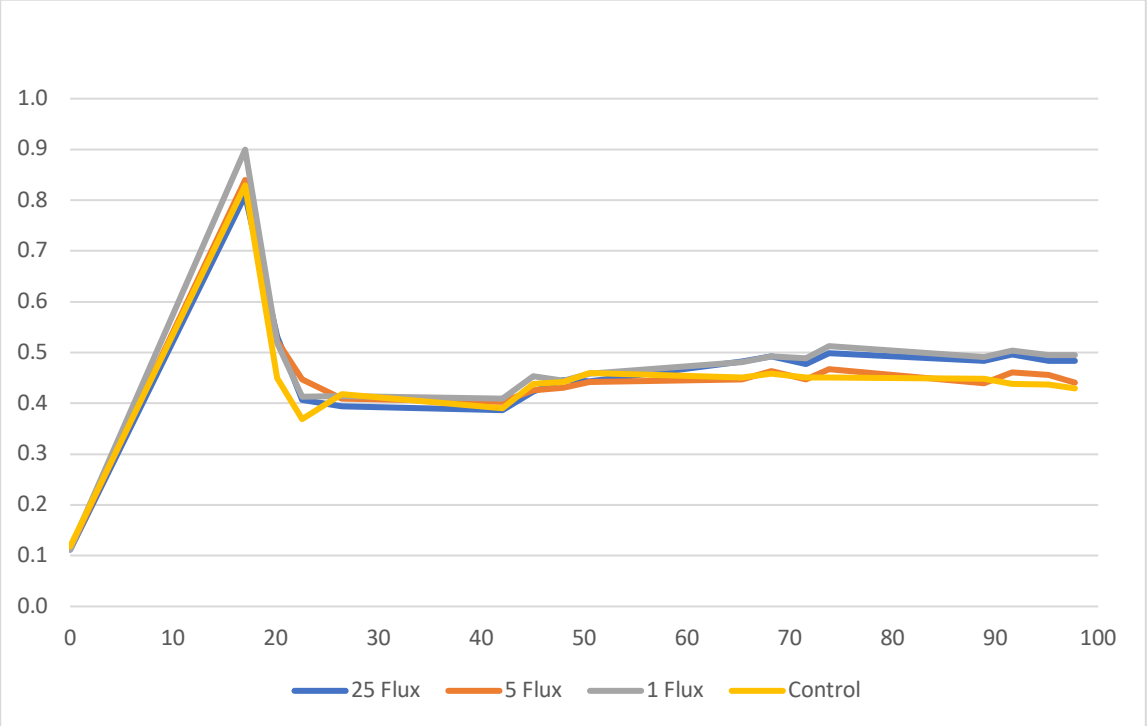


Figure 3.43. The OD curve of isolate 80 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

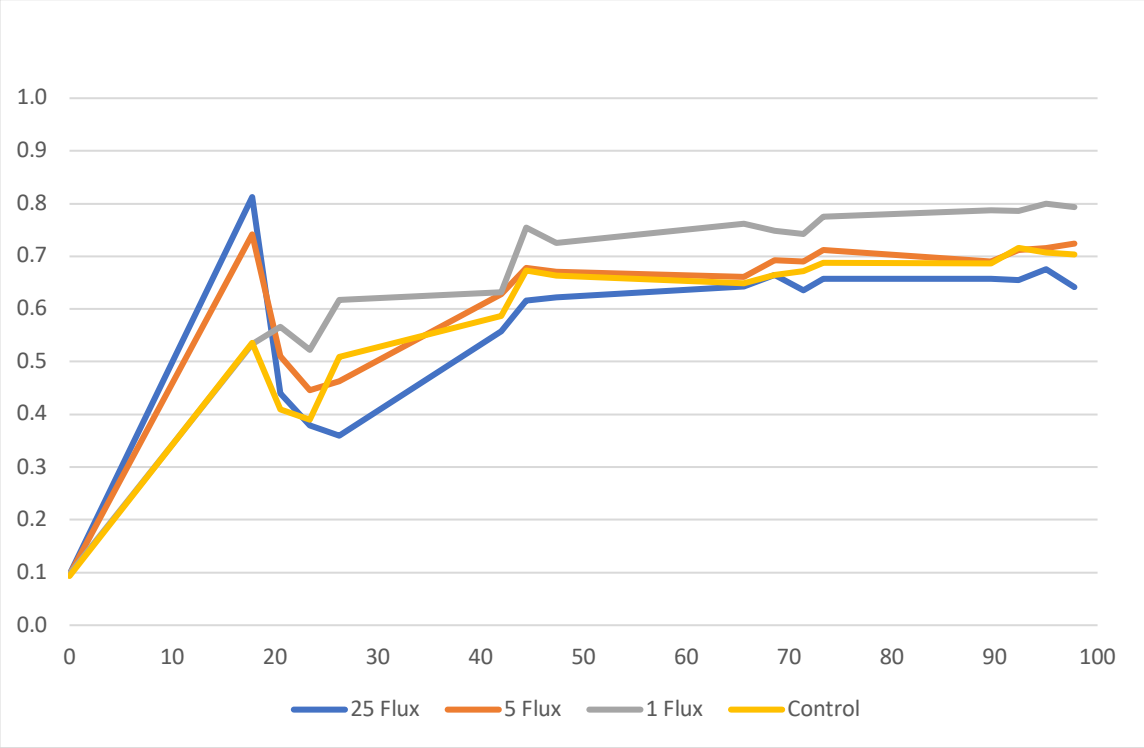


Figure 3.44. The OD curve of isolate 80 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.112	0.114	0.113	0.118	1.630E-23	0.007	0.010	0.013
17.03	0.810	0.840	0.900	0.829	4.580E-03	0.077	0.102	0.132
20.13	0.531	0.523	0.519	0.449	6.986E-04	0.086	0.114	0.148
22.58	0.406	0.447	0.413	0.368	3.937E-06	0.042	0.055	0.072
26.43	0.394	0.409	0.415	0.418	6.260E-01	0.044	0.059	0.077
42.08	0.387	0.403	0.410	0.390	7.495E-04	0.032	0.043	0.056
45.07	0.423	0.426	0.453	0.438	1.365E-05	0.043	0.057	0.074
47.97	0.446	0.431	0.444	0.442	1.134E-05	0.039	0.052	0.067
50.55	0.443	0.443	0.459	0.459	1.680E-03	0.040	0.054	0.069
65.37	0.482	0.447	0.481	0.451	3.085E-07	0.036	0.048	0.062
68.18	0.493	0.464	0.492	0.458	5.528E-06	0.040	0.053	0.069
71.60	0.478	0.447	0.488	0.451	2.404E-05	0.041	0.054	0.070
73.82	0.499	0.467	0.513	0.451	6.149E-07	0.042	0.056	0.072
88.87	0.483	0.440	0.492	0.449	3.516E-07	0.041	0.054	0.070
91.60	0.496	0.460	0.503	0.438	2.457E-07	0.046	0.061	0.079
95.18	0.484	0.456	0.495	0.437	6.389E-08	0.042	0.055	0.072
97.73	0.484	0.441	0.495	0.430	2.560E-08	0.042	0.055	0.072

Table 3.95. The mean ODs at each assessment time for isolate 80 in the presence of three concentrations of fluxapyroxad LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The cross-screening test with isolate 80 and the fungicide fluxapyroxad were run on two occasions. In both trials (Fig. 3.43, and 3.44 respectively), the control and treatments reached maximum OD value recorded around 17 hours followed by a fall in OD values. Then the ODs with the control and treatments started to level off until the test was stopped in the first trial. In the second trial, the ODs started to increase gradually until 44.42 hours, when it begun to plateau out. In both tests, with treatments 1 and 25 ppm ODs were significantly higher than the control from 73.08 hours onwards (Table 3.95 and 3.96 respectively).

The only statistically significant difference compared to the control was seen with 1 ppm in the first trial (Table 3.97) However, there was a consistent trend to increased growth with 1 and 5 ppm fluxapyroxad in both trials and with 25ppm in the first trial (Table 3.98).

Ass.(h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.096	0.096	0.097	0.093	4.5E-09	0.015	0.020	0.026
15.95	0.813	0.742	0.534	0.536	6.0E-06	0.164	0.217	0.282
18.95	0.440	0.510	0.567	0.410	2.0E-01	0.119	0.158	0.204
21.62	0.379	0.446	0.523	0.390	1.8E-02	0.109	0.145	0.188
24.55	0.360	0.463	0.617	0.509	1.1E-01	0.134	0.178	0.230
40.50	0.558	0.629	0.632	0.587	2.1E-01	0.133	0.176	0.228
43.07	0.616	0.679	0.754	0.673	1.7E-02	0.148	0.197	0.256
45.70	0.622	0.671	0.726	0.663	1.6E-02	0.154	0.204	0.264
47.83	0.643	0.661	0.762	0.649	5.3E-04	0.121	0.161	0.209
64.70	0.664	0.693	0.748	0.665	2.3E-04	0.128	0.170	0.221
66.82	0.635	0.690	0.742	0.672	1.3E-03	0.131	0.174	0.226
70.10	0.657	0.712	0.775	0.688	6.6E-05	0.128	0.169	0.220
72.43	0.657	0.690	0.787	0.687	5.7E-06	0.119	0.158	0.204
88.40	0.655	0.712	0.786	0.716	3.2E-05	0.127	0.169	0.219
90.53	0.676	0.716	0.800	0.707	9.6E-05	0.129	0.172	0.223
92.78	0.641	0.724	0.794	0.704	3.6E-05	0.134	0.178	0.231

Table 3.96. The mean ODs at each assessment time for isolate 80 in the presence of three concentrations of fluxapyroxad LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Flux. 25	34.09
Flux. 5	33.22
Flux. 1	35.85
Control	32.27
LSD5%	2.48
LSD1%	3.29
LSD0.1%	4.27

Treatment	"Growth"
Flux. 25	45.73
Flux. 5	49.34
Flux. 1	53.05
Control	46.40
LSD5%	8.652
LSD1%	11.485
LSD0.1%	14.895

Table 3.97. and 3.98. The mean "growth" of isolate 80 in treatments with 25, 5, and 1 ppm of fluxapyroxad over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

3.2.4.5. Isolate 96

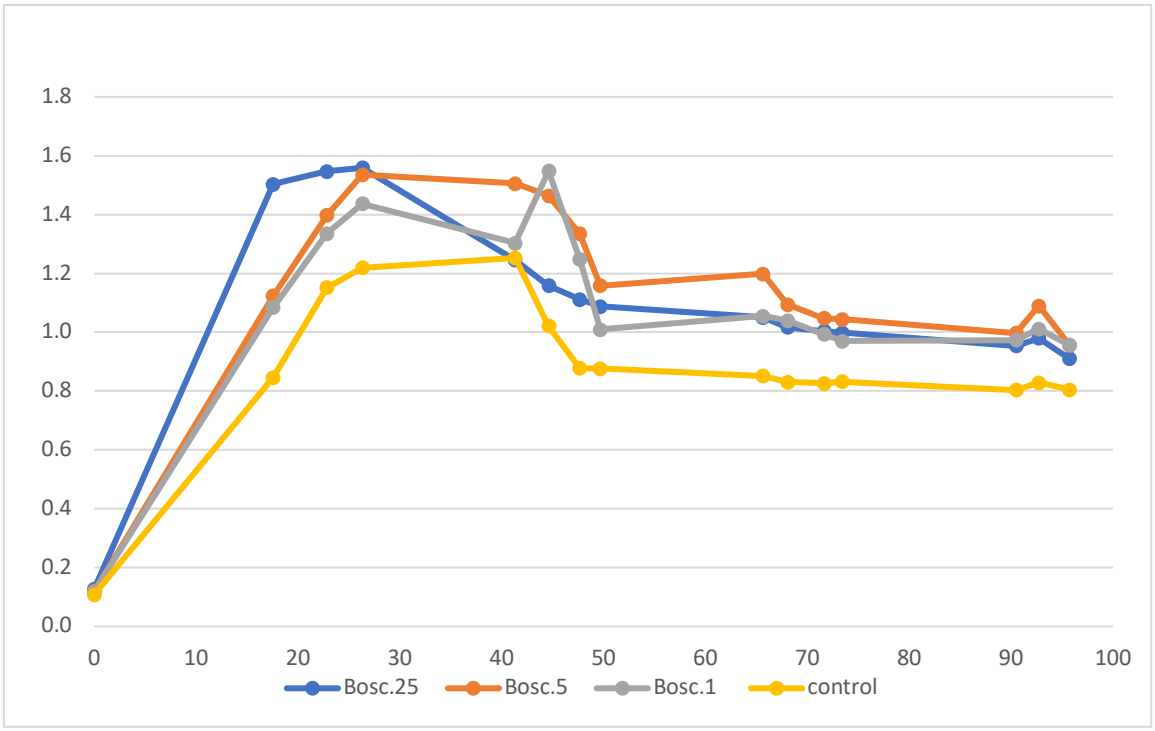


Figure 3.45. The OD curve of isolate 96 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

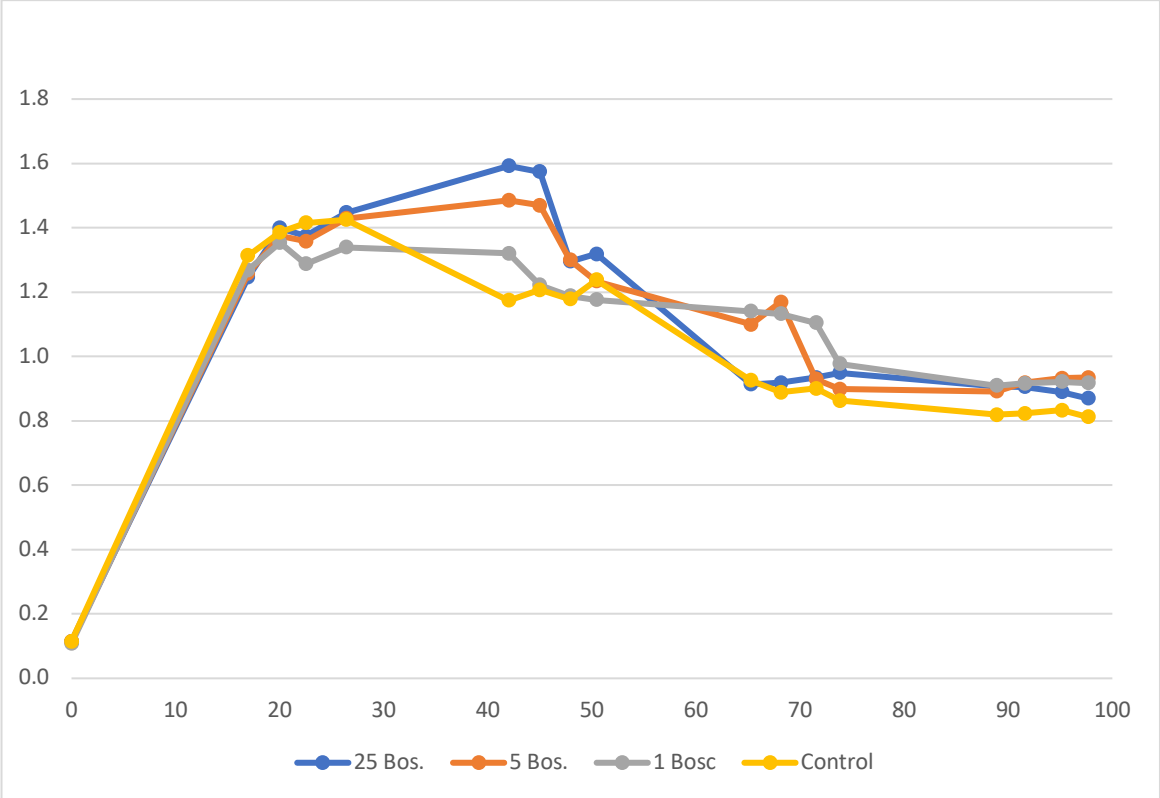


Figure 3.46. The OD curve of isolate 96 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

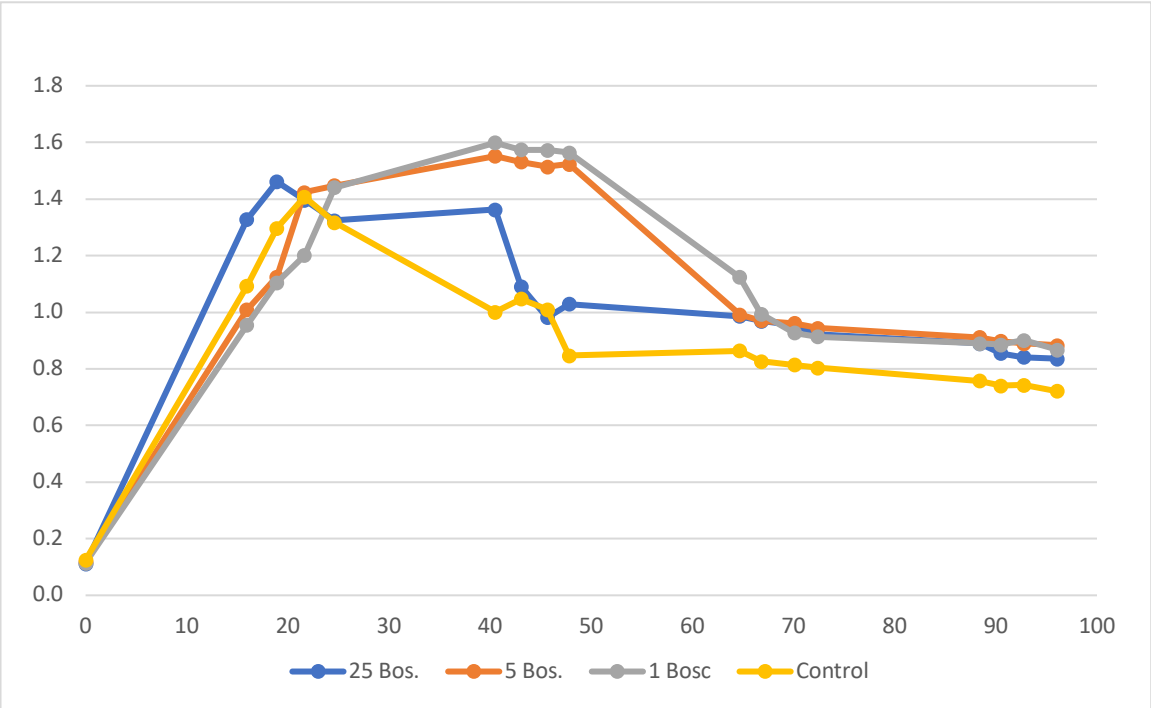


Figure 3.47. The OD curve of isolate 96 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Bosc.25	Bosc.5	Bosc.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.128	0.120	0.119	0.109	8.7E-30	0.005	0.006	0.008
17.55	1.504	1.124	1.085	0.846	9.8E-21	0.140	0.186	0.242
22.83	1.547	1.398	1.335	1.151	1.2E-18	0.179	0.238	0.310
26.32	1.559	1.536	1.437	1.219	8.5E-17	0.212	0.282	0.367
41.32	1.246	1.505	1.303	1.253	7.9E-10	0.293	0.390	0.508
44.62	1.158	1.464	1.548	1.021	1.3E-11	0.262	0.349	0.454
47.65	1.111	1.335	1.247	0.878	2.9E-10	0.256	0.341	0.444
49.67	1.088	1.158	1.009	0.876	1.5E-16	0.145	0.193	0.251
65.63	1.050	1.199	1.055	0.851	3.5E-18	0.131	0.175	0.228
68.05	1.050	1.199	1.055	0.851	3.1E-19	0.116	0.154	0.200
71.63	1.017	1.094	1.040	0.830	5.8E-20	0.106	0.141	0.184
73.42	1.004	1.047	0.993	0.826	6.9E-21	0.104	0.138	0.180
90.50	0.998	1.044	0.970	0.831	2.4E-25	0.077	0.102	0.133
92.73	0.953	0.998	0.974	0.804	2.0E-21	0.098	0.130	0.170
95.75	0.980	1.090	1.011	0.829	1.6E-28	0.067	0.089	0.116

Table 3.99. The mean ODs at each assessment time for isolate 96 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Three cross screening tests with the isolate 96 and the fungicide boscalid showed a similar growth curve as the one seen with this strain in the "30-minute test". The OD values during the course of the experiment were similar in all the three tests. After a rapid increase within the first 24 hours or so, the OD values then remained at these peak values for 16 hours before declining and eventually levelling out at 50 –70 hours. Moreover, the OD values of the control were lower

than those of the treatments in the three experiments particularly after the peak values had been reached until the levelling out occurred towards the end of the experiment (Fig. 3.45,46, and 47 respectively). Many of these differences were statistically significant particularly in the first and third runs (Table 3.99-101).

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0	0.115	0.114	0.108	0.115	9.59E-26	0.005	0.007	0.009
16.95	1.246	1.258	1.268	1.313	4.17E-14	0.175	0.232	0.301
20.03	1.399	1.374	1.354	1.386	2.06E-17	0.156	0.207	0.269
22.52	1.375	1.358	1.288	1.415	1.68E-13	0.182	0.241	0.313
26.40	1.447	1.429	1.339	1.425	2.49E-12	0.196	0.260	0.338
42.00	1.592	1.485	1.321	1.175	4.73E-07	0.298	0.395	0.513
45.00	1.575	1.470	1.222	1.207	2.07E-07	0.319	0.423	0.549
47.92	1.296	1.300	1.188	1.178	9.44E-07	0.273	0.362	0.470
50.45	1.319	1.234	1.177	1.238	3.00E-05	0.317	0.420	0.545
65.30	0.914	1.099	1.140	0.925	1.33E-11	0.179	0.238	0.308
68.15	0.918	1.169	1.132	0.889	5.04E-12	0.180	0.239	0.310
71.53	0.934	0.929	1.104	0.900	1.51E-10	0.166	0.220	0.285
73.80	0.950	0.898	0.977	0.863	2.59E-12	0.143	0.190	0.247
88.87	0.907	0.892	0.910	0.819	3.52E-14	0.119	0.158	0.205
91.60	0.905	0.919	0.916	0.823	4.63E-15	0.116	0.154	0.200
95.17	0.890	0.933	0.922	0.833	3.35E-17	0.104	0.137	0.178
97.70	0.870	0.934	0.918	0.812	1.96E-17	0.102	0.135	0.175

Table 3.100. The mean ODs at each assessment time for isolate 96 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.111	0.118	0.115	0.125	9.1E-22	0.006	0.008	0.010
15.93	1.327	1.008	0.954	1.092	1.4E-05	0.172	0.228	0.296
18.92	1.461	1.125	1.105	1.297	2.2E-04	0.220	0.292	0.378
21.62	1.397	1.423	1.201	1.408	2.6E-04	0.227	0.302	0.391
24.60	1.325	1.447	1.441	1.317	1.2E-01	0.293	0.389	0.505
40.48	1.362	1.552	1.599	1.001	3.5E-03	0.310	0.412	0.534
43.07	1.091	1.532	1.574	1.047	3.0E-03	0.293	0.389	0.505
45.67	0.981	1.514	1.573	1.010	1.2E-07	0.238	0.317	0.411
47.80	1.030	1.523	1.564	0.846	3.7E-08	0.233	0.310	0.402
64.68	0.986	0.991	1.124	0.864	7.8E-06	0.127	0.169	0.219
66.80	0.970	0.970	0.994	0.827	2.4E-05	0.109	0.145	0.188
70.08	0.953	0.961	0.927	0.814	2.2E-05	0.087	0.115	0.149
72.38	0.923	0.945	0.913	0.804	2.0E-06	0.073	0.097	0.125
88.38	0.890	0.912	0.890	0.757	6.4E-07	0.075	0.099	0.129
90.52	0.855	0.898	0.885	0.741	1.4E-06	0.076	0.101	0.131
92.75	0.841	0.890	0.902	0.743	4.3E-07	0.076	0.101	0.131
96.08	0.836	0.883	0.868	0.721	1.9E-05	0.088	0.117	0.151

Table 3. 101. The mean ODs at each assessment time for isolate 96 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Bosc.25	92.86
Bosc.5	95.84
Bosc.1	88.30
Control	72.84
LSD5%	7.190
LSD1%	9.571
LSD0.1%	12.465

Treatment	"Growth"
Bosc.25	95.59
Bosc.5	95.54
Bosc.1	93.49
Control	88.05
LSD5%	11.37
LSD1%	15.09
LSD0.1%	19.57

Treatment	"Growth"
Bosc.25	87.63
Bosc.5	93.93
Bosc.1	94.78
Control	73.69
LSD5%	9.02
LSD1%	11.97
LSD0.1%	15.53

Table 3.102. and 3.103 and 3.104. The mean "growth" of isolate 96 in treatments with 25, 5, and 1 ppm of boscalid over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

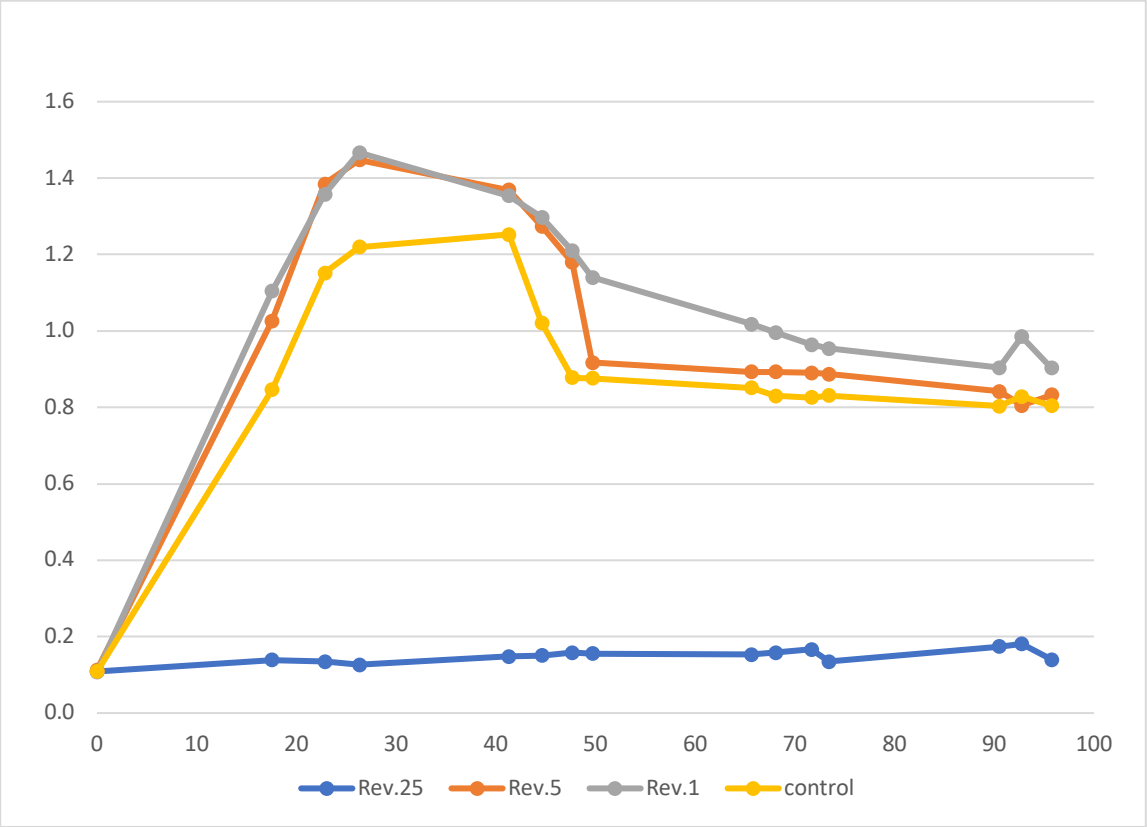


Figure 3.48. The OD curve of isolate 96 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

The cross-screening test with the isolate 96 and fungicide Revysol® was run on three occasions. In the first two experiments (Fig. 3.48 and 3.49 respectively) the OD values with the 25 ppm Revysol® treatment remained around the initial value in the first run or only slightly above in the second and had lowest growth in the third run (Fig. 3.50) indicating an inhibitory effect of this dose of Revysol® on the growth of this isolate. The OD values of this treatment were significantly lower than that of the control at the 0.1% level over the 4 experimental days in first 2 runs (Table 3.105, 3.106) and within first 24 hours in the third run (Table 3.107).

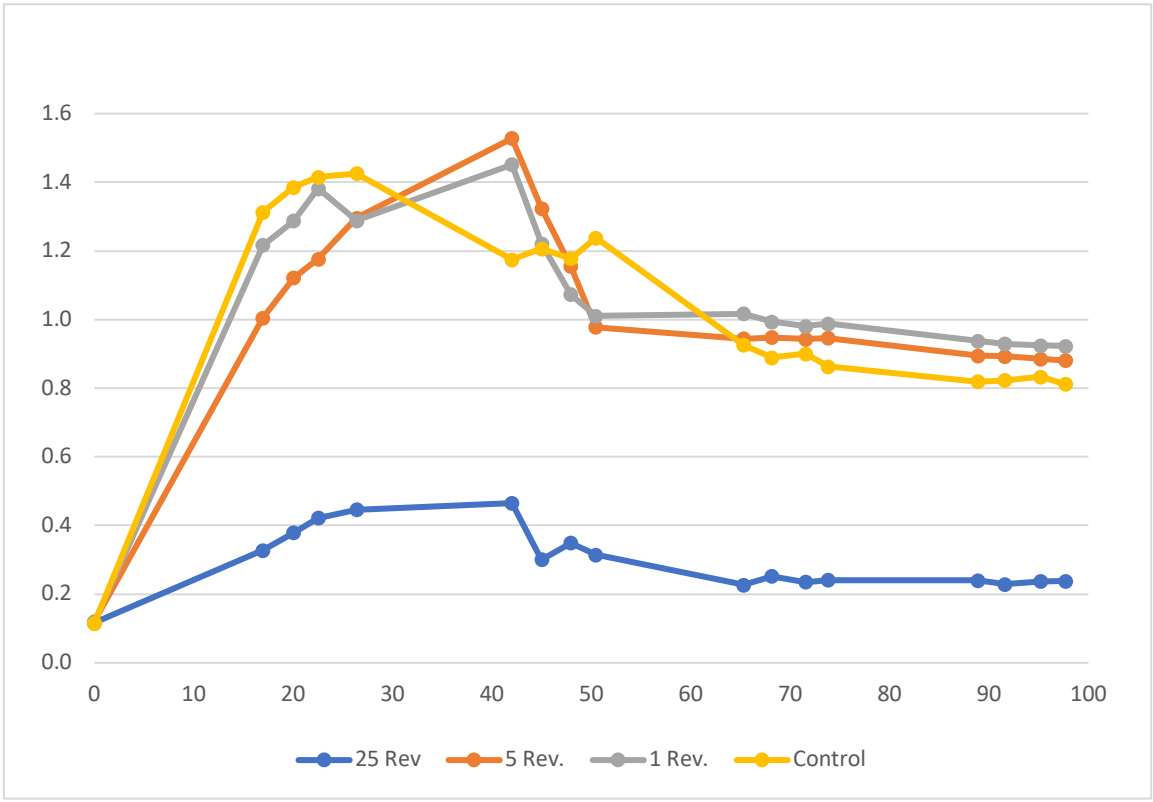


Figure 3.49. The OD curve of isolate 96 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

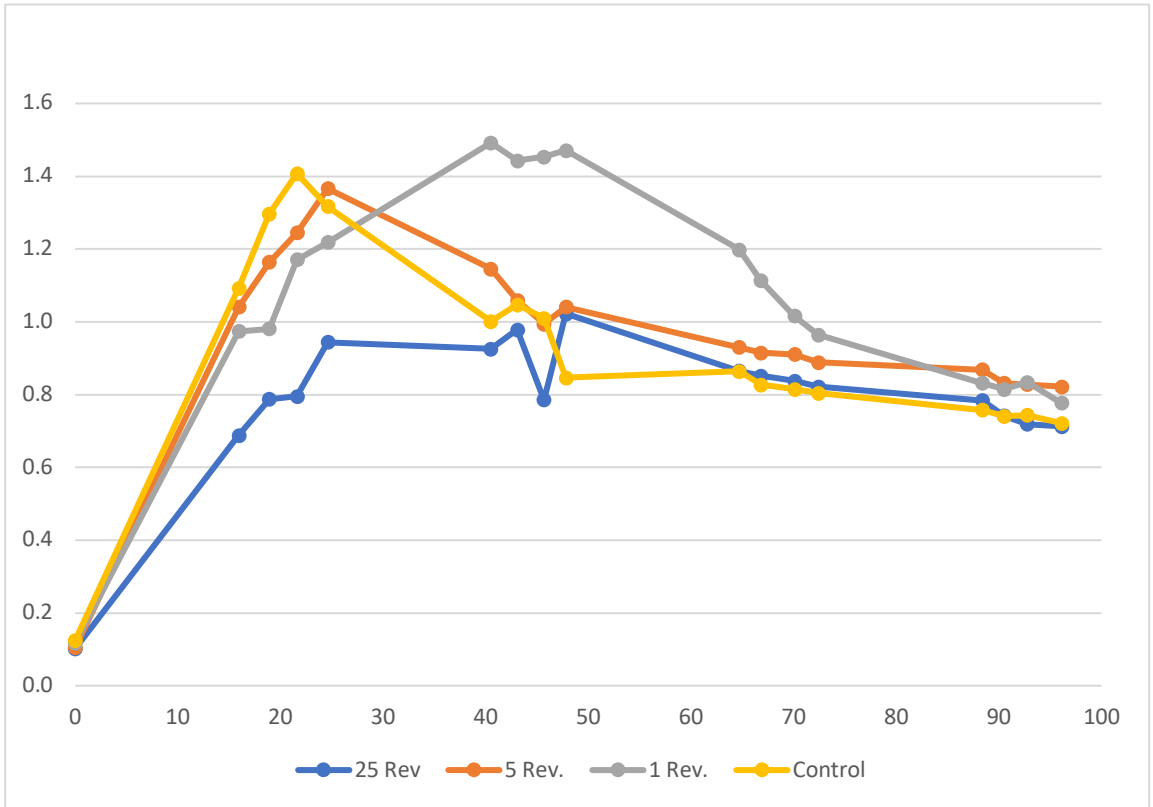


Figure 3.50. The OD curve of isolate 96 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Rev.25	Rev.5	Rev.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.109	0.113	0.107	0.109	8.7E-30	0.005	0.006	0.008
17.55	0.139	1.026	1.104	0.846	9.8E-21	0.140	0.186	0.242
22.83	0.135	1.384	1.358	1.151	1.2E-18	0.179	0.238	0.310
26.32	0.127	1.447	1.467	1.219	8.5E-17	0.212	0.282	0.367
41.32	0.148	1.369	1.354	1.253	7.9E-10	0.293	0.390	0.508
44.62	0.151	1.274	1.298	1.021	1.3E-11	0.262	0.349	0.454
47.65	0.158	1.180	1.210	0.878	2.9E-10	0.256	0.341	0.444
49.67	0.156	0.917	1.140	0.876	1.5E-16	0.145	0.193	0.251
65.63	0.154	0.893	1.018	0.851	3.5E-18	0.131	0.175	0.228
68.05	0.154	0.893	1.018	0.851	3.1E-19	0.116	0.154	0.200
71.63	0.158	0.893	0.996	0.830	5.8E-20	0.106	0.141	0.184
73.42	0.166	0.890	0.964	0.826	6.9E-21	0.104	0.138	0.180
90.50	0.135	0.887	0.954	0.831	2.4E-25	0.077	0.102	0.133
92.73	0.174	0.842	0.904	0.804	2.0E-21	0.098	0.130	0.170
95.75	0.181	0.805	0.985	0.829	1.6E-28	0.067	0.089	0.116

Table 3.105. The mean ODs at each assessment time for isolate 96 in the presence of three concentrations of Revysol® LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.117	0.120	0.117	0.115	9.6E-26	0.005	0.007	0.009
16.95	0.327	1.005	1.217	1.313	4.2E-14	0.175	0.232	0.301
20.03	0.379	1.121	1.287	1.386	2.1E-17	0.156	0.207	0.269
22.52	0.422	1.177	1.382	1.415	1.7E-13	0.182	0.241	0.313
26.40	0.446	1.296	1.288	1.425	2.5E-12	0.196	0.260	0.338
42.00	0.465	1.528	1.451	1.175	4.7E-07	0.298	0.395	0.513
45.00	0.300	1.324	1.222	1.207	2.1E-07	0.319	0.423	0.549
47.92	0.349	1.155	1.073	1.178	9.4E-07	0.273	0.362	0.470
50.45	0.315	0.978	1.010	1.238	3.0E-05	0.317	0.420	0.545
65.30	0.227	0.944	1.017	0.925	1.3E-11	0.179	0.238	0.308
68.15	0.252	0.948	0.993	0.889	5.0E-12	0.180	0.239	0.310
71.53	0.235	0.943	0.981	0.900	1.5E-10	0.166	0.220	0.285
73.80	0.241	0.946	0.989	0.863	2.6E-12	0.143	0.190	0.247
88.87	0.240	0.895	0.937	0.819	3.5E-14	0.119	0.158	0.205
91.60	0.229	0.893	0.930	0.823	4.6E-15	0.116	0.154	0.200
95.17	0.238	0.886	0.924	0.833	3.4E-17	0.104	0.137	0.178
97.70	0.238	0.881	0.922	0.812	2.0E-17	0.102	0.135	0.175

Table 3.106. The mean ODs at each assessment time for isolate 96 in the presence of three concentrations of Revysol® LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

With the 1 and 5 ppm doses, the OD values increased more slowly in the second experiment than the first one which resulted in the peak value being reached later than the control (24.52 hours) at 42 hours. Lower OD values compared to the control were observed in the first 24 hours of the experiment and in the case of the 5ppm treatment these were statistically

significant. In the third experiment (Table. 3.107, 3.110), the initial lower increase with treatments 1 and 5 ppm Revysol® which was statistically significant in case of 1 ppm within first 20 hours, led to a delayed peak value for 1 ppm until 40.48 hours which was nevertheless the highest peak OD value of this experiment (1.4). In the first trial (Fig. 3.48) however this initial delay with the 1 and 5 ppm treatments was not observed. In fact, throughout the experiment the OD values for these treatments exceeded those of the control significantly for both treatments for the first 26 hours of the experiment and with 1ppm mostly throughout the experiment. (Table 3.105). Peak values for the 1 and 5 ppm treatments and also the control was reached at 26 hours.

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.102	0.105	0.117	0.125	9.1E-22	0.006	0.008	0.010
15.93	0.688	1.041	0.974	1.092	1.4E-05	0.172	0.228	0.296
18.92	0.787	1.164	0.981	1.297	2.2E-04	0.220	0.292	0.378
21.62	0.795	1.245	1.171	1.408	2.6E-04	0.227	0.302	0.391
24.60	0.944	1.366	1.219	1.317	1.2E-01	0.293	0.389	0.505
40.48	0.925	1.146	1.492	1.001	3.5E-03	0.310	0.412	0.534
43.07	0.978	1.059	1.442	1.047	3.0E-03	0.293	0.389	0.505
45.67	0.785	0.994	1.454	1.010	1.2E-07	0.238	0.317	0.411
47.80	1.023	1.041	1.471	0.846	3.7E-08	0.233	0.310	0.402
64.68	0.865	0.930	1.198	0.864	7.8E-06	0.127	0.169	0.219
66.80	0.852	0.915	1.113	0.827	2.4E-05	0.109	0.145	0.188
70.08	0.838	0.911	1.016	0.814	2.2E-05	0.087	0.115	0.149
72.38	0.821	0.889	0.964	0.804	2.0E-06	0.073	0.097	0.125
88.38	0.784	0.869	0.832	0.757	6.4E-07	0.075	0.099	0.129
90.52	0.741	0.831	0.815	0.741	1.4E-06	0.076	0.101	0.131
92.75	0.719	0.828	0.834	0.743	4.3E-07	0.076	0.101	0.131
96.08	0.712	0.822	0.777	0.721	1.9E-05	0.088	0.117	0.151

Table 3.107. The mean ODs at each assessment time for isolate 96 in the presence of three concentrations of Revysol® LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Rev. 25	3.47
Rev. 5	82.25
Rev. 1	89.21
Control	72.84
LSD5%	7.190
LSD1%	9.571
LSD0.1%	12.465

Treatment	"Growth"
Rev. 25	17.93
Rev. 5	85.35
Rev. 1	89.76
Control	88.05
LSD5%	11.37
LSD1%	15.09
LSD0.1%	19.57

Treatment	"Growth"
Rev. 25	65.87
Rev. 5	81.28
Rev. 1	90.45
Control	73.69
LSD5%	9.02
LSD1%	11.97
LSD0.1%	15.53

Table 3.108 and 3.109. and 3.110. The mean "growth" of isolate 96 in treatments with 25, 5, and 1 ppm of Revysol® over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

The “growth” of the treatments with 1 ppm Revysol® in the first and third trial and 5 ppm Revysol® in the first trial were significantly greater than that of the control (Table 3.108 and 3.110 respectively.) suggesting that the treatments with lower doses of Revysol® enhance the “growth” of this isolate. However, at higher doses such as 25ppm, there is an inhibitory effect.

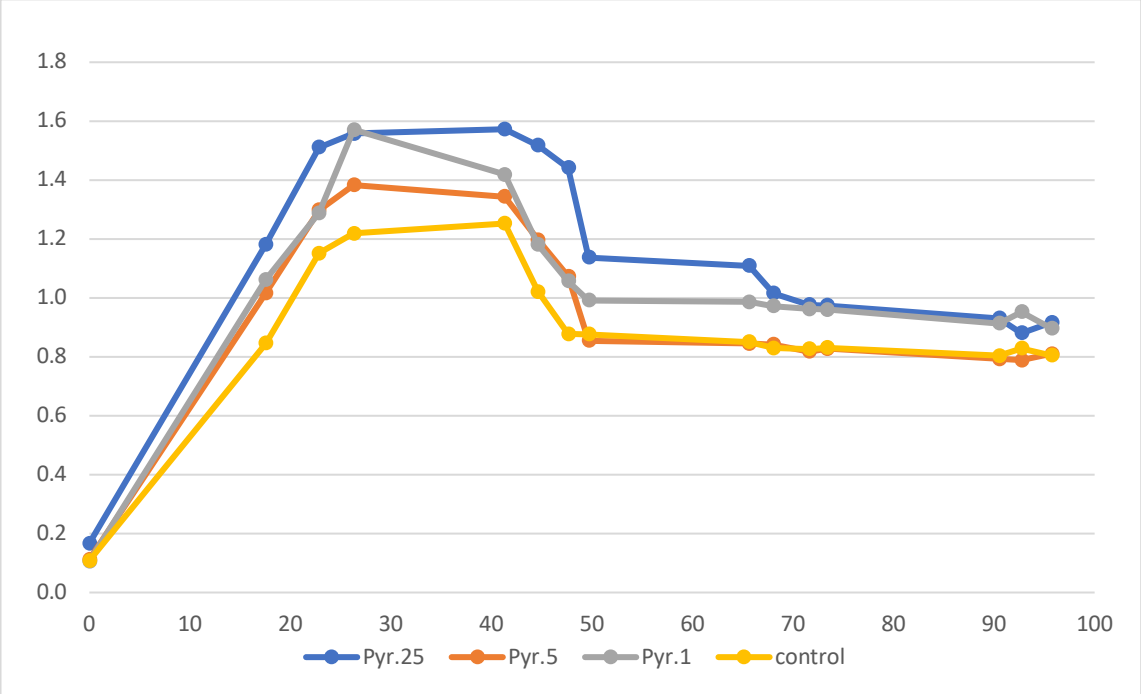


Figure 3.51. The OD curve of isolate 96 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

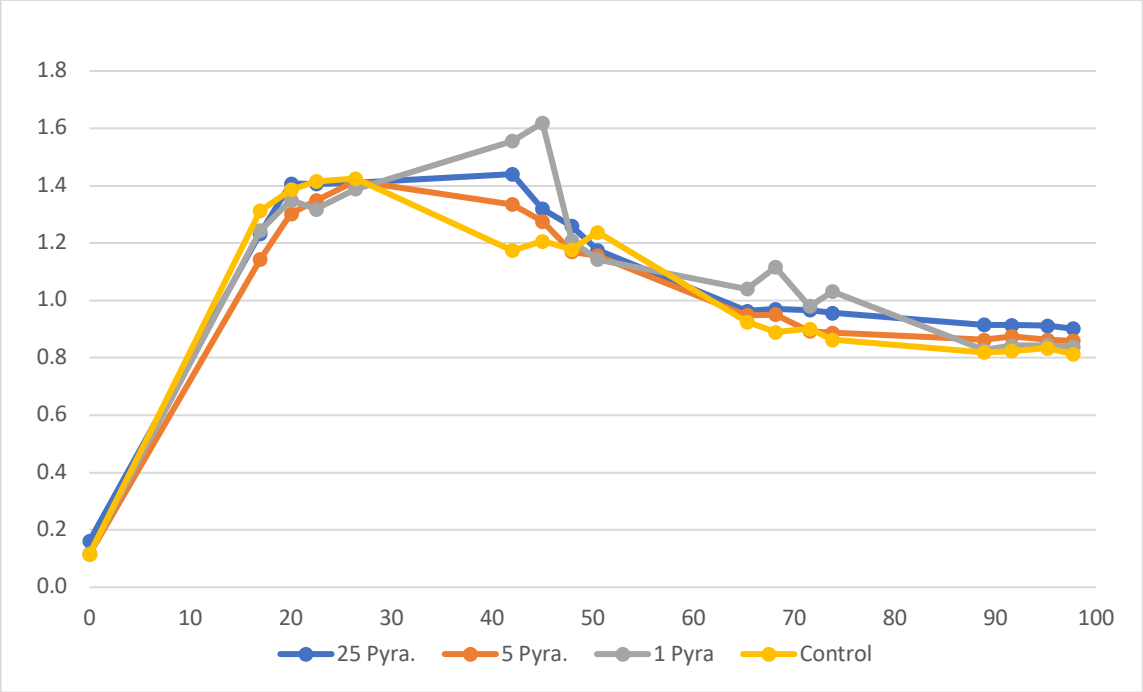


Figure 3.52. The OD curve of isolate 96 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

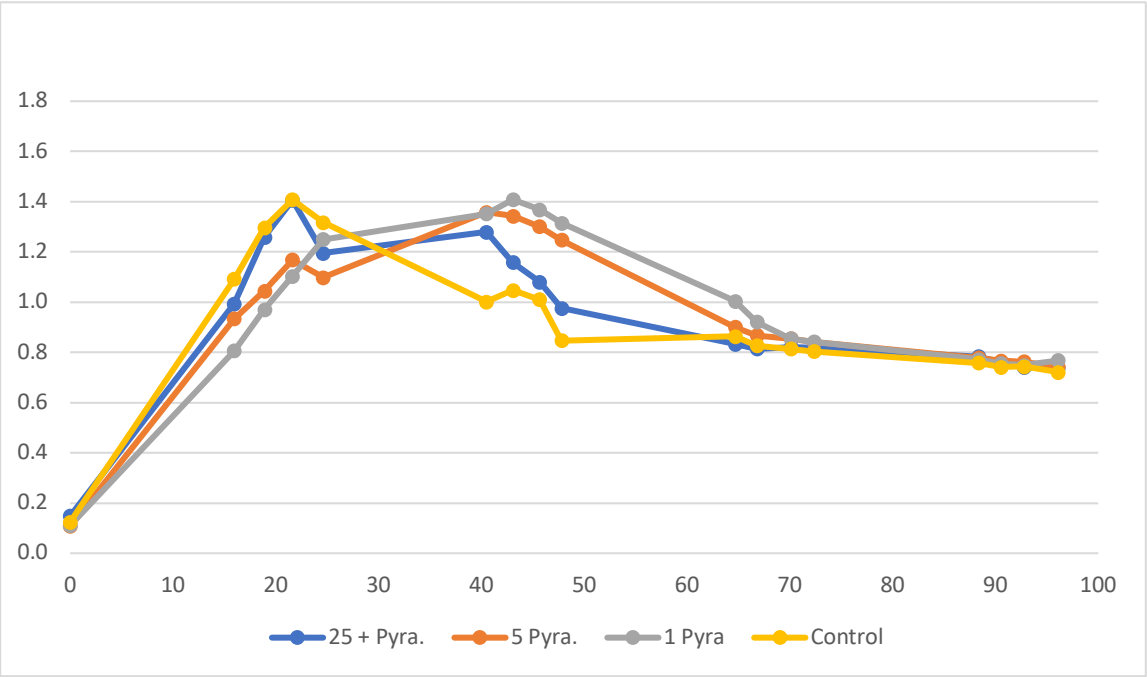


Figure 3.53. The OD curve of isolate 96 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Pyra.25	Pyra.5	Pyar.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.167	0.112	0.106	0.109	8.7E-30	0.005	0.006	0.008
17.55	1.181	1.017	1.063	0.846	9.8E-21	0.140	0.186	0.242
22.83	1.511	1.299	1.288	1.151	1.2E-18	0.179	0.238	0.310
26.32	1.558	1.383	1.571	1.219	8.5E-17	0.212	0.282	0.367
41.32	1.573	1.343	1.419	1.253	7.9E-10	0.293	0.390	0.508
44.62	1.518	1.197	1.181	1.021	1.3E-11	0.262	0.349	0.454
47.65	1.441	1.074	1.057	0.878	2.9E-10	0.256	0.341	0.444
49.67	1.138	0.855	0.992	0.876	1.5E-16	0.145	0.193	0.251
65.63	1.110	0.845	0.986	0.851	3.5E-18	0.131	0.175	0.228
68.05	1.110	0.845	0.986	0.851	3.1E-19	0.116	0.154	0.200
71.63	1.016	0.842	0.972	0.830	5.8E-20	0.106	0.141	0.184
73.42	0.977	0.818	0.962	0.826	6.9E-21	0.104	0.138	0.180
90.50	0.974	0.828	0.960	0.831	2.4E-25	0.077	0.102	0.133
92.73	0.931	0.793	0.914	0.804	2.0E-21	0.098	0.130	0.170
95.75	0.881	0.788	0.953	0.829	1.6E-28	0.067	0.089	0.116

Table 3.111. The mean ODs at each assessment time for isolate 96 in the presence of three concentrations of pyraclostrobin LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The cross-screening test with isolate 96 and the fungicide pyraclostrobin was run three times. All tests depicted are similar growth curve to the one shown in 30-minute test with this isolate. The peak OD values for the controls in the second and third experiment were somewhat higher (1.4) than in the first (1.2) but the "plateau values were similar in all three cases. The peaks were reached at around 26 hours in first two experiments although with I ppm in the second

trial a higher OD value was observed at 45.00 hours. In the third experiment the earlier Peak with the control and 25 ppm observed at 21.62 and later at 40.48- and 43.07-hours maximum OD values with 5 and 1 ppm respectively were recorded. Thereafter the OD values gradually declined until 70.08 hours when with all treatments and the control the OD value started to level off. in the second and third trial but in the first trial after the fall, the ODs started to level off from 49.67 hours onwards (Fig. 3.51-53 respectively).

Ass. (h)	Pyra.25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.162	0.116	0.117	0.115	9.6E-26	0.005	0.007	0.009
16.95	1.233	1.143	1.244	1.313	4.2E-14	0.175	0.232	0.301
20.03	1.407	1.303	1.349	1.386	2.1E-17	0.156	0.207	0.269
22.52	1.407	1.349	1.318	1.415	1.7E-13	0.182	0.241	0.313
26.40	1.411	1.416	1.389	1.425	2.5E-12	0.196	0.260	0.338
42.00	1.441	1.334	1.555	1.175	4.7E-07	0.298	0.395	0.513
45.00	1.319	1.276	1.619	1.207	2.1E-07	0.319	0.423	0.549
47.92	1.259	1.170	1.208	1.178	9.4E-07	0.273	0.362	0.470
50.45	1.175	1.157	1.143	1.238	3.0E-05	0.317	0.420	0.545
65.30	0.963	0.949	1.040	0.925	1.3E-11	0.179	0.238	0.308
68.15	0.970	0.951	1.116	0.889	5.0E-12	0.180	0.239	0.310
71.53	0.967	0.893	0.980	0.900	1.5E-10	0.166	0.220	0.285
73.80	0.956	0.888	1.031	0.863	2.6E-12	0.143	0.190	0.247
88.87	0.916	0.863	0.827	0.819	3.5E-14	0.119	0.158	0.205
91.60	0.915	0.876	0.843	0.823	4.6E-15	0.116	0.154	0.200
95.17	0.912	0.863	0.844	0.833	3.4E-17	0.104	0.137	0.178
97.70	0.902	0.859	0.838	0.812	2.0E-17	0.102	0.135	0.175

Table 3.112. The mean ODs at each assessment time for isolate 96 in the presence of three concentrations of pyraclostrobin LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In the first trial with the 25 and 1 ppm treatments, the OD values were generally statistically greater than the control mostly at 0.1 and 1% respectively throughout the experiment and with 5 ppm the ODs were numerically higher (Fig. 3.51, Table 3.111).

In the second trial, the OD value with 1 ppm was occasionally statistically significant but at assessments where there appeared to be a deviation from the curve and therefore it has to be questioned whether these points are not anomalies (Fig. 3.52, Table 3.112).

In the third trial, because the treatment with 1 and 5 ppm grew more slowly, they have reached the maximum OD value when the control and 25 ppm already passed that point and had started to decline reflected in significantly lower values with 1ppm until 21.06 hours (Fig. 3.53, Table 3.113).

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	SM+B	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.148	0.108	0.111	0.125	9.076E-22	0.006	0.008	0.010
15.93	0.993	0.933	0.807	1.092	1.401E-05	0.172	0.228	0.296
18.92	1.259	1.045	0.971	1.297	2.232E-04	0.220	0.292	0.378
21.62	1.407	1.168	1.104	1.408	2.638E-04	0.227	0.302	0.391
24.60	1.195	1.098	1.250	1.317	1.210E-01	0.293	0.389	0.505
40.48	1.279	1.358	1.352	1.001	3.515E-03	0.310	0.412	0.534
43.07	1.158	1.343	1.408	1.047	2.950E-03	0.293	0.389	0.505
45.67	1.080	1.300	1.369	1.010	1.222E-07	0.238	0.317	0.411
47.80	0.976	1.248	1.315	0.846	3.720E-08	0.233	0.310	0.402
64.68	0.834	0.901	1.004	0.864	7.832E-06	0.127	0.169	0.219
66.80	0.814	0.868	0.922	0.827	2.439E-05	0.109	0.145	0.188
70.08	0.822	0.855	0.855	0.814	2.214E-05	0.087	0.115	0.149
72.38	0.819	0.841	0.842	0.804	2.041E-06	0.073	0.097	0.125
88.38	0.784	0.778	0.772	0.757	6.373E-07	0.075	0.099	0.129
90.52	0.759	0.766	0.755	0.741	1.385E-06	0.076	0.101	0.131
92.75	0.741	0.763	0.749	0.743	4.347E-07	0.076	0.101	0.131
96.08	0.737	0.742	0.768	0.721	1.890E-05	0.088	0.117	0.151

Table 3.113. The mean ODs at each assessment time for isolate 96 in the presence of three concentrations of pyraclostrobin LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Pyra. 25	90.76
Pyra. 5	78.08
Pyra. 1	87.64
Control	72.84
LSD5%	7.190
LSD1%	9.571
LSD0.1%	12.465

Treatment	"Growth"
Pyra. 25	88.54
Pyra. 5	88.02
Pyra. 1	93.97
Control	88.05
LSD5%	11.37
LSD1%	15.09
LSD0.1%	19.57

Treatment	"Growth"
Pyra. 25	73.78
Pyra. 5	80.03
Pyra. 1	81.56
Control	73.69
LSD5%	9.02
LSD1%	11.97
LSD0.1%	15.53

Table 3.114. and 3.115 and 3.116. The mean "growth" of isolate 96 in treatments with 25, 5, and 1 ppm of pyraclostrobin over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

Comparing the treatments, with the 25 ppm treatment the OD values decreased faster than with other two doses 1 and 5 ppm however there were significantly higher OD values than with the control from 40.5 hours onwards and these differences were statistically significant between 40.5 and 47.8 hours (Table 3.113).

With the "growth" parameter, the 25 and 1ppm dose of pyraclostrobin showed a statistically significant increase compared to the control in the first experiment. The 5 ppm dose was also greater than the control and approached statistical significance at the 5% level (Table 3.114). In the second run, the 1ppm dose and in the third all three doses showed a greater value than the control although not statistically significant. In the second trial the increase was largely a

result of the influence of possible anomalies and therefore should not be given too much weight (Table 3.115). Nonetheless in the first and third experiment there is good evidence that the growth of this isolate is enhanced in the presence of pyraclostrobin (Table 3.114, 3.116).

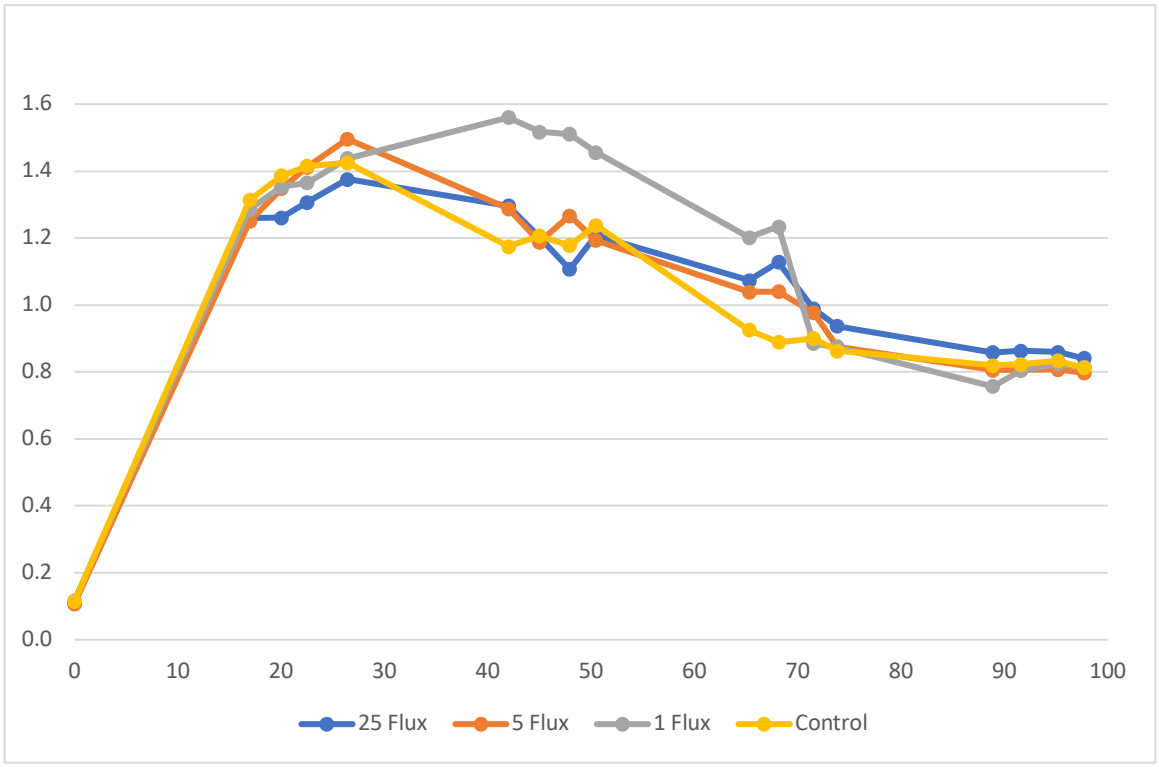


Figure 3.54. The OD curve of isolate 96 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

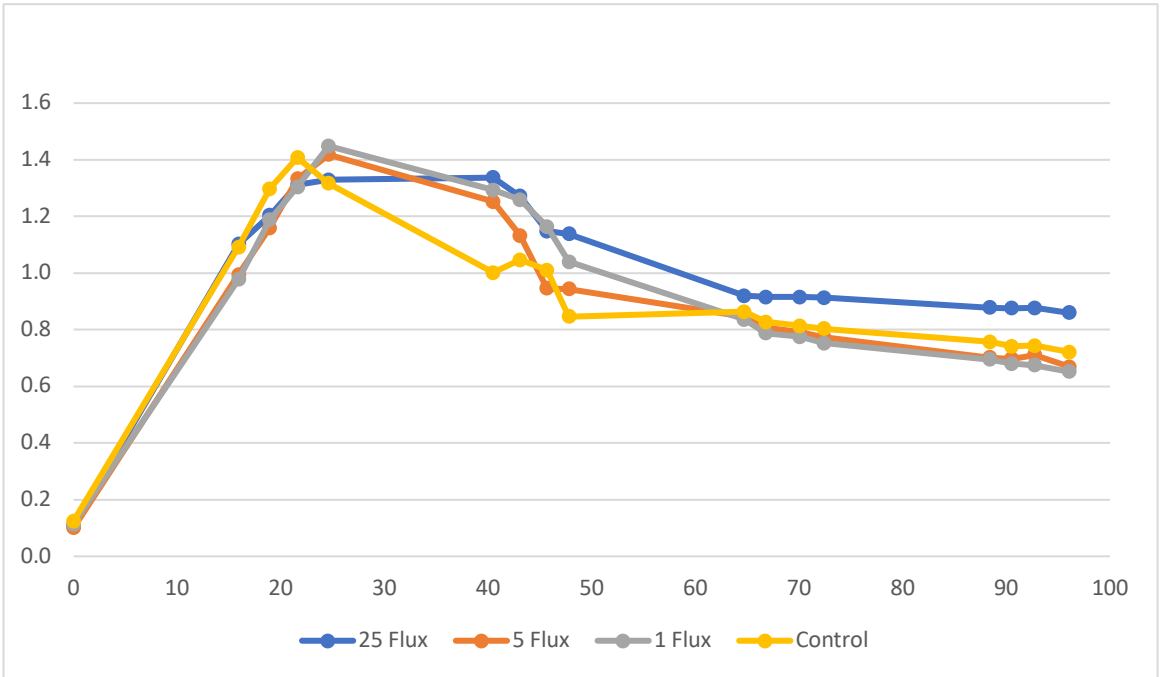


Figure 3.55. The OD curve of isolate 96 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.113	0.108	0.117	0.115	9.6E-26	0.005	0.007	0.009
16.95	1.259	1.250	1.286	1.313	4.2E-14	0.175	0.232	0.301
20.03	1.261	1.348	1.352	1.386	2.1E-17	0.156	0.207	0.269
22.52	1.307	1.412	1.365	1.415	1.7E-13	0.182	0.241	0.313
26.40	1.376	1.496	1.438	1.425	2.5E-12	0.196	0.260	0.338
42.00	1.296	1.288	1.560	1.175	4.7E-07	0.298	0.395	0.513
45.00	1.202	1.189	1.517	1.207	2.1E-07	0.319	0.423	0.549
47.92	1.107	1.266	1.510	1.178	9.4E-07	0.273	0.362	0.470
50.45	1.208	1.193	1.456	1.238	3.0E-05	0.317	0.420	0.545
65.30	1.074	1.039	1.202	0.925	1.3E-11	0.179	0.238	0.308
68.15	1.128	1.040	1.234	0.889	5.0E-12	0.180	0.239	0.310
71.53	0.989	0.977	0.885	0.900	1.5E-10	0.166	0.220	0.285
73.80	0.937	0.876	0.873	0.863	2.6E-12	0.143	0.190	0.247
88.87	0.858	0.805	0.757	0.819	3.5E-14	0.119	0.158	0.205
91.60	0.863	0.806	0.805	0.823	4.6E-15	0.116	0.154	0.200
95.17	0.859	0.807	0.824	0.833	3.4E-17	0.104	0.137	0.178
97.70	0.841	0.797	0.815	0.812	2.0E-17	0.102	0.135	0.175

Table 3.117. The mean ODs at each assessment time for isolate 96 in the presence of three concentrations of fluxapyroxad LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.107	0.100	0.113	0.125	9.1E-22	0.006	0.008	0.010
15.93	1.102	0.995	0.978	1.092	1.4E-05	0.172	0.228	0.296
18.92	1.204	1.160	1.188	1.297	2.2E-04	0.220	0.292	0.378
21.62	1.312	1.332	1.305	1.408	2.6E-04	0.227	0.302	0.391
24.60	1.329	1.418	1.448	1.317	1.2E-01	0.293	0.389	0.505
40.48	1.337	1.253	1.292	1.001	3.5E-03	0.310	0.412	0.534
43.07	1.271	1.132	1.259	1.047	3.0E-03	0.293	0.389	0.505
45.67	1.148	0.946	1.164	1.010	1.2E-07	0.238	0.317	0.411
47.80	1.138	0.943	1.040	0.846	3.7E-08	0.233	0.310	0.402
64.68	0.920	0.845	0.837	0.864	7.8E-06	0.127	0.169	0.219
66.80	0.915	0.807	0.787	0.827	2.4E-05	0.109	0.145	0.188
70.08	0.915	0.792	0.775	0.814	2.2E-05	0.087	0.115	0.149
72.38	0.913	0.773	0.753	0.804	2.0E-06	0.073	0.097	0.125
88.38	0.878	0.702	0.695	0.757	6.4E-07	0.075	0.099	0.129
90.52	0.876	0.697	0.680	0.741	1.4E-06	0.076	0.101	0.131
92.75	0.877	0.711	0.675	0.743	4.3E-07	0.076	0.101	0.131
96.08	0.860	0.669	0.652	0.721	1.9E-05	0.088	0.117	0.151

Table 3.118. The mean ODs at each assessment time for isolate 96 in the presence of three concentrations of fluxapyroxad LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The cross-screening test with the isolate 96 and the fungicide fluxapyroxad showed the same growth curve as the one in 30-minute test. The maximum OD value was observed between 24

and 26 hours except with 1 ppm in the first experiment where the peak value was observed at the next assessment at 42.00 hours (Fig. 3.54 and 3.55 respectively). After reaching the peak with all treatments and the control, the ODs started to drop down until the test was stopped. In the first run, the OD values with the 5 and 25 ppm fluxapyroxad treatments were generally slightly higher than the control. However, with the 1 ppm treatment the OD values were consistently higher than the control from 24.52 to 66.75 hours and in many cases the differences were statistically significant (Fig. 3.54, Table 3.117). In the second run the OD values of the treatments with 1 and 5 ppm were in general higher than those of the control. In this run, instead of 1 ppm, 25 ppm showed consistently highest OD values from the occurrence of the peak value until the test was stopped (Fig. 3.55, Table 3.118).

Treatment	"Growth"
Flux. 25	90.70
Flux. 5	90.97
Flux. 1	97.67
Control	88.05
LSD 5%	11.37
LSD 1%	15.09
LSD 0.1%	19.57

Treatment	"Growth"
Flux. 25	85.74
Flux. 5	77.23
Flux. 1	77.73
Control	73.69
LSD 5%	9.02
LSD 1%	11.97
LSD 0.1%	15.53

Table 3.119. and 3.120. The mean "growth" of isolate 96 in treatments with 25, 5, and 1 ppm of fluxapyroxad over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

The "growth" value recorded for the 1ppm treatment in the first run was considerably greater than the control although not quite statistically significant at the 5% level but in the second trial the 25 ppm was statistically significant at 1% level providing some evidence that the growth of isolate 96 in the presence fluxapyroxad was enhanced (Table 3.119, 3.120).

3.2.4.6 Isolate 124

The cross-screening test with the isolate 124 was run in 2 occasions. In both experiments, presented the same growth curve to 30-minute test with the isolate 124, OD values had a rapid initial increase until peak OD values were achieved. The OD value with all treatments reached the peak and the control at 17.53 hours in the first experiment (Fig. 3.56) and in the second experiment peak OD values with the control and 1 ppm were recorded at 16.97 hours and with 5 and 25 ppm at 22.53 hour (Fig. 3.57).

The highest peak OD value was observed with 5 and 25 ppm in both experiments (0.7 in the first and 0.8 in the second).

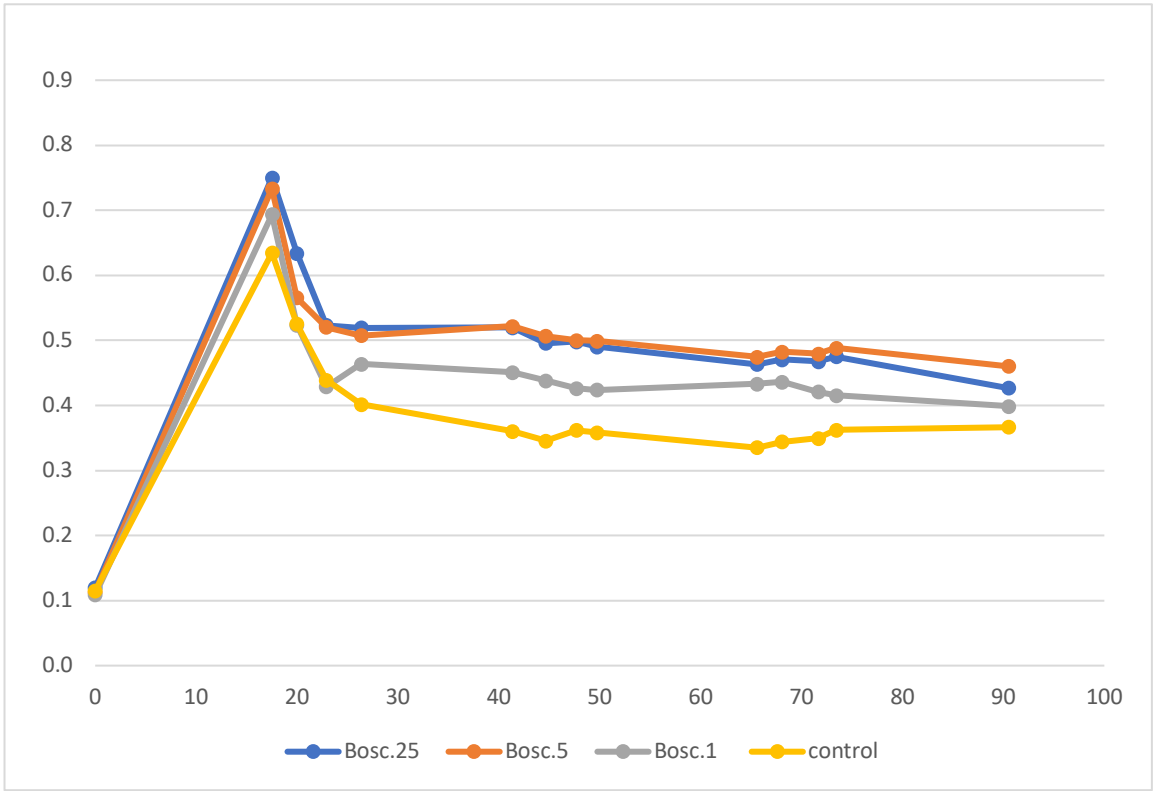


Figure 3.56. The OD curve of isolate 124 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

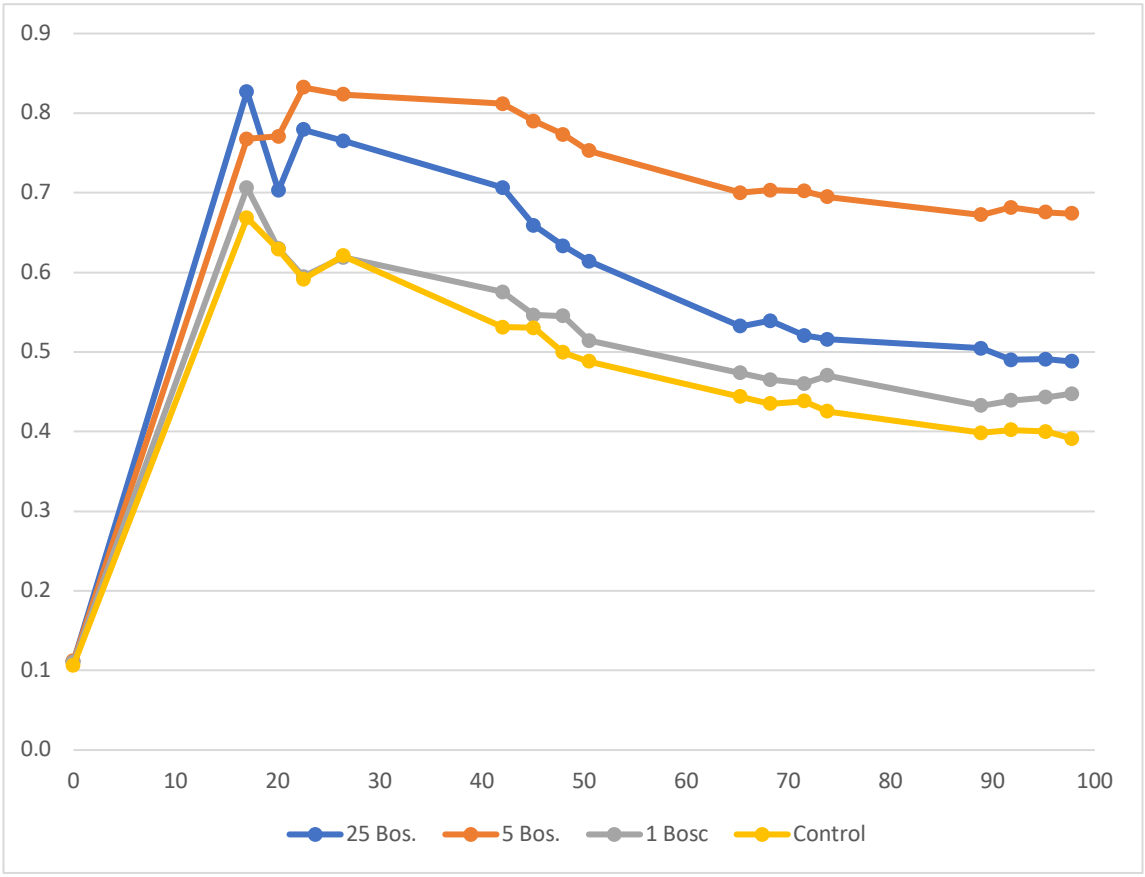


Figure 3.57. The OD curve of isolate 124 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Bosc.25	Bosc.5	Bosc.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.120	0.114	0.109	0.115	2.2E-08	0.016	0.021	0.028
17.53	0.750	0.733	0.693	0.634	6.3E-09	0.101	0.134	0.175
19.98	0.634	0.566	0.524	0.525	1.9E-07	0.089	0.118	0.154
22.88	0.523	0.521	0.429	0.439	6.0E-07	0.082	0.109	0.143
26.33	0.519	0.507	0.464	0.402	4.4E-06	0.085	0.113	0.147
41.33	0.520	0.522	0.451	0.360	2.3E-06	0.106	0.141	0.183
44.65	0.496	0.507	0.438	0.345	1.4E-06	0.116	0.155	0.201
47.67	0.498	0.500	0.427	0.362	2.7E-07	0.100	0.133	0.173
49.68	0.490	0.499	0.424	0.358	8.3E-06	0.110	0.146	0.191
65.60	0.463	0.475	0.433	0.335	2.5E-08	0.099	0.132	0.172
68.07	0.471	0.483	0.436	0.344	7.5E-07	0.103	0.136	0.178
71.65	0.468	0.479	0.421	0.350	1.5E-06	0.105	0.140	0.182
73.43	0.475	0.489	0.416	0.362	1.4E-05	0.108	0.143	0.187
90.52	0.427	0.461	0.399	0.367	7.1E-05	0.123	0.163	0.213

Table 3.121. The mean ODs at each assessment time for isolate 124 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0	0.111	0.112	0.110	0.107	9.3E-27	0.008	0.011	0.015
16.97	0.827	0.768	0.706	0.669	2.8E-20	0.106	0.140	0.182
20.07	0.703	0.771	0.630	0.629	1.7E-22	0.087	0.115	0.150
22.53	0.779	0.832	0.595	0.591	4.5E-21	0.086	0.114	0.148
26.42	0.765	0.823	0.619	0.621	3.2E-18	0.095	0.127	0.164
42.03	0.707	0.812	0.576	0.531	1.1E-14	0.100	0.133	0.172
45.05	0.659	0.790	0.546	0.530	1.0E-14	0.098	0.130	0.169
47.95	0.633	0.773	0.545	0.499	2.1E-18	0.083	0.110	0.143
50.48	0.614	0.753	0.514	0.488	1.5E-18	0.081	0.108	0.140
65.30	0.532	0.700	0.474	0.444	3.4E-19	0.078	0.103	0.134
68.23	0.539	0.703	0.465	0.435	5.0E-15	0.092	0.121	0.158
71.53	0.521	0.702	0.461	0.438	6.6E-19	0.077	0.103	0.133
73.82	0.516	0.695	0.471	0.426	5.2E-19	0.078	0.104	0.134
88.88	0.505	0.672	0.433	0.398	1.9E-19	0.078	0.104	0.134
91.78	0.490	0.682	0.439	0.402	2.9E-19	0.080	0.106	0.138
95.20	0.491	0.675	0.443	0.400	1.0E-18	0.082	0.109	0.141
97.75	0.488	0.674	0.447	0.391	3.3E-17	0.087	0.116	0.150

Table 3.122. The mean ODs at each assessment time for isolate 124 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In the first trial, there was a quick fall in OD values until 22.88 hours with treatment and it continued with the control until 41.33. then all treatments and the control started to level off until the test was stopped (Fig. 3.56) The lowest OD value was recorded with the control over the course of both experiments led to significant differences between the control and 25 ppm

from 17.53 hours and 5 ppm from 41.33 hours mostly at 1% level until the experiment was finished. (Table 121).

In the second trial, with the control OD value had the lowest initial increase, lowest peak, and the greatest decline reflected in significantly greater OD values with 5 and 25 ppm at 0.1 and 5% level respectively from the middle of first day until the test was stopped (Fig. 3.57, Table 3.122).

Considering the average "growth" in both experiments, it suggests that the "growth" of strain 124 is enhanced in the presence of boscalid especially at rates 5 and 25 ppm (Table 3.123, 3.124).

Treatment	"Growth"
Bosc.25	32.91
Bosc.5	33.66
Bosc.1	29.22
Control	23.60
LSD5%	7.37
LSD1%	9.81
LSD0.1%	12.78

Treatment	"Growth"
Bosc.25	46.36
Bosc.5	56.55
Bosc.1	38.03
Control	35.74
LSD5%	6.44
LSD1%	8.55
LSD0.1%	11.09

Table 3.123. and 3.124. The mean "growth" of isolate 124 in treatments with 25, 5, and 1 ppm of boscalid over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

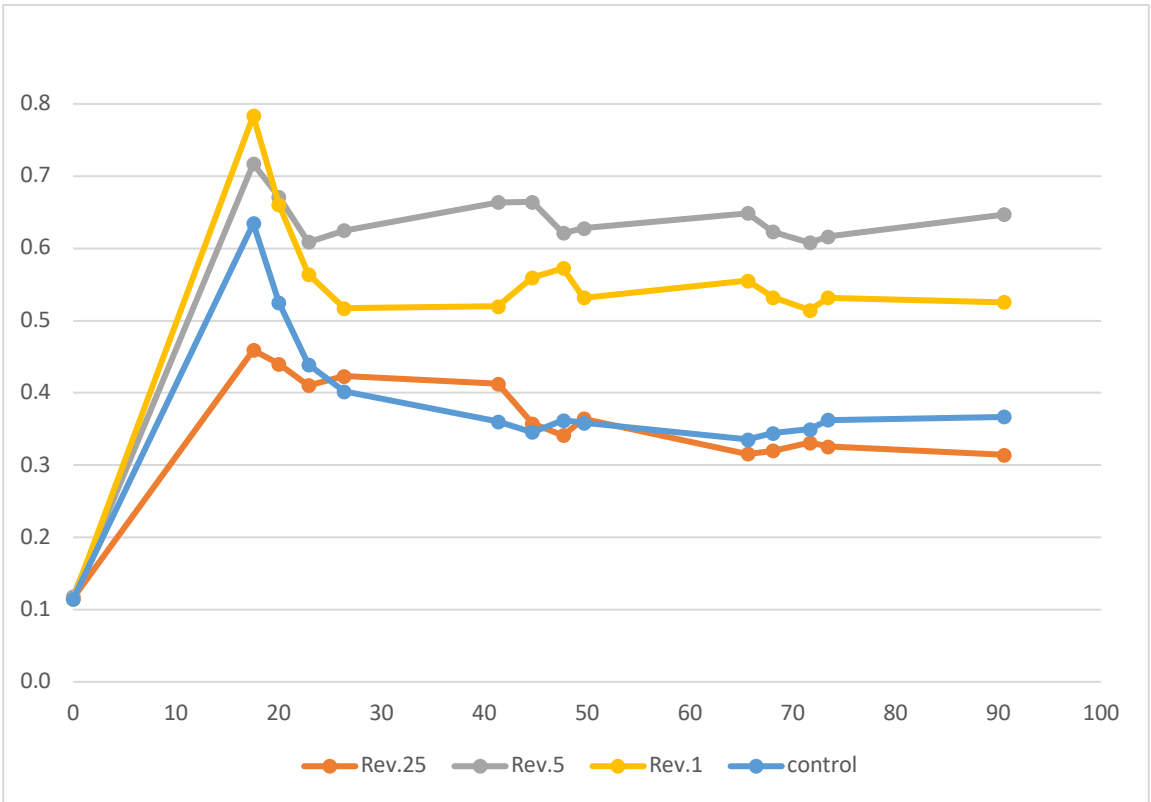


Figure 3.58. The OD curve of isolate 124 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

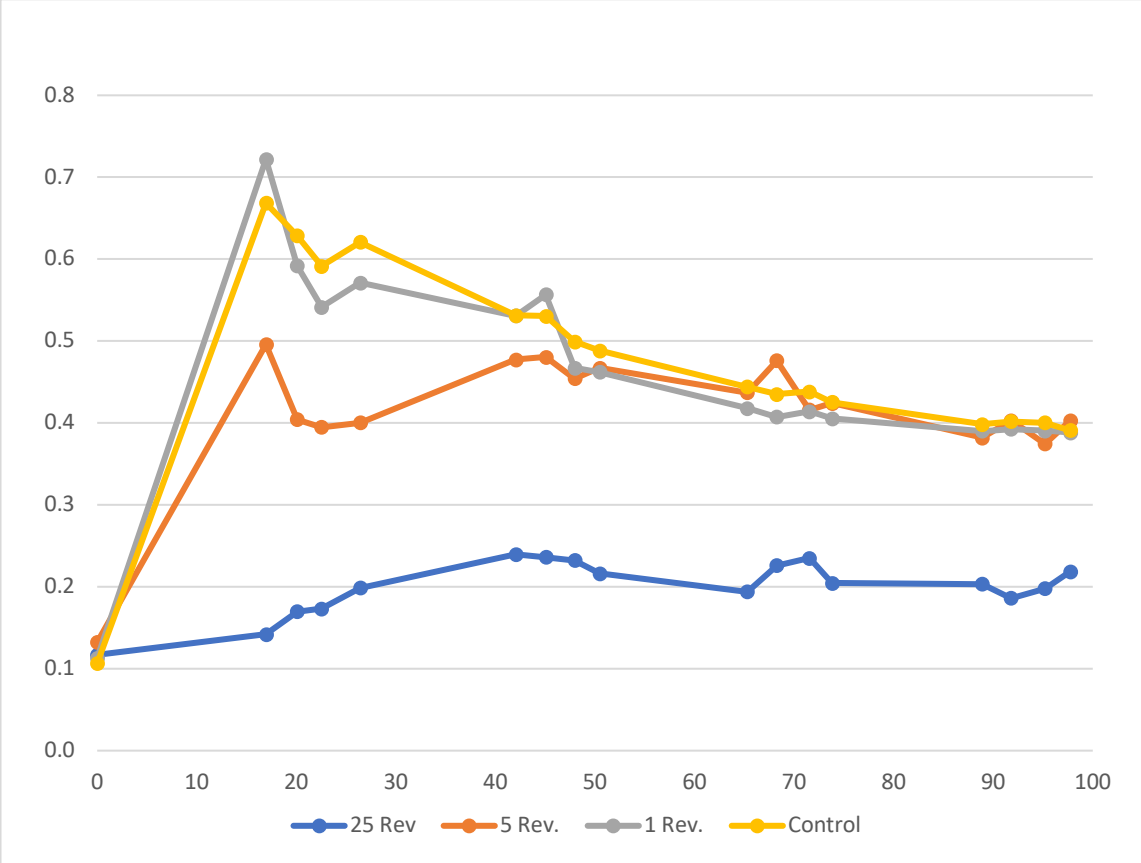


Figure 3.59. The OD curve of isolate 124 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Rev.25	Rev.5	Rev.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.116	0.118	0.115	0.115	2.2E-08	0.016	0.021	0.028
17.53	0.459	0.717	0.783	0.634	6.3E-09	0.101	0.134	0.175
19.98	0.440	0.671	0.661	0.525	1.9E-07	0.089	0.118	0.154
22.88	0.410	0.609	0.564	0.439	6.0E-07	0.082	0.109	0.143
26.33	0.423	0.625	0.517	0.402	4.4E-06	0.085	0.113	0.147
41.33	0.413	0.664	0.520	0.360	2.3E-06	0.106	0.141	0.183
44.65	0.357	0.664	0.560	0.345	1.4E-06	0.116	0.155	0.201
47.67	0.341	0.621	0.573	0.362	2.7E-07	0.100	0.133	0.173
49.68	0.364	0.628	0.532	0.358	8.3E-06	0.110	0.146	0.191
65.60	0.316	0.649	0.555	0.335	2.5E-08	0.099	0.132	0.172
68.07	0.320	0.623	0.532	0.344	7.5E-07	0.103	0.136	0.178
71.65	0.331	0.608	0.515	0.350	1.5E-06	0.105	0.140	0.182
73.43	0.326	0.616	0.532	0.362	1.4E-05	0.108	0.143	0.187
90.52	0.314	0.647	0.525	0.367	7.1E-05	0.123	0.163	0.213

Table 3.125. The mean ODs at each assessment time for isolate 124 in the presence of three concentrations of Revysol® LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The cross-screening test with isolate 124 and Fungicide Revysol® was run twice. In both experiments, treatments and the control achieved a peak value around 17 hours or so. The highest peak observed was with 1 ppm and the lowest with 25 ppm in both tests.

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0	0.117	0.132	0.113	0.107	9.3E-27	0.008	0.011	0.015
16.97	0.142	0.496	0.722	0.669	2.8E-20	0.106	0.140	0.182
20.07	0.170	0.404	0.592	0.629	1.7E-22	0.087	0.115	0.150
22.53	0.173	0.395	0.541	0.591	4.5E-21	0.086	0.114	0.148
26.42	0.199	0.400	0.571	0.621	3.2E-18	0.095	0.127	0.164
42.03	0.240	0.478	0.531	0.531	1.1E-14	0.100	0.133	0.172
45.05	0.236	0.481	0.557	0.530	1.0E-14	0.098	0.130	0.169
47.95	0.232	0.454	0.467	0.499	2.1E-18	0.083	0.110	0.143
50.48	0.216	0.467	0.462	0.488	1.5E-18	0.081	0.108	0.140
65.30	0.194	0.437	0.418	0.444	3.4E-19	0.078	0.103	0.134
68.23	0.226	0.476	0.407	0.435	5.0E-15	0.092	0.121	0.158
71.53	0.235	0.416	0.414	0.438	6.6E-19	0.077	0.103	0.133
73.82	0.205	0.424	0.405	0.426	5.2E-19	0.078	0.104	0.134
88.88	0.204	0.382	0.390	0.398	1.9E-19	0.078	0.104	0.134
91.78	0.186	0.403	0.392	0.402	2.9E-19	0.080	0.106	0.138
95.20	0.198	0.375	0.391	0.400	1.0E-18	0.082	0.109	0.141
97.75	0.219	0.403	0.388	0.391	3.3E-17	0.087	0.116	0.150

Table 3.126. The mean ODs at each assessment time for isolate 124 in the presence of three concentrations of Revysol® LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Rev.25	21.03
Rev.5	43.18
Rev.1	37.12
Control	23.60
LSD5%	7.37
LSD1%	9.81
LSD0.1%	12.78

Treatment	"Growth"
Rev.25	7.52
Rev.5	27.14
Rev.1	34.02
Control	35.74
LSD5%	6.44
LSD1%	8.55
LSD0.1%	11.09

Table 3.127. and 3.128. The mean "growth" of isolate 124 in treatments with 25, 5, and 1 ppm of Revysol® over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

In the first trial (Fig. 3.58) after reaching the peak, with the treatments and the control OD value started to decrease. Although the fall with 1 ppm was greater than the one with 5 ppm but OD values with both were significantly higher than the control until the test was finished. With 1 and 5 ppm the OD values started to plateau out after first 24 hours, whereas the fall with the control and 25 ppm was greater and continued until the test was stopped resulted in statistically significant differences between 1 and 5 ppm and the control especially 5 ppm at 0.1% level (Table. 3.125).

However, in the second trial the order curve of the treatments with 1 and 5 ppm and the control in terms of OD values following the maxima was reversed compared to the first trial. Although the highest peak OD value was observed with 1 ppm, but the decline was greater than the one

with the control afterwards (Fig. 3.59). Moreover, with 5 ppm the OD increased slower than the control and 1 ppm resulted in significantly lower OD values with 5 ppm within first 24 hours and numerically with 1 ppm in most timings (Table 3.126). With the 25 ppm there was no increase with the OD value over the experimental time reflecting in very significantly lower ODs compared to the control at the 0.1% level.

Since with treatments somewhat contradictory results were obtained, with a clear statistically significant increase in "growth" being observed with the 1 and 5 ppm in the first trial (Table 3.127) whereas in the second trial (Table 3.128) there was a significant reduction with the 5 and 25 ppm in the second trial, it is difficult to recognize a consistent effect of Revysol® on the isolate 124 over the course of both experiments.

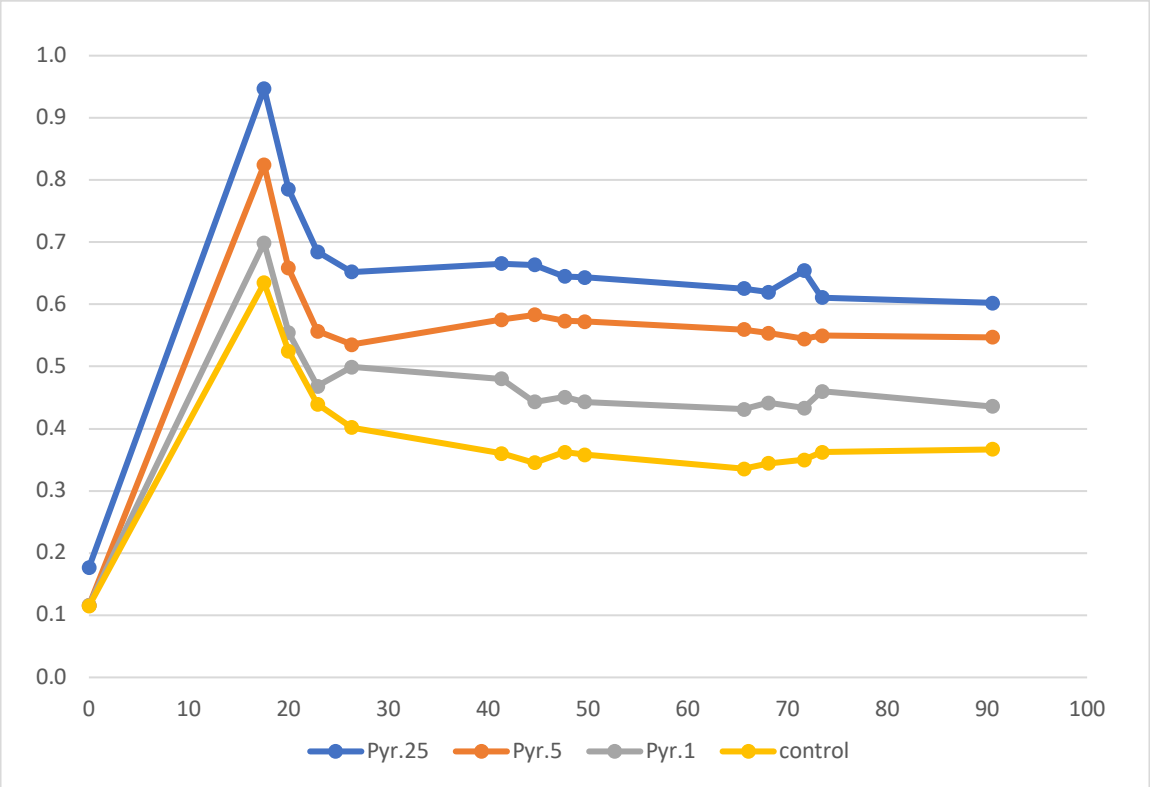


Figure 3.60. The OD curve of isolate 124 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

The cross-screening test with the isolate 124 and the fungicide pyraclostrobin was run twice. In both cases a similar growth curve was obtained to that seen with this isolate in the 30-minute test. The OD increased until reaching peak values between 15 and 17 hours then fell before levelling off from 26 hours onwards (Fig. 3.60).

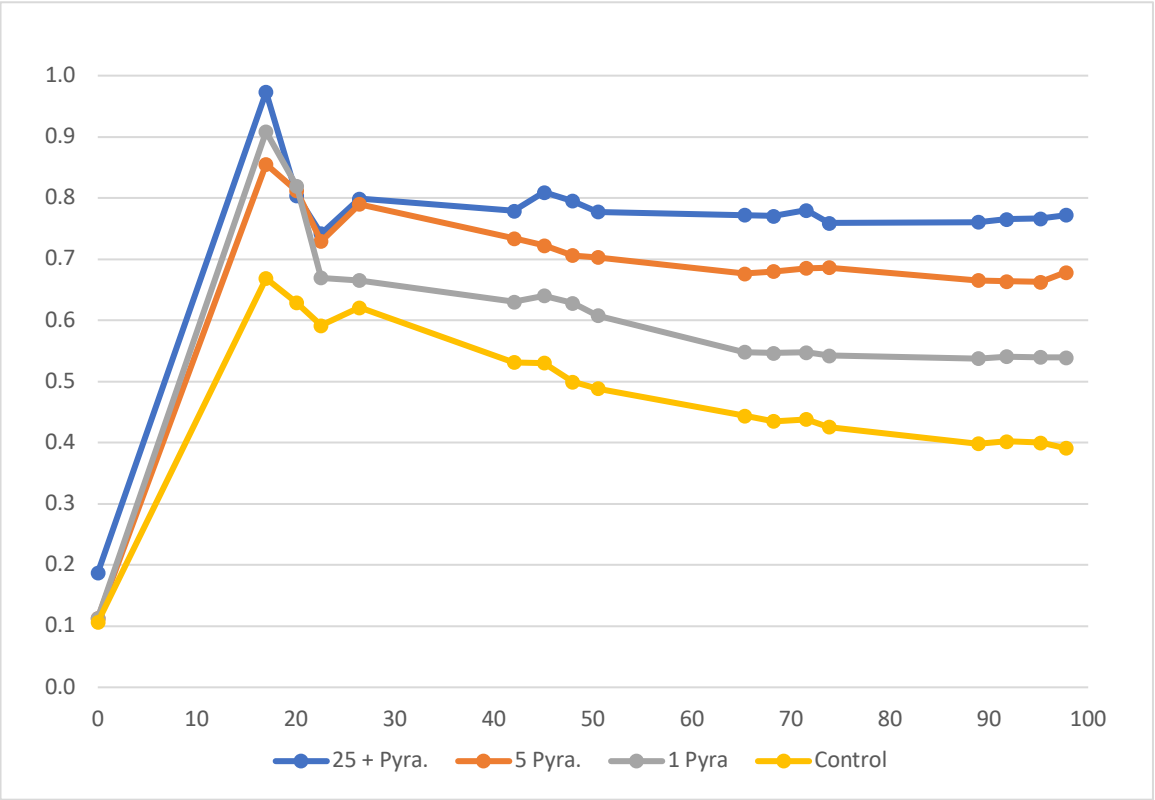


Figure 3.61. The OD curve of isolate 124 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.177	0.116	0.116	0.115	2.2E-08	0.016	0.021	0.028
17.53	0.947	0.825	0.699	0.634	6.3E-09	0.101	0.134	0.175
19.98	0.786	0.658	0.555	0.525	1.9E-07	0.089	0.118	0.154
22.88	0.685	0.557	0.469	0.439	6.0E-07	0.082	0.109	0.143
26.33	0.652	0.535	0.499	0.402	4.4E-06	0.085	0.113	0.147
41.33	0.665	0.575	0.480	0.360	2.3E-06	0.106	0.141	0.183
44.65	0.663	0.583	0.443	0.345	1.4E-06	0.116	0.155	0.201
47.67	0.645	0.573	0.451	0.362	2.7E-07	0.100	0.133	0.173
49.68	0.643	0.573	0.443	0.358	8.3E-06	0.110	0.146	0.191
65.60	0.625	0.560	0.432	0.335	2.5E-08	0.099	0.132	0.172
68.07	0.620	0.554	0.441	0.344	7.5E-07	0.103	0.136	0.178
71.65	0.654	0.544	0.433	0.350	1.5E-06	0.105	0.140	0.182
73.43	0.611	0.550	0.460	0.362	1.4E-05	0.108	0.143	0.187
90.52	0.602	0.547	0.436	0.367	7.1E-05	0.123	0.163	0.213

Table 3.129. The mean ODs at each assessment time for isolate 124 in the presence of three concentrations of pyraclostrobin LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Moreover, in both runs the highest peak was recorded with 25 ppm (Fig. 3.60 and 3.61 respectively) and generally all treatments showed a higher OD value than the one with the control over the course of the experiment. This resulted in the significant enhancement of the

OD value with treatments 5 and 25 ppm from 41.33 hours onwards in the first run (Table 3.129) and with all treatments over the whole experimental time in the second run (Table 3.130).

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0	0.187	0.113	0.113	0.107	9.3E-27	0.008	0.011	0.015
16.97	0.973	0.855	0.908	0.669	2.8E-20	0.106	0.140	0.182
20.07	0.804	0.812	0.819	0.629	1.7E-22	0.087	0.115	0.150
22.53	0.742	0.729	0.669	0.591	4.5E-21	0.086	0.114	0.148
26.42	0.799	0.790	0.665	0.621	3.2E-18	0.095	0.127	0.164
42.03	0.779	0.734	0.630	0.531	1.1E-14	0.100	0.133	0.172
45.05	0.809	0.723	0.640	0.530	1.0E-14	0.098	0.130	0.169
47.95	0.795	0.706	0.628	0.499	2.1E-18	0.083	0.110	0.143
50.48	0.778	0.703	0.608	0.488	1.5E-18	0.081	0.108	0.140
65.30	0.772	0.676	0.548	0.444	3.4E-19	0.078	0.103	0.134
68.23	0.770	0.680	0.547	0.435	5.0E-15	0.092	0.121	0.158
71.53	0.780	0.685	0.548	0.438	6.6E-19	0.077	0.103	0.133
73.82	0.759	0.687	0.542	0.426	5.2E-19	0.078	0.104	0.134
88.88	0.761	0.665	0.538	0.398	1.9E-19	0.078	0.104	0.134
91.78	0.765	0.664	0.541	0.402	2.9E-19	0.080	0.106	0.138
95.20	0.766	0.663	0.540	0.400	1.0E-18	0.082	0.109	0.141
97.75	0.772	0.679	0.539	0.391	3.3E-17	0.087	0.116	0.150

Table 3.130. The mean ODs at each assessment time for isolate 124 in the presence of three concentrations of pyraclostrobin LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Pyra.25	41.20
Pyra.5	39.09
Pyra.1	30.50
Control	23.60
LSD5%	7.37
LSD1%	9.81
LSD0.1%	12.78

Treatment	"Growth"
Pyra.25	54.53
Pyra.5	54.67
Pyra.1	46.29
Control	35.74
LSD5%	6.44
LSD1%	8.55
LSD0.1%	11.09

Table 3.131. and 3.132. The mean "growth" of isolate 124 in treatments with 25, 5, and 1 ppm of pyraclostrobin over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

Accordingly, the "growth" of treatments with all doses was pyraclostrobin was markedly enhanced in both trials with statistical significance at the 0.1% level for the 5 and 25 ppm treatments in both trials and with the 1 ppm treatment at the 1% level in the second trial. It suggests that the "growth" of strain 124 is enhanced in the presence of pyraclostrobin (Table 3.131, 3.132).

The cross-screening test with the isolate 124 and the fluxapyroxad was run once. The growth curve was similar to the one with other fungicides showing an initial increase to a smaller peak compared to the tests with boscalid and pyraclostrobin at 16.97 hours (Fig. 3.62).

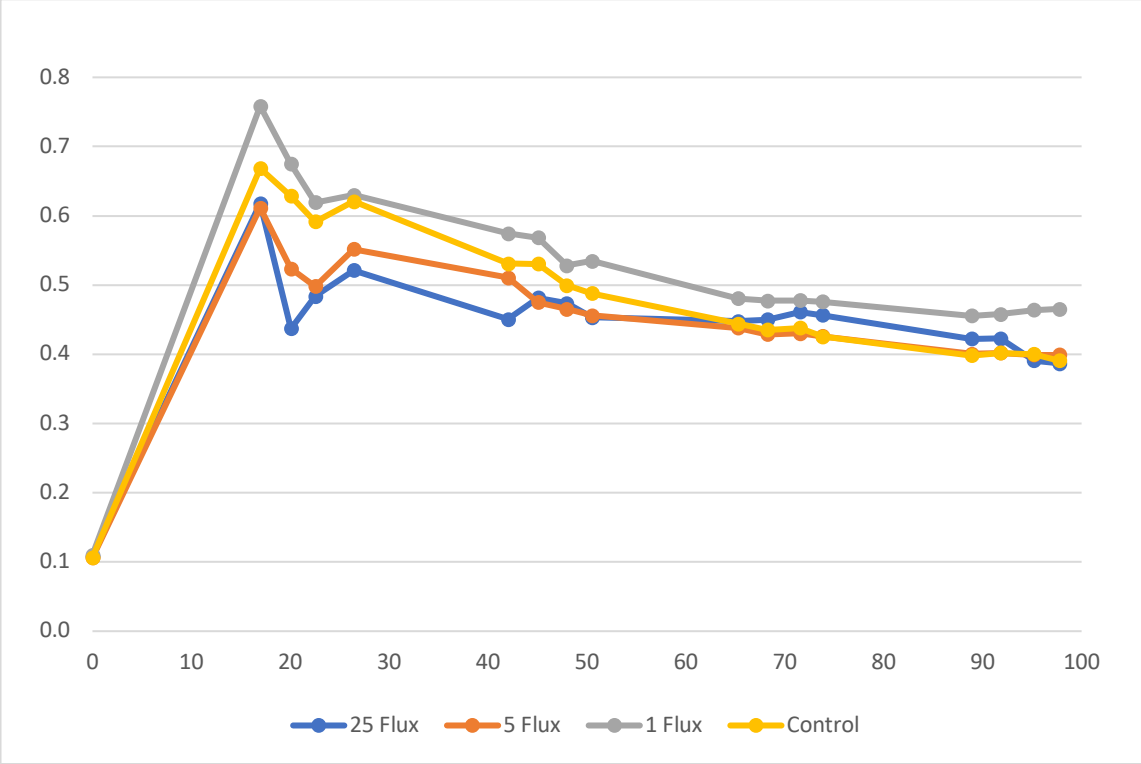


Figure 3.62. The OD curve of isolate 124 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0	0.108	0.106	0.110	0.107	9.3E-27	0.008	0.011	0.015
16.97	0.618	0.612	0.759	0.669	2.8E-20	0.106	0.140	0.182
20.07	0.437	0.524	0.675	0.629	1.7E-22	0.087	0.115	0.150
22.53	0.484	0.498	0.619	0.591	4.5E-21	0.086	0.114	0.148
26.42	0.521	0.552	0.630	0.621	3.2E-18	0.095	0.127	0.164
42.03	0.451	0.511	0.575	0.531	1.1E-14	0.100	0.133	0.172
45.05	0.482	0.475	0.568	0.530	1.0E-14	0.098	0.130	0.169
47.95	0.474	0.466	0.528	0.499	2.1E-18	0.083	0.110	0.143
50.48	0.453	0.456	0.535	0.488	1.5E-18	0.081	0.108	0.140
65.30	0.448	0.438	0.481	0.444	3.4E-19	0.078	0.103	0.134
68.23	0.450	0.429	0.477	0.435	5.0E-15	0.092	0.121	0.158
71.53	0.461	0.430	0.478	0.438	6.6E-19	0.077	0.103	0.133
73.82	0.457	0.426	0.476	0.426	5.2E-19	0.078	0.104	0.134
88.88	0.422	0.401	0.456	0.398	1.9E-19	0.078	0.104	0.134
91.78	0.423	0.402	0.458	0.402	2.9E-19	0.080	0.106	0.138
95.20	0.391	0.399	0.464	0.400	1.0E-18	0.082	0.109	0.141
97.75	0.387	0.399	0.465	0.391	3.3E-17	0.087	0.116	0.150

Table 3.133. The mean ODs at each assessment time for isolate 124 in the presence of three concentrations of fluxapyroxad LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The 1 ppm treatment was the only treatment that achieved a higher peak than the control and also showed a numerically higher OD value compared to the control over the experimental

period. After the peak, the OD value with both the treatments and the control started to decline until 20 to 23 hours. Then, after a short increase the OD started to level off slowly towards the end of the test (Table 3.133).

None of the treatments fluxapyroxad showed significantly greater "growth" than the control but with the 1 ppm treatment which was considerably higher than the control, which suggests that fluxapyroxad did not markedly enhance the growth of strain 124 (Table 134).

Treatment	"Growth"
Flux. 25	32.63
Flux. 5	33.15
Flux. 1	39.59
Control	35.74
LSD5%	6.44
LSD1%	8.55
LSD0.1%	11.09

Table 3.134. The mean "growth" of isolate 124 in treatments with 25, 5, and 1 ppm of fluxapyroxad over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

3.2.4.7. Isolate 180

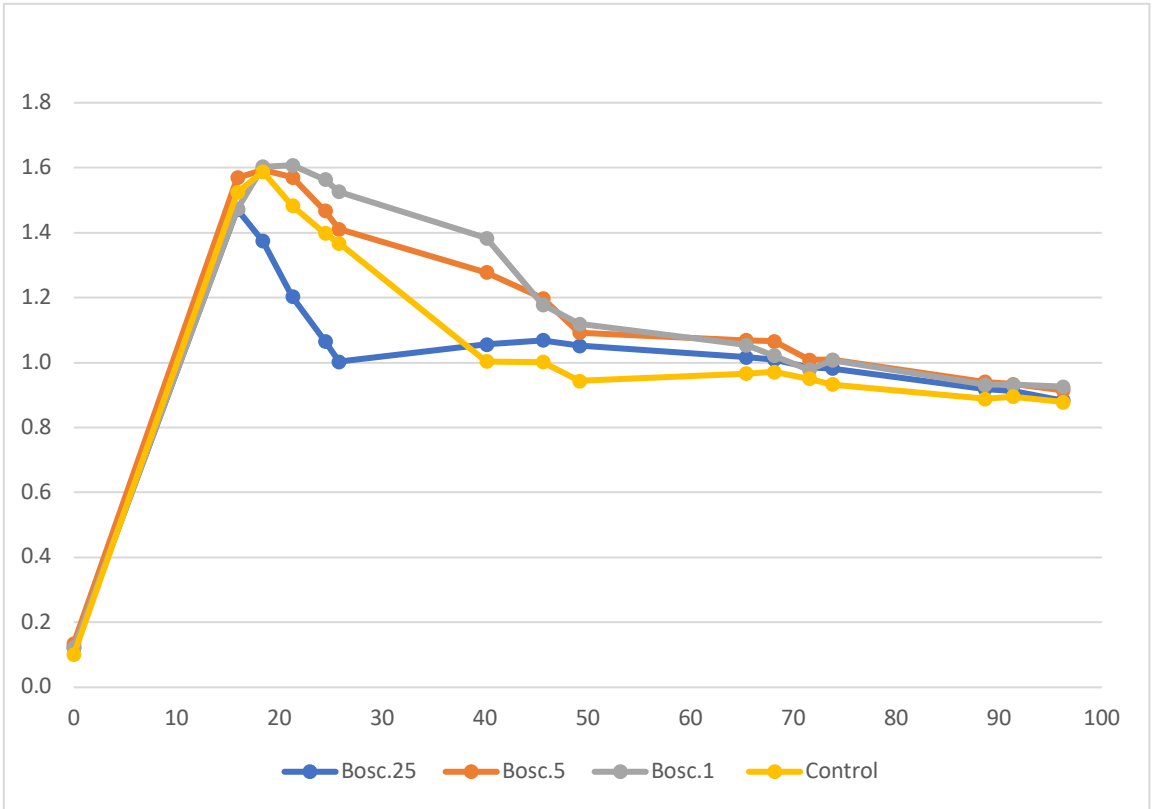


Figure 3.63. The OD curve of isolate 180 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Bosc.25	Bosc.5	Bosc.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.122	0.136	0.117	0.101	9.3E-21	0.007	0.010	0.012
15.92	1.472	1.570	1.509	1.524	8.5E-34	0.091	0.120	0.154
18.38	1.374	1.593	1.504	1.588	4.6E-22	0.162	0.212	0.273
21.27	1.203	1.571	1.386	1.483	9.3E-16	0.213	0.279	0.359
24.45	1.066	1.467	1.335	1.398	8.4E-17	0.193	0.253	0.325
25.78	1.002	1.411	1.309	1.367	8.3E-19	0.166	0.217	0.279
40.13	1.056	1.278	1.186	1.004	2.5E-24	0.109	0.142	0.183
45.62	1.068	1.198	1.097	1.002	1.0E-37	0.056	0.073	0.094
49.17	1.068	1.198	1.097	1.002	5.7E-32	0.063	0.083	0.107
65.40	1.052	1.092	1.033	0.944	1.9E-30	0.068	0.089	0.114
68.10	1.017	1.068	0.973	0.966	8.8E-31	0.064	0.084	0.108
71.53	1.010	1.066	0.977	0.971	1.3E-35	0.049	0.065	0.083
73.78	0.986	1.008	0.935	0.950	4.3E-32	0.058	0.076	0.098
88.62	0.982	1.008	0.935	0.933	3.8E-32	0.052	0.068	0.088
91.35	0.919	0.941	0.892	0.889	8.2E-32	0.054	0.071	0.091
96.18	0.912	0.933	0.871	0.895	8.2E-32	0.052	0.068	0.088

Table 3.135. The mean ODs at each assessment time for isolate 180 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Bosc.25	83.60
Bosc.5	93.61
Bosc.1	95.94
Control	87.29
LSD5%	4.662
LSD1%	6.205
LSD 0.1%	8.082

Table 3.136. The mean "growth" of isolate 180 in treatments with 25, 5, and 1 ppm of boscalid over the four day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

In the cross-screening test with isolate 180 and boscalid indicated a growth curve with maximum OD values observed around 18 hours, then a fall in ODs afterwards (Fig. 3.63). However, earlier peaks within first 18 hours cannot be excluded. With 25 ppm, the OD value having reached a peak at 15.92 hours, declined steeply until 25.78 hours, and then plateaued out until the end of the test. With the control the decline was more gradual resulting in significantly lower OD values with 25 ppm from the peak point until 25.78 hours (Table 3.135). Comparing the decline of the 1 and 5 ppm boscalid treatments and the control, the control showed the steepest fall resulting in statistically greater OD values with 5 and 1 ppm from 40.13 hours to 88.62 and 65.40 hours respectively.

Confirming these observations, the average "growth" of the 1 and 5 ppm boscalid treatments was statistically greater than that of the control at the 0.1% and 1% level respectively suggesting the growth of strain 180 is enhanced in the presence of boscalid (Table 3.136).

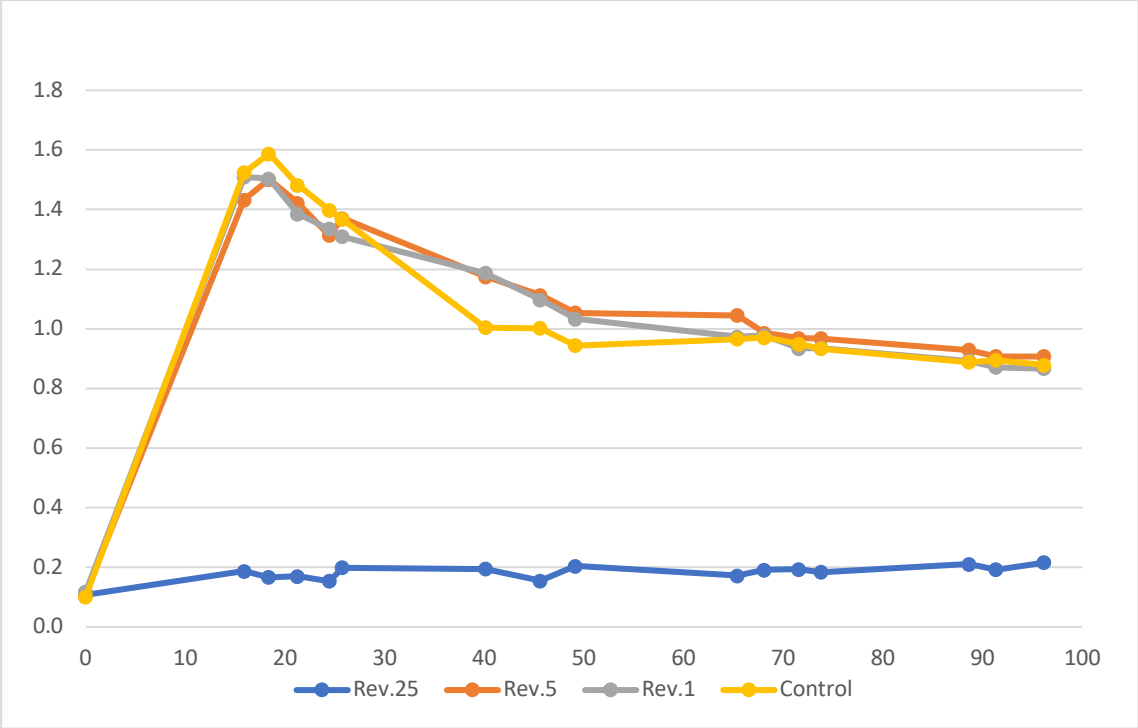


Figure 3.64. The OD curve of isolate 180 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Rev.25	Rev.5	Rev.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.108	0.115	0.117	0.101	9.3E-21	0.007	0.010	0.012
15.92	0.186	1.432	1.509	1.524	8.5E-34	0.091	0.120	0.154
18.38	0.166	1.502	1.504	1.588	4.6E-22	0.162	0.212	0.273
21.27	0.170	1.422	1.386	1.483	9.3E-16	0.213	0.279	0.359
24.45	0.154	1.313	1.335	1.398	8.4E-17	0.193	0.253	0.325
25.78	0.199	1.370	1.309	1.367	8.3E-19	0.166	0.217	0.279
40.13	0.194	1.175	1.186	1.004	2.5E-24	0.109	0.142	0.183
45.62	0.155	1.113	1.097	1.002	1.0E-37	0.056	0.073	0.094
49.17	0.155	1.113	1.097	1.002	5.7E-32	0.063	0.083	0.107
65.40	0.204	1.054	1.033	0.944	1.9E-30	0.068	0.089	0.114
68.10	0.172	1.045	0.973	0.966	8.8E-31	0.064	0.084	0.108
71.53	0.192	0.986	0.977	0.971	1.3E-35	0.049	0.065	0.083
73.78	0.193	0.969	0.935	0.950	4.3E-32	0.058	0.076	0.098
88.62	0.183	0.968	0.935	0.933	3.8E-32	0.052	0.068	0.088
91.35	0.210	0.929	0.892	0.889	8.2E-32	0.054	0.071	0.091
96.18	0.192	0.907	0.871	0.895	8.2E-32	0.052	0.068	0.088

Table 3.137. The mean ODs at each assessment time for isolate 180 in the presence of three concentrations of Revysol® LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance

Treatment	"Growth"
Rev.25	7.15
Rev.5	89.92
Rev.1	88.09
Control	87.29
LSD5%	4.662
LSD1%	6.205
LSD 0.1%	8.082

Table 3.138. The mean "growth" of isolate 180 in treatments with 25, 5, and 1 ppm of Revysol® over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

In the cross-screening test with the isolate 180, the Revysol® treatments led to peak observed OD values for 1 ppm at 15.92 hours and 5 ppm and the control in the next assessment (18.32 hours). After the recoded peak points, the values declined slowly with the treatments until the test was stopped but the control had a steeper decline until 49.17 hours, followed by a plateauing out until the experiment was stopped (Fig. 3.64). This resulted in significant higher ODs with 1 and 5 ppm between 40 and 68 hours compared to the control. The exception with this is the 25 ppm where the OD value barely increased from the value at the start of the experiment, reflected in very significant lower ODs at 0.1% level (Table 3.137).

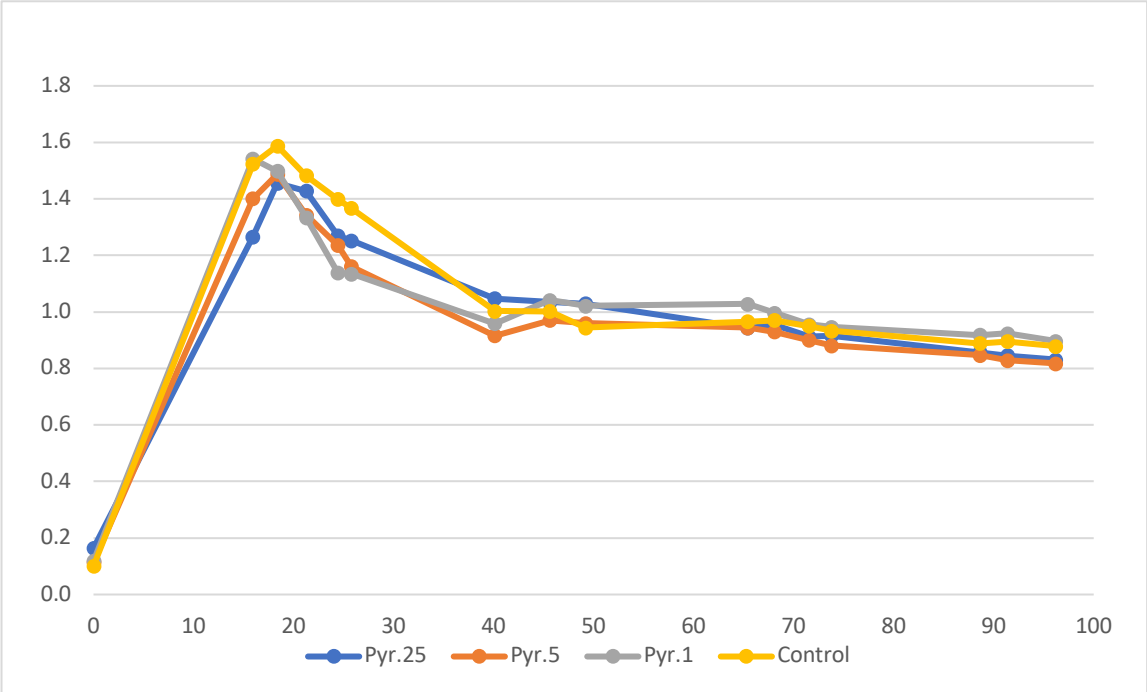


Figure 3.65. The OD curve of isolate 180 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

The average "growth" of the 1 and 5 ppm treatments exceeded that of the control although none of them reached statistical significance. The very significant lower "growth" of the 25 ppm

treatment suggests an inhibitory effect of this dose of Revysol® on the growth of isolate 180 (Table 3.138).

Ass. (h)	Pyra.25	Pyra.5	Pyra.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.164	0.114	0.118	0.101	9.3E-21	0.007	0.010	0.012
15.92	1.266	1.400	1.542	1.524	8.5E-34	0.091	0.120	0.154
18.38	1.456	1.487	1.499	1.588	4.6E-22	0.162	0.212	0.273
21.27	1.428	1.343	1.334	1.483	9.3E-16	0.213	0.279	0.359
24.45	1.270	1.236	1.138	1.398	8.4E-17	0.193	0.253	0.325
25.78	1.253	1.161	1.135	1.367	8.3E-19	0.166	0.217	0.279
40.13	1.047	0.916	0.959	1.004	2.5E-24	0.109	0.142	0.183
45.62	1.035	0.970	1.041	1.002	1.0E-37	0.056	0.073	0.094
49.17	1.035	0.970	1.041	1.002	5.7E-32	0.063	0.083	0.107
65.40	1.029	0.961	1.021	0.944	1.9E-30	0.068	0.089	0.114
68.10	0.943	0.944	1.028	0.966	8.8E-31	0.064	0.084	0.108
71.53	0.955	0.930	0.996	0.971	1.3E-35	0.049	0.065	0.083
73.78	0.912	0.900	0.955	0.950	4.3E-32	0.058	0.076	0.098
88.62	0.916	0.881	0.947	0.933	3.8E-32	0.052	0.068	0.088
91.35	0.857	0.847	0.918	0.889	8.2E-32	0.054	0.071	0.091
96.18	0.846	0.829	0.924	0.895	8.2E-32	0.052	0.068	0.088

Table 3.139. The mean ODs at each assessment time for isolate 180 in the presence of three concentrations of pyraclostrobin LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Pyra.25	78.35
Pyra.5	80.16
Pyra.1	84.89
Control	87.29
LSD5%	4.662
LSD1%	6.205
LSD 0.1%	8.082

Table 3.140. The mean "growth" of isolate 180 in treatments with 25, 5, and 1 ppm of pyraclostrobin over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

The cross-screening test with 180 and the fungicide pyraclostrobin recorded the highest observed OD values between 15 and 18 hours followed by a steep decline until 40.13 hours after which it became more gradual until the test was finished (Fig. 3.65).

Although the OD value trends with the pyraclostrobin treatments over the experimental period was similar to that with the control, the average "growth" of the 25 ppm pyraclostrobin treatment is significantly lower (Table 3.140). This apparent discrepancy is because the initial OD value for this treatment at time zero was quite high, even significantly higher than those of the other treatments and the control and this "base value" is deducted from the ODs recorded

at the other time points during the experiment (table 3.139). Based on the results observed here, the pyraclostrobin treatments tended to lead to lower (but not significantly lower) ODs than the control.

3.2.4.8. Isolate 195

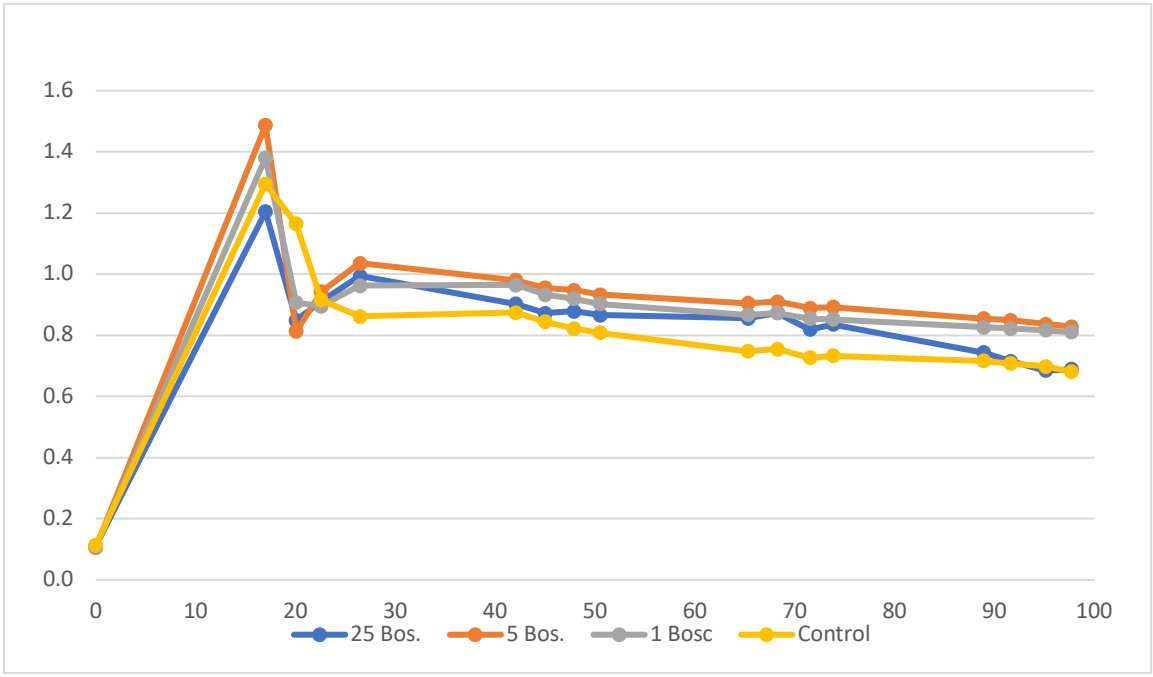


Figure 3.66. The OD curve of isolate 195 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

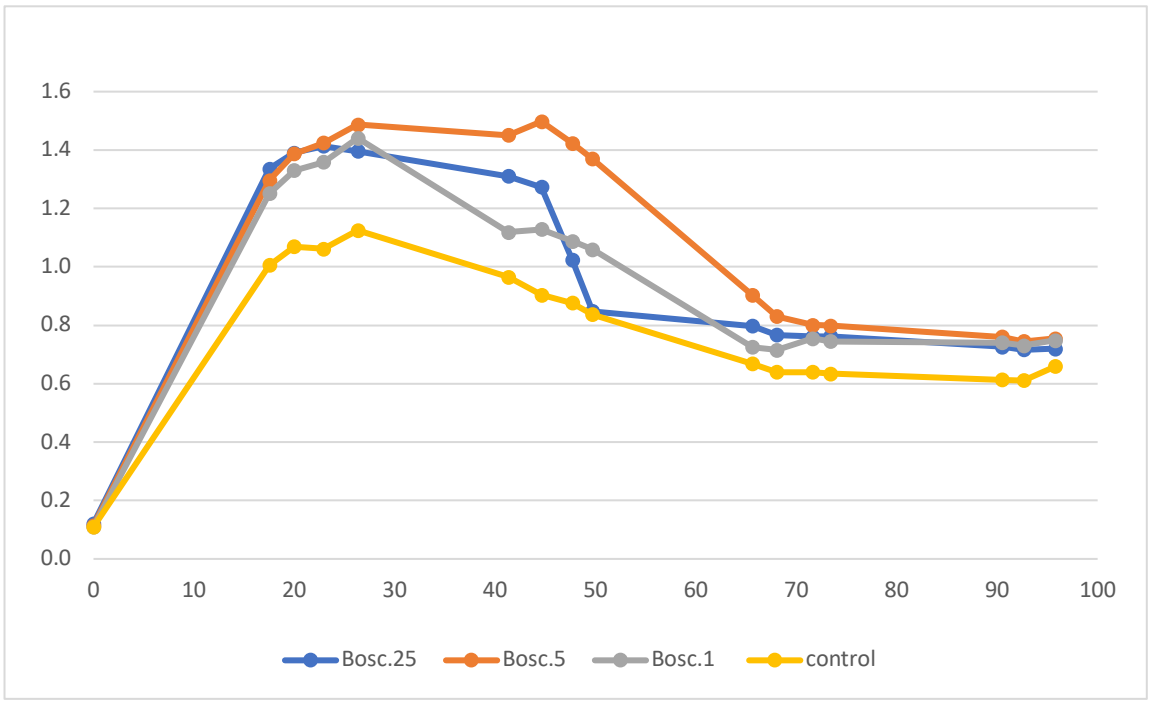


Figure 3.67. The OD curve of isolate 195 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.111	0.105	0.108	0.114	1.5E-21	0.007	0.009	0.011
16.98	1.204	1.487	1.381	1.295	4.9E-08	0.197	0.261	0.338
20.07	0.849	0.813	0.907	1.164	4.7E-06	0.242	0.321	0.416
22.57	0.911	0.943	0.896	0.915	6.4E-02	0.160	0.212	0.275
26.43	0.994	1.035	0.963	0.862	2.9E-02	0.157	0.209	0.271
42.05	0.902	0.980	0.965	0.874	2.3E-04	0.123	0.164	0.212
45.00	0.872	0.955	0.933	0.844	1.7E-04	0.119	0.158	0.204
47.90	0.878	0.948	0.919	0.822	3.2E-01	0.136	0.181	0.235
50.48	0.866	0.933	0.902	0.808	2.1E-03	0.129	0.172	0.222
65.32	0.856	0.905	0.867	0.748	1.0E-03	0.123	0.164	0.212
68.25	0.874	0.910	0.874	0.755	1.7E-03	0.124	0.164	0.213
71.53	0.819	0.890	0.855	0.727	5.4E-04	0.118	0.156	0.202
73.83	0.835	0.892	0.852	0.733	1.7E-03	0.121	0.160	0.208
88.92	0.743	0.855	0.826	0.717	1.6E-05	0.099	0.132	0.171
91.62	0.716	0.850	0.822	0.708	4.0E-06	0.098	0.130	0.169
95.13	0.685	0.837	0.817	0.698	7.5E-06	0.101	0.134	0.174
97.68	0.689	0.828	0.811	0.680	3.3E-05	0.106	0.140	0.182

Table 3.141. The mean ODs at each assessment time for isolate 195 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Bosc.25	Bosc.5	Bosc.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.121	0.115	0.110	0.110	1.4E-21	0.006	0.008	0.011
17.52	1.336	1.296	1.251	1.006	7.2E-17	0.187	0.248	0.324
19.97	1.390	1.387	1.330	1.070	2.1E-16	0.201	0.267	0.348
22.90	1.413	1.425	1.358	1.062	2.6E-13	0.234	0.311	0.406
26.32	1.396	1.487	1.441	1.125	8.2E-11	0.273	0.364	0.473
41.32	1.311	1.451	1.118	0.965	8.2E-07	0.334	0.444	0.578
44.62	1.274	1.498	1.129	0.904	3.3E-06	0.346	0.460	0.599
47.67	1.024	1.424	1.088	0.877	1.9E-06	0.320	0.426	0.555
49.68	0.848	1.370	1.060	0.837	1.3E-07	0.269	0.358	0.466
65.63	0.798	0.903	0.725	0.668	9.4E-15	0.111	0.148	0.193
68.05	0.767	0.830	0.715	0.640	1.2E-16	0.095	0.126	0.164
71.63	0.762	0.801	0.754	0.639	1.1E-16	0.089	0.118	0.154
73.42	0.762	0.798	0.745	0.635	2.7E-15	0.093	0.124	0.161
90.50	0.727	0.761	0.740	0.614	1.4E-17	0.078	0.104	0.135
92.63	0.717	0.745	0.731	0.611	1.1E-16	0.082	0.110	0.143
95.78	0.720	0.754	0.749	0.659	1.1E-16	0.053	0.071	0.092

Table 3.142. The mean ODs at each assessment time for isolate 195 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The cross-screening test with 195 and fungicide boscalid was run on 2 occasions. In the first trial, a maximum observed peak OD value with all treatments and the control was recorded in day one at 20.07 hours where the treatment with boscalid at 5 ppm achieved the highest peak (1.4) and then with all treatments the OD value started a steep decline until 20.07 hours (Fig. 3.66). Thereafter, with the treatments the OD value slightly increased again while it was

still decreasing with the control until 26.43 hours. This resulted in statistically significant differences with the 5ppm boscalid treatment at 26.43 hours and from 65.63 onwards (Table 3.141). The ODs with all treatments and the control continued a slight decline from 26.47 until the test was stopped.

In the second trial, the growth curve started with an initial increase until 26.32 where the peak OD values were reached. As in the first run, here also the treatment with 5ppm achieved the highest peak (1.4) followed by a decline until 71.63 where the ODs started to level off until the test was stopped (Fig. 3.67). With the control the OD value was significantly lower than that of all the boscalid treatments especially with 5 ppm (Table 3.142)

Treatment	"Growth"
Bosc. 25	69.69
Bosc. 5	78.43
Bosc. 1	74.70
Control	66.23
LSD5%	11.45
LSD1%	15.20
LSD 0.1%	19.71

Treatment	"Growth"
Bosc.25	79.02
Bosc.5	89.78
Bosc.1	78.27
Control	62.72
LSD5%	12.894
LSD1%	17.163
LSD 0.1%	22.353

Table 3.143. and 3.144. The mean "growth" of isolate 195 in treatments with 25, 5, and 1 ppm of boscalid over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

Confirming the results, the average "growth" of treatments in both trials was higher than that of control, although this difference didn't reach the statistical significance with 1 and 25 ppm in the first trial. Nevertheless, the experiments suggest that the growth of strain 195 is enhanced with the presence of boscalid (Table 3.143 and 3.144 respectively).

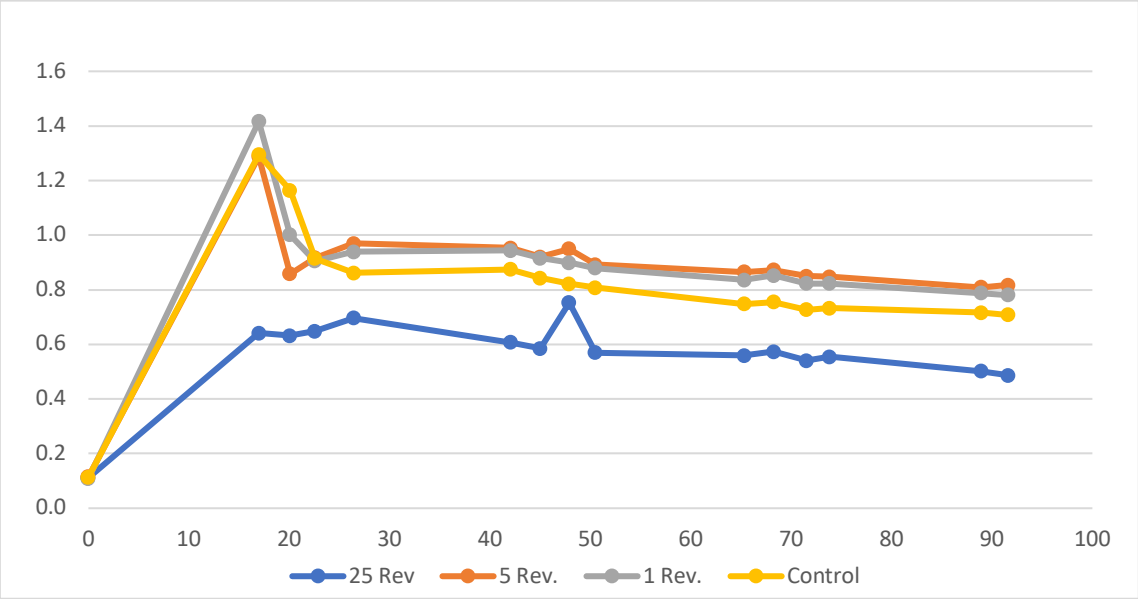


Figure 3.68. The OD curve of isolate 195 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

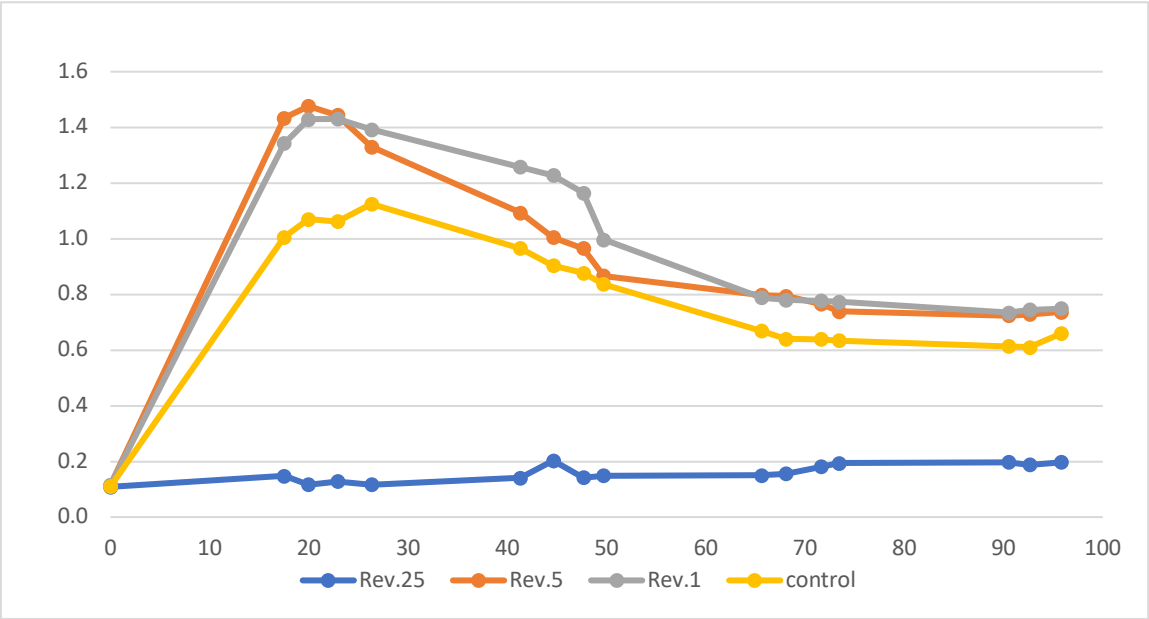


Figure 3.69. The OD curve of isolate 195 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.112	0.115	0.108	0.114	1.5E-21	0.007	0.009	0.011
16.98	0.641	1.288	1.416	1.295	4.9E-08	0.197	0.261	0.338
20.07	0.632	0.859	1.001	1.164	4.7E-06	0.242	0.321	0.416
22.57	0.648	0.916	0.905	0.915	6.4E-02	0.160	0.212	0.275
26.43	0.696	0.970	0.939	0.862	2.9E-02	0.157	0.209	0.271
42.05	0.608	0.953	0.943	0.874	2.3E-04	0.123	0.164	0.212
45.00	0.586	0.921	0.916	0.844	1.7E-04	0.119	0.158	0.204
47.90	0.753	0.950	0.900	0.822	3.2E-01	0.136	0.181	0.235
50.48	0.570	0.893	0.880	0.808	2.1E-03	0.129	0.172	0.222
65.32	0.559	0.866	0.837	0.748	1.0E-03	0.123	0.164	0.212
68.25	0.573	0.872	0.852	0.755	1.7E-03	0.124	0.164	0.213
71.53	0.541	0.850	0.823	0.727	5.4E-04	0.118	0.156	0.202
73.83	0.555	0.848	0.823	0.733	1.7E-03	0.121	0.160	0.208
88.92	0.502	0.809	0.787	0.717	1.6E-05	0.099	0.132	0.171
91.62	0.486	0.817	0.781	0.708	4.0E-06	0.098	0.130	0.169
95.13	0.472	0.785	0.770	0.698	7.5E-06	0.101	0.134	0.174
97.68	0.469	0.777	0.764	0.680	3.3E-05	0.106	0.140	0.182

Table 3.144. The mean ODs at each assessment time for isolate 195 in the presence of three concentrations of Revysol® LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The cross-screening test with the isolate 195 and fungicide Revysol® was run twice. In both trials the OD values with 25 ppm were significantly lower (in the second run the OD barely increased beyond the initial value at the start of the experiment) showing the inhibitory effect of 25 ppm on the growth of strain 195.

Ass. (h)	Rev.25	Rev.5	Rev.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.109	0.116	0.115	0.110	1.4E-21	0.006	0.008	0.011
17.52	0.148	1.434	1.343	1.006	7.2E-17	0.187	0.248	0.324
19.97	0.117	1.476	1.429	1.070	2.1E-16	0.201	0.267	0.348
22.90	0.128	1.444	1.431	1.062	2.6E-13	0.234	0.311	0.406
26.32	0.117	1.330	1.393	1.125	8.2E-11	0.273	0.364	0.473
41.32	0.141	1.093	1.257	0.965	8.2E-07	0.334	0.444	0.578
44.62	0.203	1.005	1.227	0.904	3.3E-06	0.346	0.460	0.599
47.67	0.142	0.965	1.164	0.877	1.9E-06	0.320	0.426	0.555
49.68	0.149	0.867	0.996	0.837	1.3E-07	0.269	0.358	0.466
65.63	0.150	0.797	0.789	0.668	9.4E-15	0.111	0.148	0.193
68.05	0.156	0.794	0.781	0.640	1.2E-16	0.095	0.126	0.164
71.63	0.181	0.766	0.777	0.639	1.1E-16	0.089	0.118	0.154
73.42	0.193	0.738	0.774	0.635	2.7E-15	0.093	0.124	0.161
90.50	0.197	0.724	0.734	0.614	1.4E-17	0.078	0.104	0.135
92.63	0.188	0.728	0.745	0.611	1.1E-16	0.082	0.110	0.143
95.78	0.198	0.737	0.749	0.659	1.1E-16	0.053	0.071	0.092

Table 3.145. The mean ODs at each assessment time for isolate 195 in the presence of three concentrations of Revysol® LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Rev. 25	42.54
Rev. 5	72.58
Rev. 1	73.20
Control	66.23
LSD5%	11.45
LSD1%	15.20
LSD0.1%	19.71

Treatment	"Growth"
Rev. 25	4.29
Rev. 5	77.25
Rev. 1	81.04
Control	62.72
LSD5%	12.894
LSD1%	17.163
LSD0.1%	22.353

Table 3.146. and 3.147. The mean "growth" of isolate 195 in treatments with 25, 5, and 1 ppm of Revysol® over the four day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

In the first trial, maximum observed OD values were recorded at 16.98 hours with the highest recorded peak with 1 ppm (1.4) followed by a steep decline until 26.43 hours where the ODs started to plateau until the test was stopped (Fig. 3.68, Table 3.144).

In the second run, with treatments with 1 and 5 ppm, the OD values were higher than the those with the control. The ODs of the Revysol® treatments reached peak values at 19.97 hours with the highest recorded value with 5 ppm (1.4). With the control the peak was achieved later at 26.32 hours (1.1). After the peak, the ODs started a gradual decline which continued until levelling off from 65.63 hours onwards (Fig. 3.69, Table 3.145).

Considering the "growth" parameter, the increase with 1 and 5 ppm in both trials numerically in the first (Table 3.146) and statistically significant in the second (Table 3.147) suggests the enhanced growth of the Isolate 195 in the presence of Revysol® at 1 and 5 ppm.

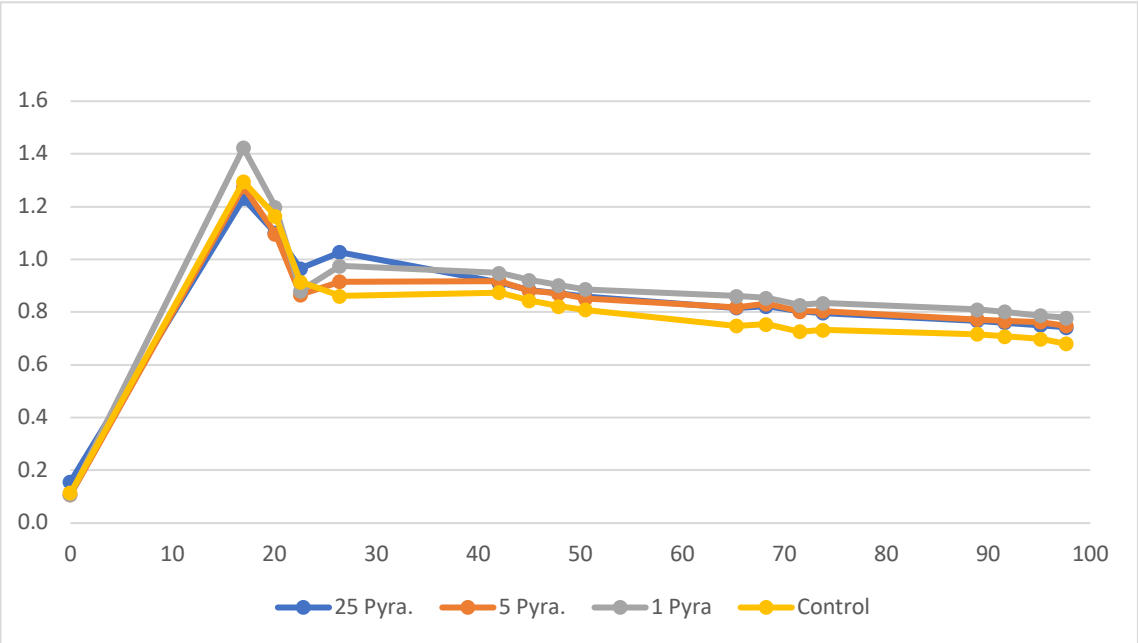


Figure 3.70. The OD curve of isolate 195 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis pyraclostrobin.

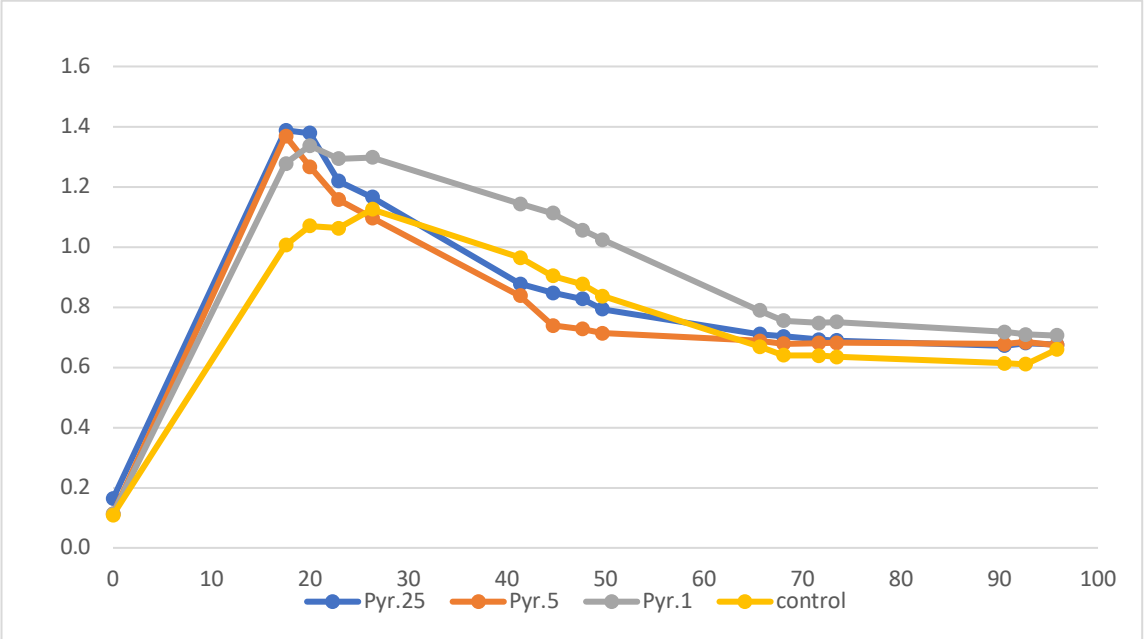


Figure 3.71. The OD curve of isolate 195 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis pyraclostrobin.

In the first cross-screening test with the isolate 195 and the fungicide pyraclostrobin. after an initial increase the OD values reached a peak at 16.98 followed by a steep decline in all treatments with and without the fungicide. Then, the ODs started to level off at 26.43 hours. Throughout the experiment the ODs of the pyraclostrobin treatments tended to be higher than those of the control although statistically significant differences were not observed (Fig. 3.70, Table 3.148).

Ass. (h)	Pyra. 25	Pyra. 5	Pyra 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.157	0.107	0.108	0.114	1.5E-21	0.007	0.009	0.011
16.98	1.231	1.276	1.423	1.295	4.9E-08	0.197	0.261	0.338
20.07	1.101	1.096	1.199	1.164	4.7E-06	0.242	0.321	0.416
22.57	0.966	0.867	0.881	0.915	6.4E-02	0.160	0.212	0.275
26.43	1.028	0.915	0.975	0.862	2.9E-02	0.157	0.209	0.271
42.05	0.913	0.918	0.948	0.874	2.3E-04	0.123	0.164	0.212
45.00	0.884	0.882	0.921	0.844	1.7E-04	0.119	0.158	0.204
47.90	0.872	0.871	0.902	0.822	3.2E-01	0.136	0.181	0.235
50.48	0.858	0.851	0.887	0.808	2.1E-03	0.129	0.172	0.222
65.32	0.816	0.819	0.862	0.748	1.0E-03	0.123	0.164	0.212
68.25	0.822	0.832	0.853	0.755	1.7E-03	0.124	0.164	0.213
71.53	0.804	0.802	0.828	0.727	5.4E-04	0.118	0.156	0.202
73.83	0.797	0.804	0.835	0.733	1.7E-03	0.121	0.160	0.208
88.92	0.767	0.773	0.810	0.717	1.6E-05	0.099	0.132	0.171
91.62	0.760	0.766	0.801	0.708	4.0E-06	0.098	0.130	0.169
95.13	0.751	0.763	0.787	0.698	7.5E-06	0.101	0.134	0.174
97.68	0.742	0.749	0.778	0.680	3.3E-05	0.106	0.140	0.182

Table 3.148. The mean ODs at each assessment time for isolate 195 in the presence of three concentrations of pyraclostrobin LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.164	0.113	0.114	0.110	1.4E-21	0.006	0.008	0.011
17.52	1.388	1.369	1.278	1.006	7.2E-17	0.187	0.248	0.324
19.97	1.379	1.267	1.337	1.070	2.1E-16	0.201	0.267	0.348
22.90	1.219	1.157	1.293	1.062	2.6E-13	0.234	0.311	0.406
26.32	1.165	1.097	1.298	1.125	8.2E-11	0.273	0.364	0.473
41.32	0.878	0.838	1.144	0.965	8.2E-07	0.334	0.444	0.578
44.62	0.848	0.739	1.112	0.904	3.3E-06	0.346	0.460	0.599
47.67	0.827	0.728	1.057	0.877	1.9E-06	0.320	0.426	0.555
49.68	0.794	0.713	1.025	0.837	1.3E-07	0.269	0.358	0.466
65.63	0.710	0.688	0.789	0.668	9.4E-15	0.111	0.148	0.193
68.05	0.703	0.678	0.755	0.640	1.2E-16	0.095	0.126	0.164
71.63	0.692	0.681	0.748	0.639	1.1E-16	0.089	0.118	0.154
73.42	0.689	0.682	0.751	0.635	2.7E-15	0.093	0.124	0.161
90.50	0.672	0.677	0.718	0.614	1.4E-17	0.078	0.104	0.135
92.63	0.680	0.684	0.709	0.611	1.1E-16	0.082	0.110	0.143
95.78	0.675	0.674	0.706	0.659	1.1E-16	0.053	0.071	0.092

Table 3.149. The mean ODs at each assessment time for isolate 195 in the presence of three concentrations of pyraclostrobin LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In the second run, with the pyraclostrobin treatments the ODs increased more quickly and showed higher peak values (around 17 to 20 hours) than the one with control (at 26.32 hours) and so the decline also began earlier and was steeper until 65.63 where the OD values with all treatments and the control started to level off (Fig. 3.71).

Treatment	"Growth"
Pyra. 25	66.79
Pyra. 5	70.47
Pyra. 1	74.92
Control	66.23
LSD5%	11.45
LSD1%	15.20
LSD0.1%	19.71

Treatment	"Growth"
Pyra. 25	64.19
Pyra. 5	65.28
Pyra. 1	76.87
Control	62.72
LSD5%	12.894
LSD1%	17.163
LSD0.1%	22.353

Table 3.150. and 3.151. The mean "growth" of isolate 195 in treatments with 25, 5, and 1 ppm of pyraclostrobin over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

The OD values with the treatments were significantly greater within first 20 hours and then with 5 and 25 ppm were numerically lower during the period of decline whereas, with 1 ppm the decrease was more gradual resulting in significant differences compared to the control until the test was stopped (Table 3.149).

In both trials higher "growth" of the pyraclostrobin treatments compared to the control were observed and at 1ppm in the second trial, the difference was statistically significant (Table 3.150 and 151 respectively). The results suggest that the growth of isolate 195 is enhanced in the presence of pyraclostrobin.

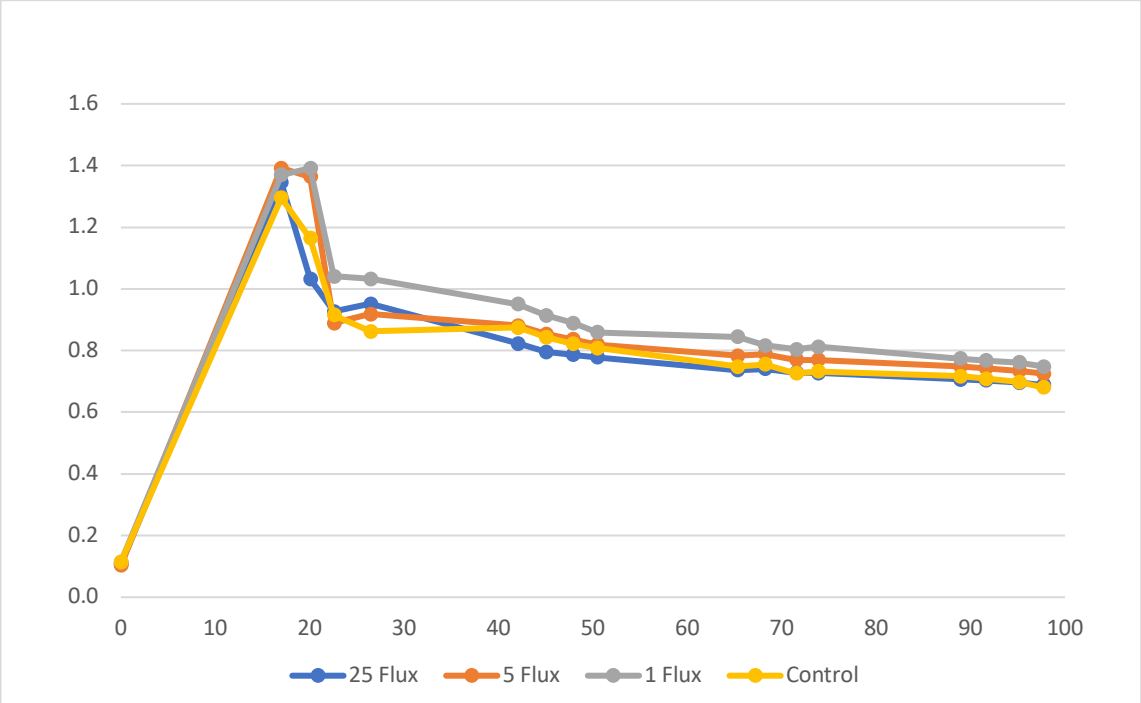


Figure 3.72. The OD curve of isolate 195 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.107	0.103	0.115	0.114	1.5E-21	0.007	0.009	0.011
16.98	1.345	1.392	1.370	1.295	4.9E-08	0.197	0.261	0.338
20.07	1.033	1.364	1.391	1.164	4.7E-06	0.242	0.321	0.416
22.57	0.926	0.889	1.041	0.915	6.4E-02	0.160	0.212	0.275
26.43	0.952	0.919	1.032	0.862	2.9E-02	0.157	0.209	0.271
42.05	0.822	0.880	0.951	0.874	2.3E-04	0.123	0.164	0.212
45.00	0.795	0.854	0.914	0.844	1.7E-04	0.119	0.158	0.204
47.90	0.786	0.836	0.889	0.822	3.2E-01	0.136	0.181	0.235
50.48	0.778	0.820	0.859	0.808	2.1E-03	0.129	0.172	0.222
65.32	0.736	0.783	0.844	0.748	1.0E-03	0.123	0.164	0.212
68.25	0.741	0.789	0.816	0.755	1.7E-03	0.124	0.164	0.213
71.53	0.728	0.770	0.804	0.727	5.4E-04	0.118	0.156	0.202
73.83	0.727	0.770	0.812	0.733	1.7E-03	0.121	0.160	0.208
88.92	0.707	0.749	0.774	0.717	1.6E-05	0.099	0.132	0.171
91.62	0.703	0.743	0.768	0.708	4.0E-06	0.098	0.130	0.169
95.13	0.696	0.734	0.761	0.698	7.5E-06	0.101	0.134	0.174
97.68	0.689	0.725	0.748	0.680	3.3E-05	0.106	0.140	0.182

Table 3.152. The mean ODs at each assessment time for isolate 195 in the presence of three concentrations of fluxapyroxad LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Flux. 25	66.56
Flux. 5	70.82
Flux. 1	74.07
Control	66.23
LSD5%	11.45
LSD1%	15.20
LSD0.1%	19.71

Table 3.153. The mean "growth" of isolate 195 in treatments with 25, 5, and 1 ppm of fluxapyroxad over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

In the cross-screening test with the isolate 195 and the fungicide fluxapyroxad, after an initial increase the OD values reached a peak at 16.98 hours followed by a steep decline. Then, the ODs started to level off at 22.57 hours (Fig. 3.72). Throughout the experiment the ODs of the 1 and 5 ppm fluxapyroxad treatments tended to be higher than those of the control although statistically significant differences were not observed (Table 3.152). These two treatments also showed higher "growth" values than the control although the differences were not statistically significant (Table 3.153).

3.2.4.9. Isolate 196

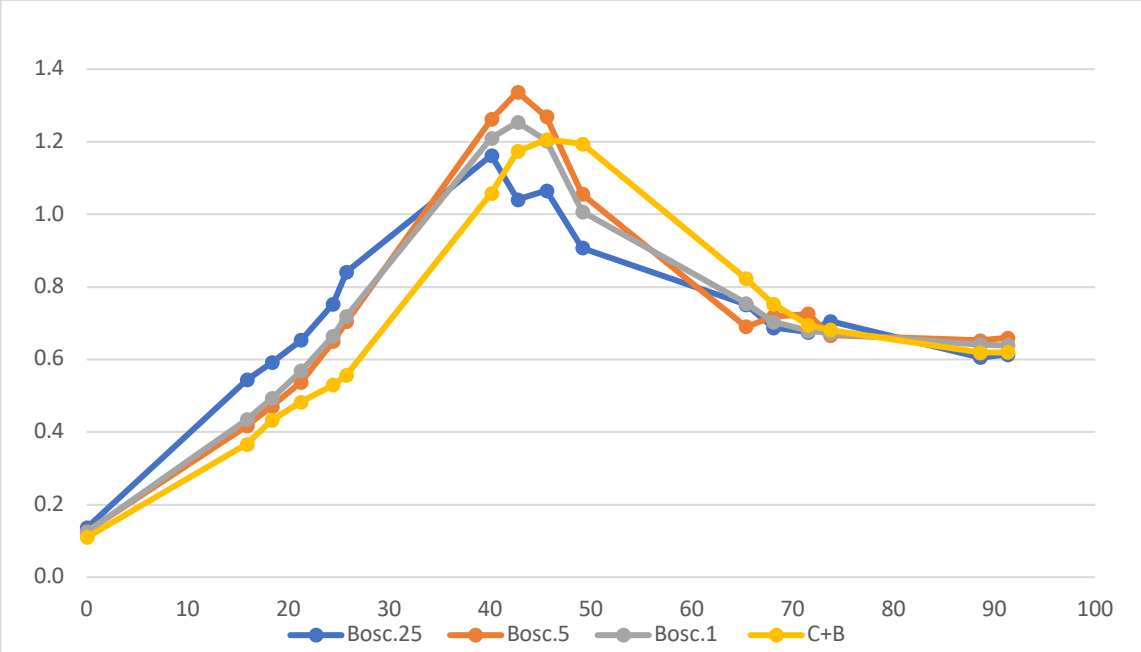


Figure 3.73. The OD curve of isolate 196 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Bosc.25	Bosc.5	Bosc.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.136	0.126	0.124	0.110	3.5E-20	0.006	0.007	0.010
15.88	0.544	0.417	0.435	0.367	4.8E-09	0.084	0.110	0.142
18.37	0.592	0.471	0.492	0.433	1.4E-07	0.103	0.136	0.174
21.25	0.653	0.538	0.568	0.483	1.8E-08	0.121	0.158	0.204
24.42	0.753	0.649	0.665	0.530	6.5E-08	0.149	0.195	0.250
25.77	0.842	0.704	0.719	0.557	2.1E-12	0.128	0.168	0.216
40.13	1.162	1.262	1.210	1.058	2.6E-02	0.250	0.328	0.422
42.75	1.040	1.337	1.254	1.174	8.5E-06	0.218	0.285	0.367
45.58	1.040	1.337	1.254	1.174	1.1E-02	0.273	0.358	0.461
49.15	1.065	1.269	1.203	1.206	1.4E-06	0.212	0.278	0.358
65.38	0.907	1.056	1.007	1.194	6.1E-03	0.195	0.256	0.329
68.07	0.751	0.690	0.755	0.823	8.3E-03	0.148	0.194	0.250
71.52	0.688	0.718	0.704	0.753	1.7E-03	0.122	0.160	0.206
73.72	0.675	0.725	0.679	0.695	1.3E-03	0.118	0.155	0.199
88.60	0.706	0.667	0.673	0.681	2.4E-03	0.106	0.139	0.179
91.33	0.606	0.652	0.641	0.619	2.4E-03	0.110	0.144	0.185

Table 3.154. The mean ODs at each assessment time for isolate 196 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In the cross-screening test with the isolate 196 and treatments with the fungicide boscalid after an initial increase the OD values reached a peak with 25 ppm boscalid at 40.13 hours, in the next assessment with the 1 and 5 ppm at 52.75 hours, and in the assessment afterwards with the

control at 45.58 hours. A steep decline followed before "levelling out" from around 70 hours until the test was finished (Fig. 3.73). The peak values with the boscalid treatments were reached earlier than the control and this led to significantly higher OD values with 25 ppm up to 25.77 hours (Table 3.154). The earliest onset of the steep decline occurring with 25 ppm boscalid resulted in numerically lower OD values, The average "growth" of isolate showed no appreciable differences between the control and treatments (Table 3.155).

Treatment	"Growth"
Bosc.25	53.96
Bosc.5	55.17
Bosc.1	54.80
Control	54.35
LSD5%	9.069
LSD1%	12.071
LSD 0.1%	15.722

Table 3.155. The mean "growth" of isolate 196 in treatments with 25, 5, and 1 ppm of boscalid over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

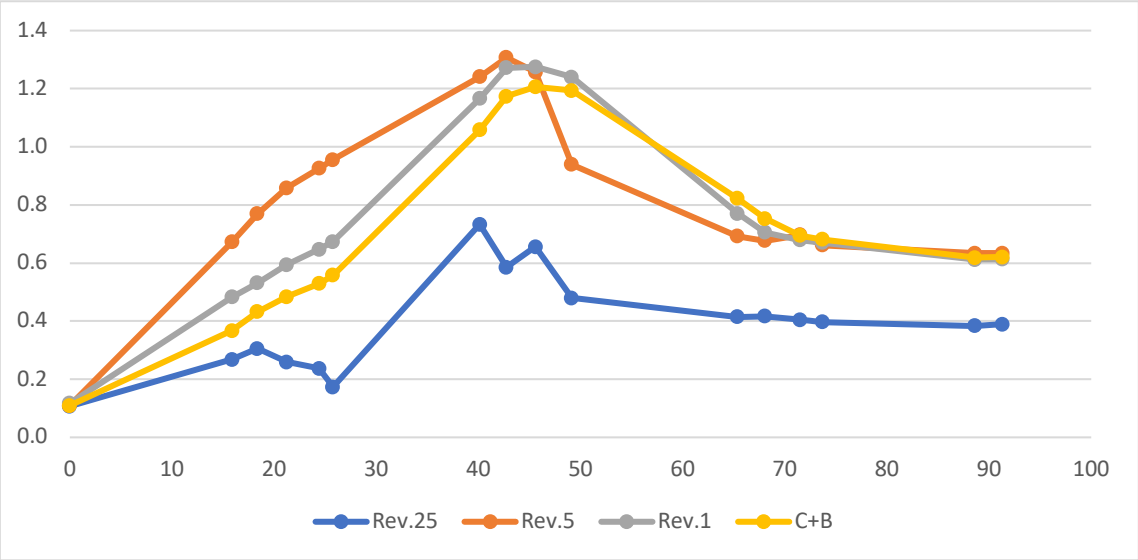


Figure 3.74. The OD curve of isolate 196 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

In the cross-screening test with the isolate 196 and the fungicide Revysol® the OD values reached a peak between 42 and 46 hours with the highest recorded peak with the 5 ppm treatment reflected in statistically significant values during the first 25.77 hours at the 0.1 % level. The 25 ppm Revysol® treatment showed the lowest peak OD (0.7) occurring at 40.13 hours (Fig. 3.75). That this treatment also had much lower ODs than the other treatments and the control is reflected in statistically significantly lower ODs throughout the whole experiment

(Table 3.156). After reaching the peak, OD values started to decline before levelling out at around 70 hours until the test was stopped.

Ass. (h)	Rev.25	Rev.5	Rev.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.107	0.110	0.117	0.110	3.5E-20	0.006	0.007	0.010
15.88	0.268	0.673	0.483	0.367	4.8E-09	0.084	0.110	0.142
18.37	0.306	0.769	0.531	0.433	1.4E-07	0.103	0.136	0.174
21.25	0.259	0.858	0.594	0.483	1.8E-08	0.121	0.158	0.204
24.42	0.237	0.926	0.647	0.530	6.5E-08	0.149	0.195	0.250
25.77	0.173	0.956	0.673	0.557	2.1E-12	0.128	0.168	0.216
40.13	0.733	1.241	1.166	1.058	2.6E-02	0.250	0.328	0.422
42.75	0.585	1.307	1.272	1.174	8.5E-06	0.218	0.285	0.367
45.58	0.585	1.307	1.272	1.174	1.1E-02	0.273	0.358	0.461
49.15	0.656	1.257	1.276	1.206	1.4E-06	0.212	0.278	0.358
65.38	0.480	0.939	1.240	1.194	6.1E-03	0.195	0.256	0.329
68.07	0.415	0.693	0.770	0.823	8.3E-03	0.148	0.194	0.250
71.52	0.416	0.677	0.706	0.753	1.7E-03	0.122	0.160	0.206
73.72	0.404	0.697	0.679	0.695	1.3E-03	0.118	0.155	0.199
88.60	0.397	0.662	0.670	0.681	2.4E-03	0.106	0.139	0.179
91.33	0.384	0.634	0.612	0.619	2.4E-03	0.110	0.144	0.185

Table 3.156. The mean ODs at each assessment time for isolate 196 in the presence of three concentrations of Revysol® LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The "growth" values recorded for 1 and 5 ppm treatments were greater than the control although not quite statistically significant at the 5% level, providing some evidence that the growth of strain 196 in the presence of 1 and 5 ppm Revysol® was enhanced (Table 3.157). Once again, the 25ppm Revysol® treatment inhibited the growth of this isolate.

Treatment	"Growth"
Rev.25	25.32
Rev.5	61.39
Rev.1	57.69
Control	54.35
LSD5%	9.069
LSD1%	12.071
LSD 0.1%	15.722

Table 3.157. The mean "growth" of isolate 196 in treatments with 25, 5, and 1 ppm of Revysol® over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

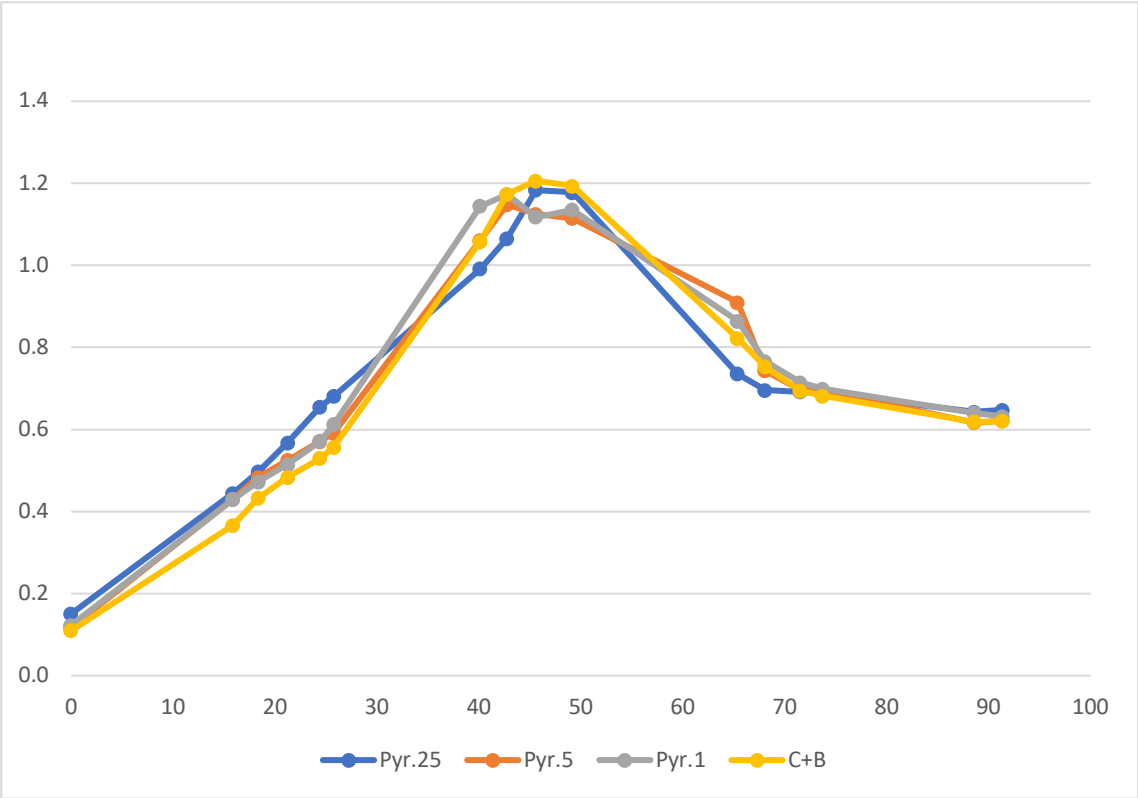


Figure 3.75. The OD curve of isolate 196 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Pyra.25	Pyra.5	Pyra.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.151	0.117	0.123	0.110	3.5E-20	0.006	0.007	0.010
15.88	0.445	0.431	0.430	0.367	4.8E-09	0.084	0.110	0.142
18.37	0.497	0.484	0.473	0.433	1.4E-07	0.103	0.136	0.174
21.25	0.568	0.525	0.515	0.483	1.8E-08	0.121	0.158	0.204
24.42	0.655	0.571	0.572	0.530	6.5E-08	0.149	0.195	0.250
25.77	0.681	0.591	0.613	0.557	2.1E-12	0.128	0.168	0.216
40.13	0.991	1.061	1.145	1.058	2.6E-02	0.250	0.328	0.422
42.75	1.066	1.148	1.173	1.174	8.5E-06	0.218	0.285	0.367
45.58	1.066	1.148	1.173	1.174	1.1E-02	0.273	0.358	0.461
49.15	1.183	1.125	1.118	1.206	1.4E-06	0.212	0.278	0.358
65.38	1.178	1.115	1.135	1.194	6.1E-03	0.195	0.256	0.329
68.07	0.736	0.910	0.865	0.823	8.3E-03	0.148	0.194	0.250
71.52	0.696	0.744	0.766	0.753	1.7E-03	0.122	0.160	0.206
73.72	0.692	0.696	0.714	0.695	1.3E-03	0.118	0.155	0.199
88.60	0.689	0.695	0.699	0.681	2.4E-03	0.106	0.139	0.179
91.33	0.643	0.617	0.641	0.619	2.4E-03	0.110	0.144	0.185

Table 3.158. The mean ODs at each assessment time for isolate 196 in the presence of three concentrations of pyraclostrobin LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Through the cross-screening with the fungicide pyraclostrobin, the ODs of the isolate 196 were more or less similar in the control and treatments, reaching the maxmimum observed OD value between 42.75 and 45.58 hours followed by a fall in ODs until 70 hours or so when there was

a “levelling out” of the OD values until the experiment was stopped (Fig. 3.75). This was reflected in the similar average “growth” of strain in the pyraclostrobin treatments and the control suggesting that there was no effect of pyraclostrobin over the experimental period (Table 3.158, 3.159).

Treatment	“Growth”
Pyra.25	51.63
Pyra.5	54.83
Pyra.1	55.37
Control	54.35
LSD5%	9.069
LSD1%	12.071
LSD 0.1%	15.722

Table 3.159. The mean “growth” of isolate 196 in treatments with 25, 5, and 1 ppm of pyraclostrobin over the four-day experimental period. Significant increases in “growth” compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

3.2.4.10. Isolate 201

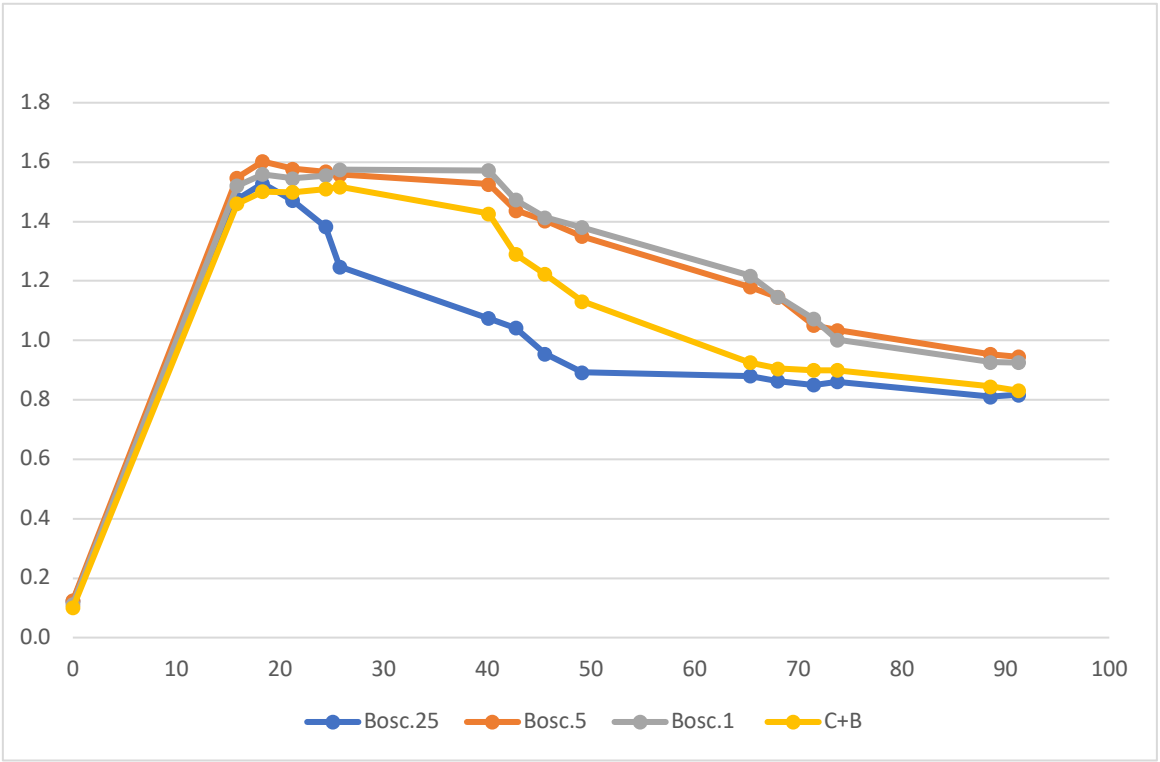


Figure 3.76. The OD curve of isolate 201 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

The cross-screening test with the isolate 201 and the fungicide boscalid was run three times. In all three experiments, with 25 ppm boscalid, the OD values were the lowest increase of all the

treatments and control. The maximum observed OD values with the 25 ppm were recorded between 18 and 26 hours followed by a fall until levelling off at around 70 hours. This is reflected in numerically in the first and third experiment (Fig. 3.76 and 3.78) and statistically in the second test (Fig. 3.77) lower "growth" than the control.

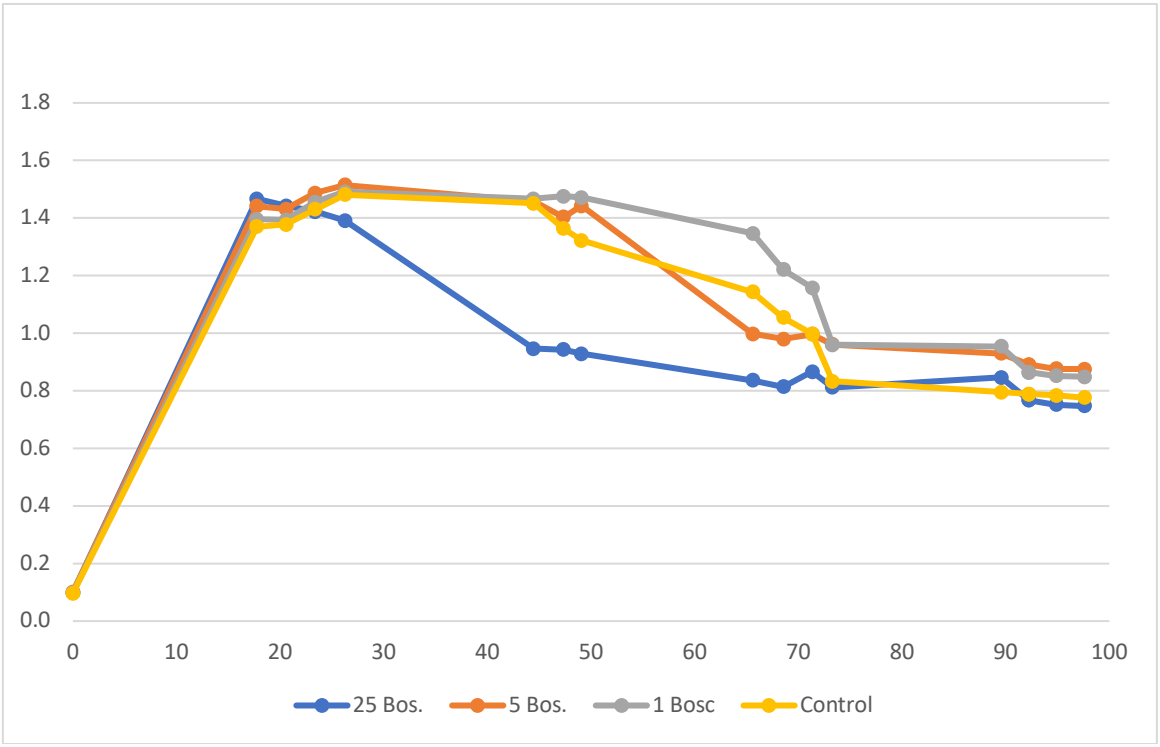


Figure 3.77. The OD curve of isolate 201 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

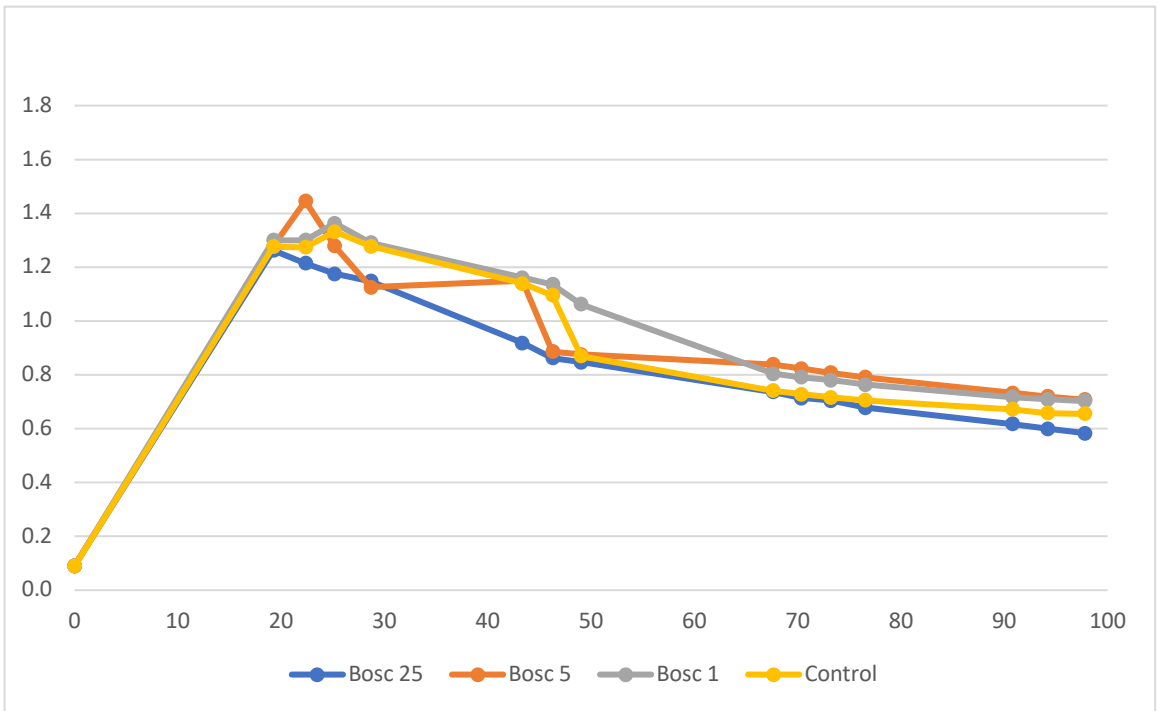


Figure 3.78. The OD curve of isolate 201 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

In the first two trials, after reaching peaks, with the 1 and 5 ppm boscalid treatments and the control OD values started to level off until 44.43 hours (the control and 5 ppm treatment) and 49.08 hours (the 1 ppm treatment), where they started to decline before levelling out at around 70 hours until the experiment was stopped. In the third run however, following the attainment of peak values (19.25 hours for the 25ppm treatment, 22.37 hours for the 5 ppm treatment and 25.17 hours for the 1 ppm treatment and the control), there was a steady decline in OD values for the remainder of the experiment.

In the first run (Table 3.160), with the 1 and 5 ppm treatments, the OD values were higher than those of the control from 15,85 hours onwards leading to significant differences from 49.13 onwards until the end of the experiment in the case of 5 ppm treatment and until 71.48 hours with 1 ppm treatment.

In the second run (Table 3.161), the OD values of the 1ppm treatment are consistently higher than those of the control although these differences were only statistically significant on one occasion (65.58 hours). The 5ppm treatment and the control did not show any consistent trend until 73.30 hours after which the 5ppm treatment tended to have higher ODs than the control.

In the third trial (Table 3.162), after an initial growth peak OD values were observed between 19.25 and 25.17 hours followed by a decline until the test was stopped. Similar to the second run, in the third run again the OD values of the 1ppm treatment were consistently higher than those of the control and those of the 5ppm treatment were only consistently higher than the control later in the experiment (from 43.32 hours onwards).

Ass. (h)	Bosc.25	Bosc.5	Bosc.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.122	0.124	0.113	0.100	1.3E-10	0.013	0.017	0.022
15.85	1.475	1.547	1.520	1.460	3.4E-06	0.219	0.291	0.379
18.30	1.528	1.602	1.559	1.502	3.3E-07	0.210	0.280	0.365
21.20	1.471	1.578	1.545	1.499	9.5E-07	0.221	0.294	0.383
24.38	1.383	1.568	1.555	1.509	6.0E-07	0.223	0.297	0.387
25.77	1.248	1.558	1.575	1.517	6.8E-07	0.245	0.326	0.425
40.10	1.075	1.526	1.572	1.427	1.1E-07	0.254	0.339	0.441
42.73	1.042	1.437	1.473	1.290	1.2E-07	0.224	0.298	0.389
45.55	0.955	1.404	1.414	1.223	5.5E-08	0.208	0.277	0.361
49.13	0.893	1.350	1.380	1.131	9.8E-07	0.215	0.286	0.373
65.35	0.880	1.180	1.218	0.926	2.5E-06	0.185	0.247	0.321
68.05	0.863	1.146	1.147	0.905	8.5E-06	0.177	0.235	0.307
71.48	0.850	1.051	1.072	0.900	3.6E-07	0.140	0.186	0.242
73.75	0.861	1.035	1.003	0.900	3.1E-07	0.130	0.173	0.226
88.55	0.811	0.954	0.927	0.845	6.2E-08	0.114	0.152	0.198
91.27	0.817	0.946	0.926	0.831	6.2E-08	0.114	0.151	0.197

Table 3.160. The mean ODs at each assessment time for isolate 201 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The “growth” of the 1 and 5 ppm treatments and the control is consistently higher in all three trials (Table 3.163, 3.164, and 3.165 respectively). Although these differences are only occasionally statistically significant, this suggests that the “growth” of isolate 201 in the presence of 1 and 5 ppm boscalid is enhanced while the consistently lower “growth” of the 25 ppm boscalid treatment compared to the control in the first two runs, suggests that these treatments tend to be inhibitory to the “growth” of this isolate.

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.098	0.102	0.099	0.096	1.5E-09	0.009	0.012	0.015
17.77	1.467	1.441	1.396	1.370	1.5E-12	0.167	0.221	0.287
20.55	1.442	1.432	1.394	1.378	1.6E-15	0.142	0.189	0.245
23.37	1.423	1.487	1.455	1.430	2.3E-16	0.132	0.175	0.228
26.23	1.391	1.514	1.492	1.481	1.4E-14	0.139	0.184	0.239
44.43	0.947	1.459	1.467	1.451	2.9E-09	0.229	0.304	0.394
47.33	0.943	1.404	1.476	1.363	2.1E-09	0.224	0.297	0.386
49.08	0.928	1.443	1.471	1.323	3.8E-10	0.221	0.294	0.381
65.58	0.836	0.997	1.347	1.144	3.0E-09	0.202	0.268	0.347
68.57	0.814	0.980	1.222	1.055	1.3E-07	0.191	0.254	0.330
71.35	0.866	0.996	1.158	0.998	2.4E-07	0.165	0.219	0.284
73.30	0.812	0.961	0.960	0.833	7.1E-09	0.096	0.127	0.165
89.55	0.846	0.929	0.955	0.795	6.3E-13	0.080	0.107	0.138
92.25	0.767	0.891	0.864	0.788	5.8E-10	0.087	0.115	0.149
94.88	0.751	0.877	0.851	0.784	1.3E-10	0.083	0.111	0.144
97.62	0.748	0.876	0.848	0.776	2.4E-11	0.084	0.112	0.145

Table 3.161. The mean ODs at each assessment time for isolate 201 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.091	0.090	0.090	0.089	1.7E-06	0.002	0.003	0.004
19.25	1.263	1.278	1.300	1.276	1.4E-34	0.078	0.104	0.135
22.37	1.214	1.445	1.300	1.274	1.4E-17	0.134	0.178	0.232
25.17	1.175	1.279	1.363	1.332	5.2E-11	0.194	0.259	0.337
28.68	1.148	1.126	1.290	1.278	2.3E-11	0.184	0.245	0.320
43.32	0.919	1.148	1.160	1.140	2.5E-07	0.207	0.276	0.359
46.27	0.862	0.887	1.136	1.095	1.3E-07	0.192	0.255	0.332
49.03	0.847	0.876	1.063	0.870	6.1E-07	0.172	0.229	0.299
67.62	0.736	0.838	0.804	0.742	2.6E-11	0.105	0.140	0.182
70.32	0.714	0.823	0.791	0.727	1.4E-10	0.108	0.143	0.187
73.20	0.704	0.807	0.780	0.716	8.3E-11	0.106	0.141	0.184
76.52	0.678	0.791	0.764	0.706	3.0E-10	0.109	0.145	0.188
90.78	0.618	0.732	0.718	0.672	1.5E-09	0.110	0.147	0.191
94.18	0.600	0.720	0.709	0.658	3.2E-09	0.108	0.144	0.188
97.77	0.583	0.708	0.703	0.656	3.0E-09	0.109	0.146	0.190

Table 3.162. The mean ODs at each assessment time for isolate 201 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Bosc.25	77.65
Bosc.5	99.57
Bosc.1	100.87
Control	89.82
LSD5%	14.063
LSD1%	18.719
LSD 0.1%	24.380

Treatment	"Growth"
Bosc.25	82.77
Bosc.5	100.17
Bosc.1	104.53
Control	96.56
LSD5%	10.560
LSD1%	14.018
LSD 0.1%	18.180

Treatment	"Growth"
Bosc.25	69.97
Bosc.5	77.81
Bosc.1	81.27
Control	68.72
LSD5%	10.03
LSD1%	13.36
LSD 0.1%	17.39

Table 3.163. and 3.164 and 3.165. The mean "growth" of isolate 201 in treatments with 25, 5, and 1 ppm of boscalid over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

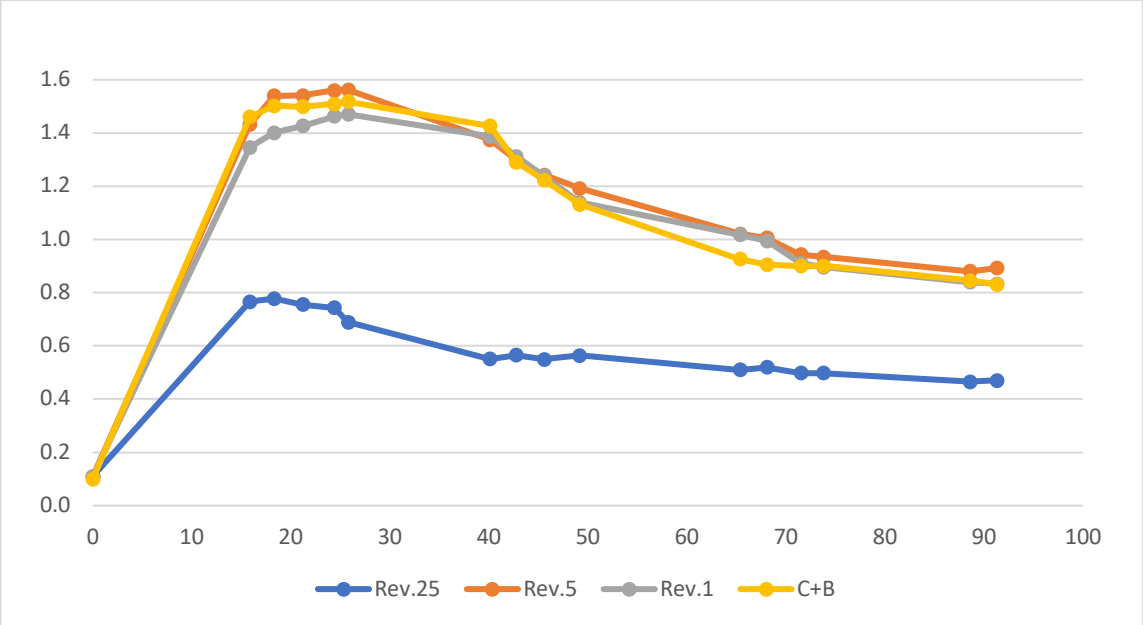


Figure 3.79. The OD curve of isolate 201 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

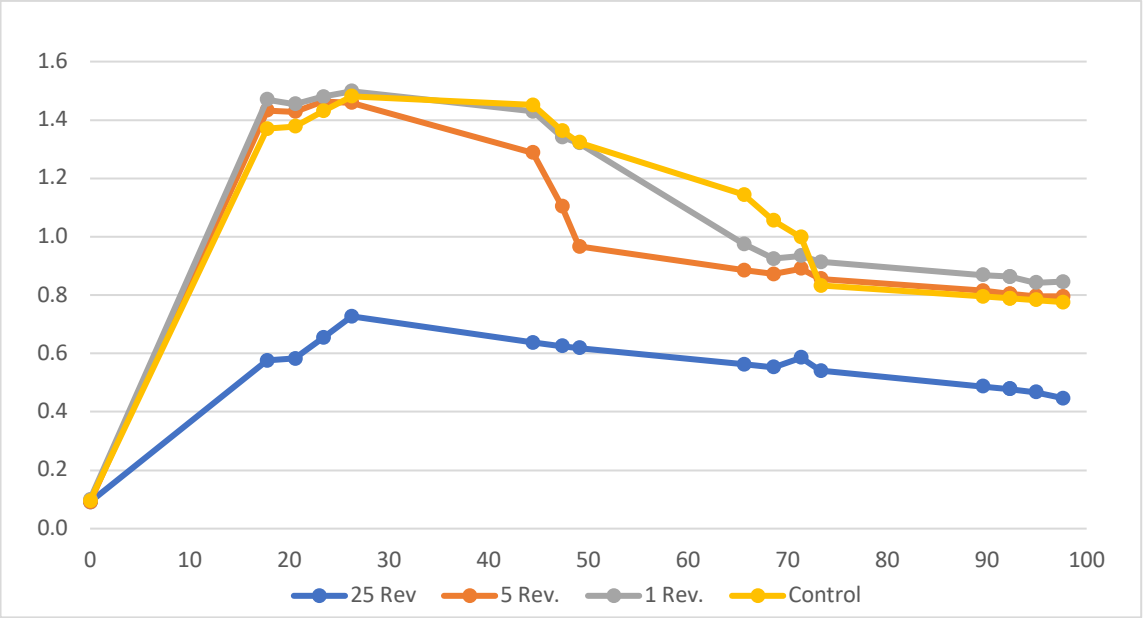


Figure 3.80. The OD curve of isolate 201 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

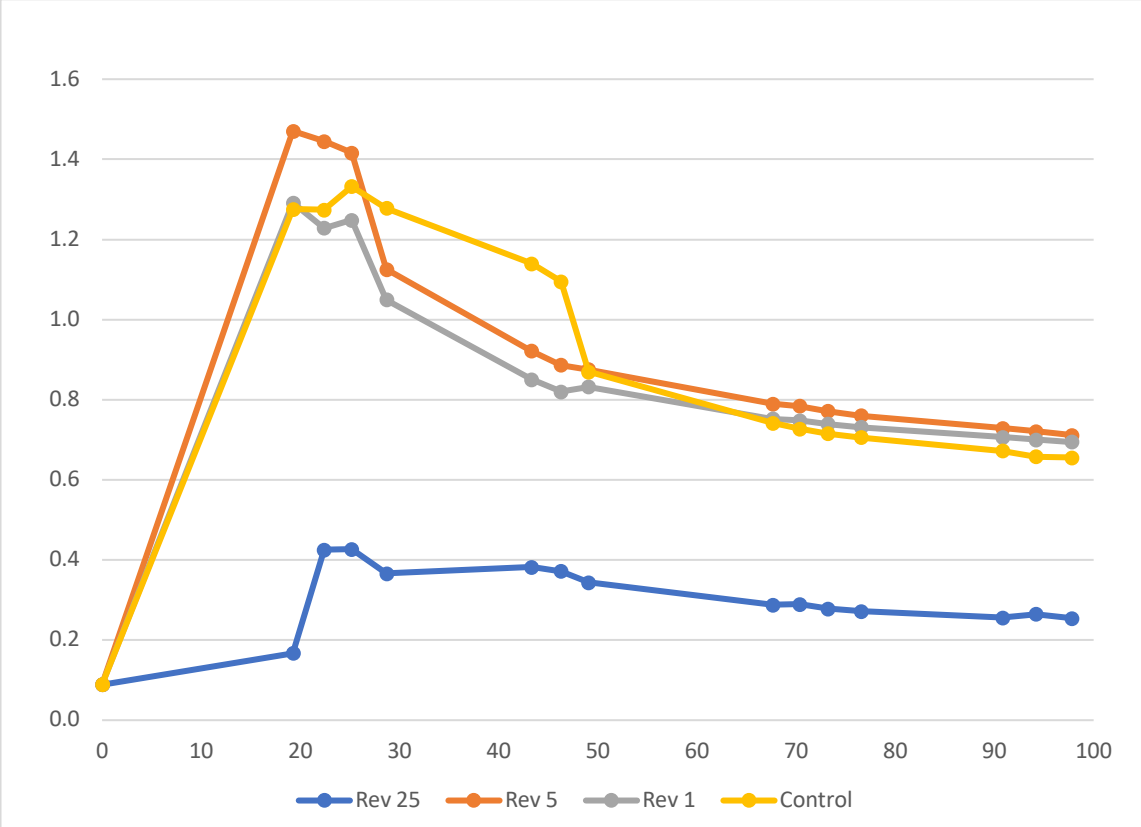


Figure 3.81. The OD curve of isolate 201 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Rev.25	Rev.5	Rev.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.110	0.109	0.110	0.100	1.3E-10	0.013	0.017	0.022
15.85	0.766	1.433	1.346	1.460	3.4E-06	0.219	0.291	0.379
18.30	0.778	1.539	1.401	1.502	3.3E-07	0.210	0.280	0.365
21.20	0.756	1.540	1.426	1.499	9.5E-07	0.221	0.294	0.383
24.38	0.743	1.560	1.463	1.509	6.0E-07	0.223	0.297	0.387
25.77	0.689	1.562	1.470	1.517	6.8E-07	0.245	0.326	0.425
40.10	0.551	1.375	1.386	1.427	1.1E-07	0.254	0.339	0.441
42.73	0.566	1.298	1.312	1.290	1.2E-07	0.224	0.298	0.389
45.55	0.550	1.242	1.239	1.223	5.5E-08	0.208	0.277	0.361
49.13	0.564	1.192	1.139	1.131	9.8E-07	0.215	0.286	0.373
65.35	0.510	1.022	1.018	0.926	2.5E-06	0.185	0.247	0.321
68.05	0.520	1.006	0.995	0.905	8.5E-06	0.177	0.235	0.307
71.48	0.498	0.943	0.911	0.900	3.6E-07	0.140	0.186	0.242
73.75	0.498	0.935	0.896	0.900	3.1E-07	0.130	0.173	0.226
88.55	0.465	0.881	0.840	0.845	6.2E-08	0.114	0.152	0.198
91.27	0.470	0.893	0.834	0.831	6.2E-08	0.114	0.151	0.197

Table 3.166. The mean ODs at each assessment time for isolate 201 in the presence of three concentrations of Revysol® LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.091	0.092	0.100	0.096	1.5E-09	0.009	0.012	0.015
17.77	0.576	1.433	1.470	1.370	1.5E-12	0.167	0.221	0.287
20.55	0.583	1.428	1.454	1.378	1.6E-15	0.142	0.189	0.245
23.37	0.654	1.464	1.480	1.430	2.3E-16	0.132	0.175	0.228
26.23	0.727	1.458	1.498	1.481	1.4E-14	0.139	0.184	0.239
44.43	0.638	1.288	1.429	1.451	2.9E-09	0.229	0.304	0.394
47.33	0.626	1.105	1.341	1.363	2.1E-09	0.224	0.297	0.386
49.08	0.619	0.966	1.321	1.323	3.8E-10	0.221	0.294	0.381
65.58	0.563	0.885	0.975	1.144	3.0E-09	0.202	0.268	0.347
68.57	0.553	0.872	0.925	1.055	1.3E-07	0.191	0.254	0.330
71.35	0.586	0.891	0.934	0.998	2.4E-07	0.165	0.219	0.284
73.30	0.541	0.856	0.914	0.833	7.1E-09	0.096	0.127	0.165
89.55	0.487	0.815	0.869	0.795	6.3E-13	0.080	0.107	0.138
92.25	0.479	0.804	0.864	0.788	5.8E-10	0.087	0.115	0.149
94.88	0.468	0.796	0.842	0.784	1.3E-10	0.083	0.111	0.144
97.62	0.446	0.796	0.846	0.776	2.4E-11	0.084	0.112	0.145

Table 3.167. The mean ODs at each assessment time for isolate 201 in the presence of three concentrations of Revysol® LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.089	0.089	0.090	0.089	1.7E-06	0.002	0.003	0.004
19.25	0.167	1.471	1.292	1.276	1.4E-34	0.078	0.104	0.135
22.37	0.425	1.445	1.228	1.274	1.4E-17	0.134	0.178	0.232
25.17	0.427	1.417	1.248	1.332	5.2E-11	0.194	0.259	0.337
28.68	0.366	1.126	1.050	1.278	2.3E-11	0.184	0.245	0.320
43.32	0.382	0.921	0.851	1.140	2.5E-07	0.207	0.276	0.359
46.27	0.372	0.887	0.820	1.095	1.3E-07	0.192	0.255	0.332
49.03	0.344	0.876	0.832	0.870	6.1E-07	0.172	0.229	0.299
67.62	0.288	0.790	0.752	0.742	2.6E-11	0.105	0.140	0.182
70.32	0.289	0.785	0.749	0.727	1.4E-10	0.108	0.143	0.187
73.20	0.278	0.771	0.740	0.716	8.3E-11	0.106	0.141	0.184
76.52	0.272	0.760	0.732	0.706	3.0E-10	0.109	0.145	0.188
90.78	0.256	0.729	0.707	0.672	1.5E-09	0.110	0.147	0.191
94.18	0.265	0.721	0.700	0.658	3.2E-09	0.108	0.144	0.188
97.77	0.254	0.712	0.695	0.656	3.0E-09	0.109	0.146	0.190

Table 3.168. The mean ODs at each assessment time for isolate 201 in the presence of three concentrations of Revysol® LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The cross-screening test with the isolate 201 and fungicide Revysol® was run three times. In all three tests, with the 25 ppm the OD value was markedly the lowest compared to other treatments and the control (1st run: Fig. 3.79, Table 3.166 and 3.169, 2nd run: Fig. 3.80, Table 3.167 and 3.170, and 3rd run: Fig. 3.80, Table 3.168, and 3.171). There was a similar tendency between the three tests. After an initial increase, with the control and 1 and 5 ppm treatments,

the maximum OD value was observed between 22 and 27 hours. A steep decline followed until it levelled off somewhat around 70 hours. Moreover, throughout most of the experiment, the OD values of the control was either higher or similar to the one with the 1 and 5 ppm treatments. The exception is the third trial, where the peak OD value with the 5 ppm treatment, was statistically greater than the peak value of the control. Although after the peak, the 5 ppm treatment declined more steeply than the control, the average "growth" of the 5 ppm treatment was numerically higher than the control. The very low "growth" value of the 25 ppm treatments (significant at the 0.1% level in all three runs), indicates the inhibitory effect of this treatment on the isolate 201.

Treatment	"Growth"
Rev.25	39.70
Rev.5	91.98
Rev.1	88.11
Control	89.82
LSD5%	14.063
LSD1%	18.719
LSD 0.1%	24.380

Treatment	"Growth"
Rev.25	43.92
Rev.5	89.21
Rev.1	97.15
Control	96.56
LSD5%	10.560
LSD1%	14.018
LSD 0.1%	18.180

Treatment	"Growth"
Rev.25	18.84
Rev.5	77.43
Rev.1	71.11
Control	68.72
LSD5%	10.03
LSD1%	13.36
LSD 0.1%	17.39

Table 3.169. and 3.170. and 3.171. The mean "growth" of isolate 201 in treatments with 25, 5, and 1 ppm of Revysol® over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

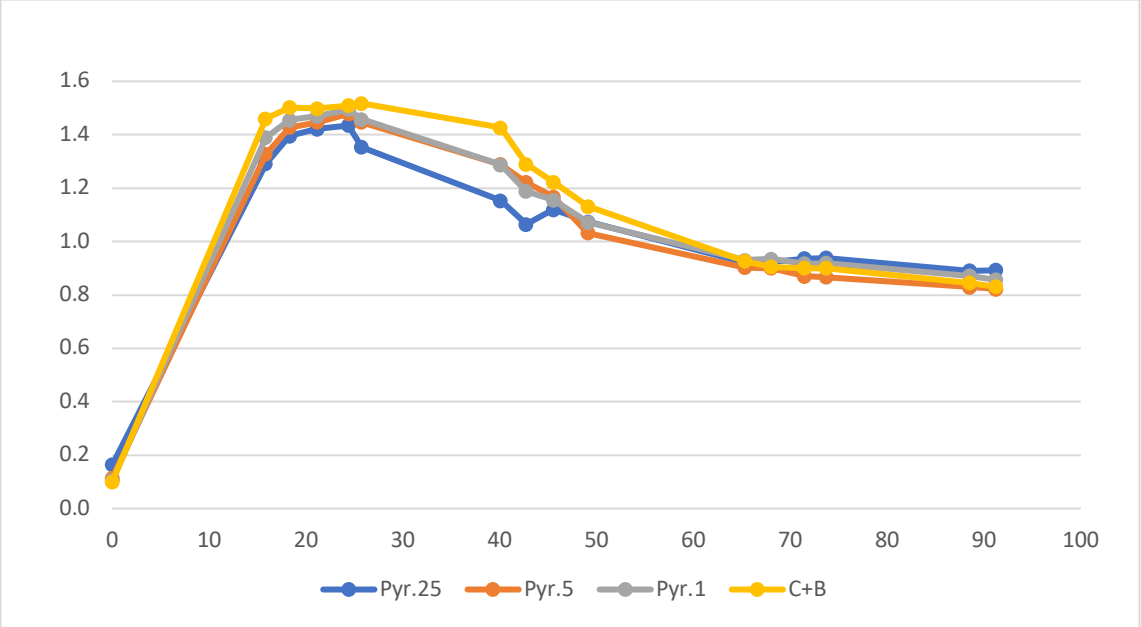


Figure 3.82. The OD curve of isolate 201 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

The cross-screening test with the isolate 201 and the fungicide pyraclostrobin was run on 2 occasions. In both runs, after an initial increase, the maximum OD values were observed

between 25 and 27 hours followed by a decline until around 73 hours or so where the ODs started to level off until the test was stopped. There was no significant reduction or increase in the OD values observed in the presence of the fungicide pyraclostrobin in either test (1st run: Fig. 3.82, Table 3.172,174 and 2nd run: Fig. 3.83, Table 3.173, 175).

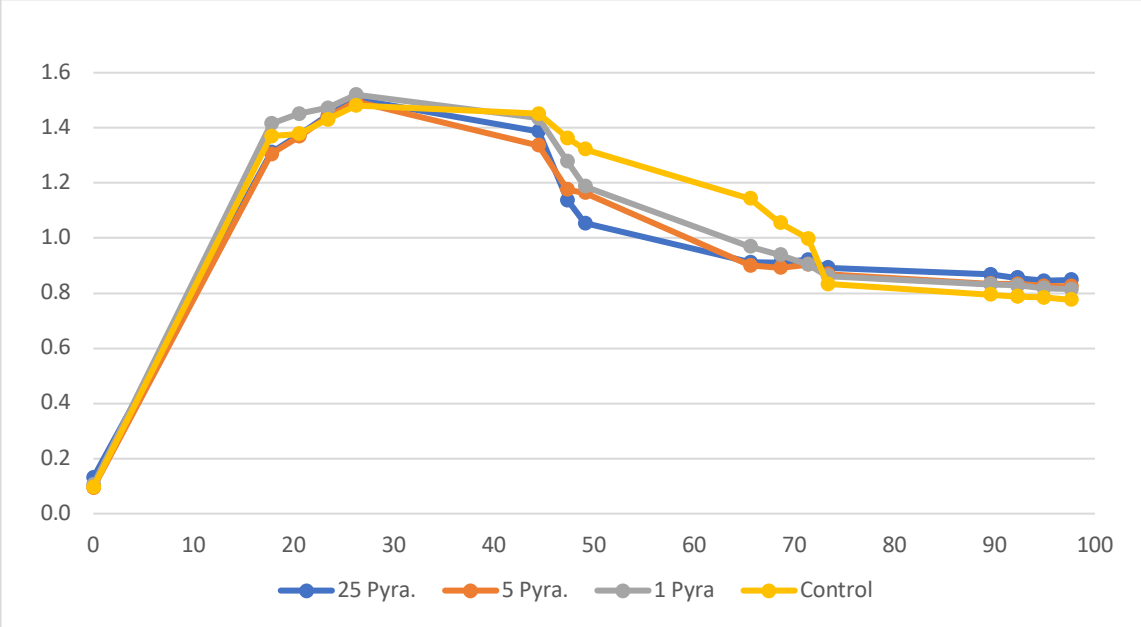


Figure 3.83. The OD curve of isolate 201 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Pyra.25	Pyra.5	Pyra.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.164	0.115	0.106	0.100	1.3E-10	0.013	0.017	0.022
15.85	1.291	1.327	1.390	1.460	3.4E-06	0.219	0.291	0.379
18.30	1.396	1.428	1.456	1.502	3.3E-07	0.210	0.280	0.365
21.20	1.422	1.447	1.470	1.499	9.5E-07	0.221	0.294	0.383
24.38	1.434	1.478	1.494	1.509	6.0E-07	0.223	0.297	0.387
25.77	1.353	1.447	1.458	1.517	6.8E-07	0.245	0.326	0.425
40.10	1.153	1.289	1.287	1.427	1.1E-07	0.254	0.339	0.441
42.73	1.065	1.221	1.188	1.290	1.2E-07	0.224	0.298	0.389
45.55	1.119	1.168	1.188	1.223	5.5E-08	0.208	0.277	0.361
49.13	1.075	1.032	1.156	1.131	9.8E-07	0.215	0.286	0.373
65.35	0.921	0.903	1.071	0.926	2.5E-06	0.185	0.247	0.321
68.05	0.924	0.900	0.930	0.905	8.5E-06	0.177	0.235	0.307
71.48	0.936	0.870	0.936	0.900	3.6E-07	0.140	0.186	0.242
73.75	0.938	0.866	0.917	0.900	3.1E-07	0.130	0.173	0.226
88.55	0.890	0.830	0.920	0.845	6.2E-08	0.114	0.152	0.198
91.27	0.893	0.822	0.871	0.831	6.2E-08	0.114	0.151	0.197

Table 3.172. The mean ODs at each assessment time for isolate 201 in the presence of three concentrations of pyraclostrobin LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.131	0.095	0.105	0.096	1.5E-09	0.009	0.012	0.015
17.77	1.311	1.305	1.415	1.370	1.5E-12	0.167	0.221	0.287
20.55	1.375	1.369	1.451	1.378	1.6E-15	0.142	0.189	0.245
23.37	1.445	1.440	1.472	1.430	2.3E-16	0.132	0.175	0.228
26.23	1.504	1.495	1.520	1.481	1.4E-14	0.139	0.184	0.239
44.43	1.387	1.336	1.435	1.451	2.9E-09	0.229	0.304	0.394
47.33	1.137	1.176	1.278	1.363	2.1E-09	0.224	0.297	0.386
49.08	1.054	1.164	1.187	1.323	3.8E-10	0.221	0.294	0.381
65.58	0.912	0.900	0.970	1.144	3.0E-09	0.202	0.268	0.347
68.57	0.910	0.893	0.939	1.055	1.3E-07	0.191	0.254	0.330
71.35	0.921	0.904	0.904	0.998	2.4E-07	0.165	0.219	0.284
73.30	0.892	0.869	0.862	0.833	7.1E-09	0.096	0.127	0.165
89.55	0.868	0.835	0.832	0.795	6.3E-13	0.080	0.107	0.138
92.25	0.855	0.834	0.828	0.788	5.8E-10	0.087	0.115	0.149
94.88	0.844	0.826	0.818	0.784	1.3E-10	0.083	0.111	0.144
97.62	0.848	0.825	0.814	0.776	2.4E-11	0.084	0.112	0.145

Table 3.173. The mean ODs at each assessment time for isolate 201 in the presence of three concentrations of pyraclostrobin LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Pyra.25	78.25
Pyra.5	83.30
Pyra.1	86.51
Control	89.82
LSD5%	14.063
LSD1%	18.719
LSD 0.1%	24.380

Treatment	"Growth"
Pyra.25	88.29
Pyra.5	91.01
Pyra.1	93.96
Control	96.56
LSD5%	10.560
LSD1%	14.018
LSD 0.1%	18.180

Table 3.174. and 3.175. The mean "growth" of isolate 201 in treatments with 25, 5, and 1 ppm of pyraclostrobin over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

The cross-screening test with the isolate 201 and the fungicide fluxapyroxad was run twice. In the first experiment, after an initial increase, the OD value with the control and 25 ppm treatment reached their maximum OD values at 26.23 hours, while with the 1 and 5 ppm treatments continued to increase until reaching the highest value at 44.43 hours. Following these maximum OD values, the OD values started to decline until 73.30 hours when the OD values started to level off until the test was stopped (Fig. 3.84). Since the decrease with the control was steeper than the one with the treatments from 44.43 hours onwards, the OD value of the 1 ppm was significantly higher than the one with the control between 49 and 71 hours (Table 3.176). All treatments showed a numerically higher "growth" value than the control and the 1ppm treatment showed the highest value for this parameter (Table 3.178).

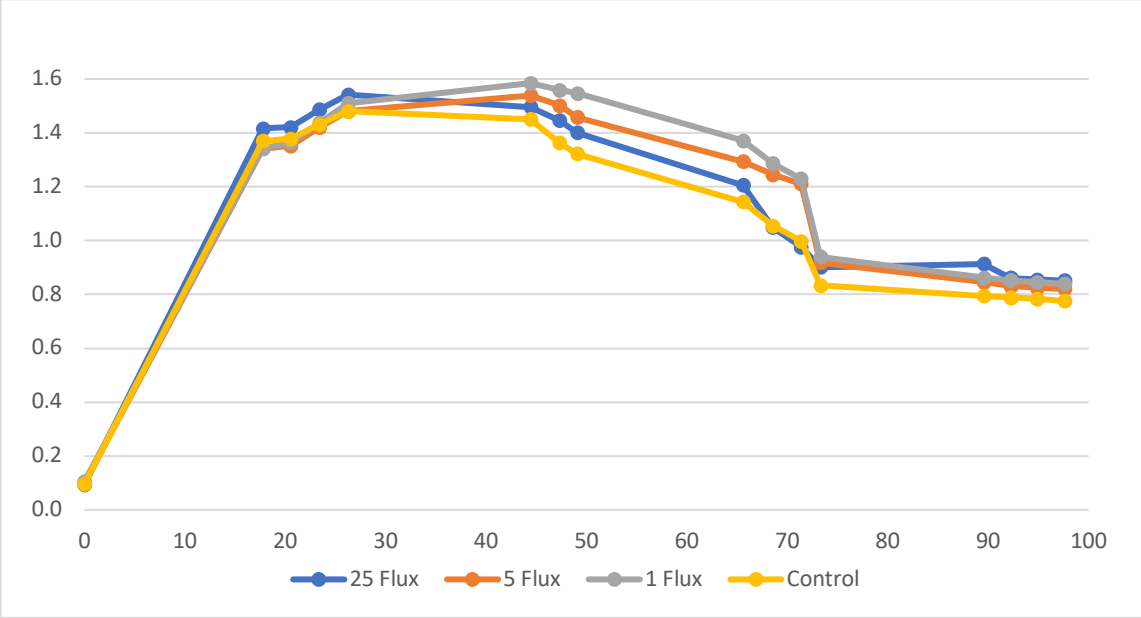


Figure 3.84. The OD curve of isolate 201 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

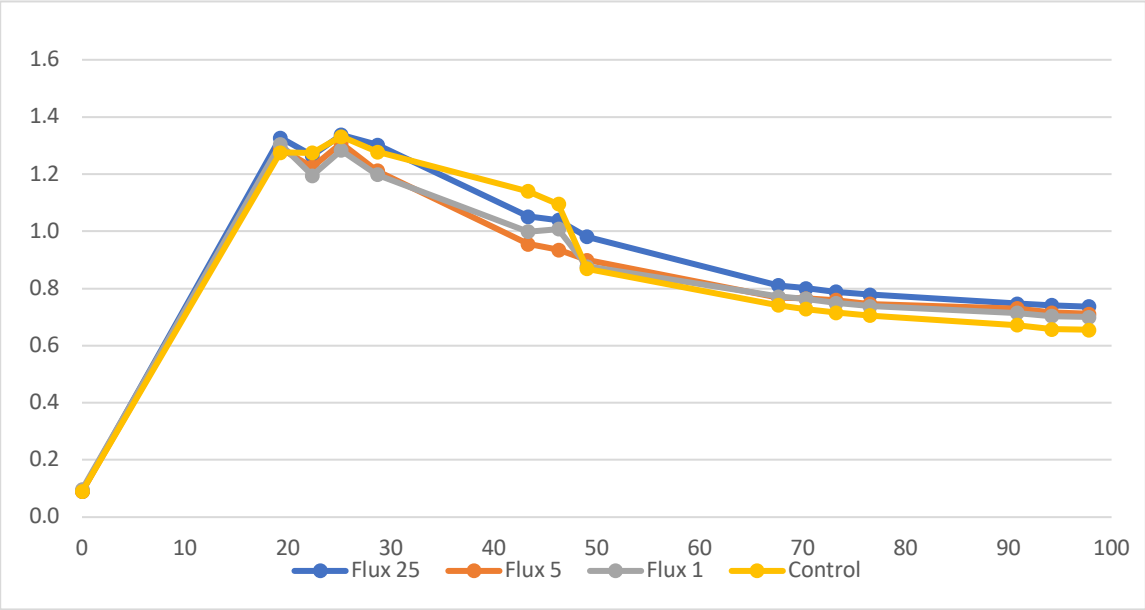


Figure 3.85. The OD curve of isolate 201 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

In the second trial (Fig. 3.85.) after the initial growth period, all treatments and the control achieved the peak point at 25.17 hours followed by a steady decline until the test was stopped. Similar to the first run, the OD values of the control was most of the time lower than those of the treatments resulting in a higher "growth" of the fluxapyroxad treatments compared to the control although statistical significance at the 5% level was only reached in the case of the 25 ppm treatment (Table 3.177). The higher "growth" of the fluxapyroxad treatments in both trials

compared to the control suggests that the "growth" of strain 201 in the presence of fluxapyroxad is enhanced (Table 3.179).

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.094	0.099	0.106	0.096	1.5E-09	0.009	0.012	0.015
17.77	1.416	1.341	1.342	1.370	1.5E-12	0.167	0.221	0.287
20.55	1.421	1.352	1.362	1.378	1.6E-15	0.142	0.189	0.245
23.37	1.486	1.420	1.437	1.430	2.3E-16	0.132	0.175	0.228
26.23	1.542	1.480	1.510	1.481	1.4E-14	0.139	0.184	0.239
44.43	1.496	1.539	1.585	1.451	2.9E-09	0.229	0.304	0.394
47.33	1.446	1.502	1.559	1.363	2.1E-09	0.224	0.297	0.386
49.08	1.400	1.458	1.548	1.323	3.8E-10	0.221	0.294	0.381
65.58	1.206	1.294	1.371	1.144	3.0E-09	0.202	0.268	0.347
68.57	1.049	1.245	1.287	1.055	1.3E-07	0.191	0.254	0.330
71.35	0.976	1.210	1.229	0.998	2.4E-07	0.165	0.219	0.284
73.30	0.902	0.918	0.939	0.833	7.1E-09	0.096	0.127	0.165
89.55	0.914	0.846	0.863	0.795	6.3E-13	0.080	0.107	0.138
92.25	0.861	0.831	0.852	0.788	5.8E-10	0.087	0.115	0.149
94.88	0.855	0.824	0.846	0.784	1.3E-10	0.083	0.111	0.144
97.62	0.852	0.821	0.840	0.776	2.4E-11	0.084	0.112	0.145

Table 3.176. The mean ODs at each assessment time for isolate 201 in the presence of three concentrations of fluxapyroxad LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.090	0.090	0.096	0.089	1.7E-06	0.002	0.003	0.004
19.25	1.328	1.289	1.305	1.276	1.4E-34	0.078	0.104	0.135
22.37	1.264	1.225	1.195	1.274	1.4E-17	0.134	0.178	0.232
25.17	1.337	1.308	1.283	1.332	5.2E-11	0.194	0.259	0.337
28.68	1.302	1.212	1.199	1.278	2.3E-11	0.184	0.245	0.320
43.32	1.052	0.955	0.999	1.140	2.5E-07	0.207	0.276	0.359
46.27	1.039	0.936	1.008	1.095	1.3E-07	0.192	0.255	0.332
49.03	0.981	0.900	0.877	0.870	6.1E-07	0.172	0.229	0.299
67.62	0.811	0.769	0.772	0.742	2.6E-11	0.105	0.140	0.182
70.32	0.801	0.765	0.764	0.727	1.4E-10	0.108	0.143	0.187
73.20	0.789	0.759	0.750	0.716	8.3E-11	0.106	0.141	0.184
76.52	0.778	0.746	0.739	0.706	3.0E-10	0.109	0.145	0.188
90.78	0.747	0.730	0.715	0.672	1.5E-09	0.110	0.147	0.191
94.18	0.741	0.716	0.703	0.658	3.2E-09	0.108	0.144	0.188
97.77	0.737	0.711	0.700	0.656	3.0E-09	0.109	0.146	0.190

Table 3.177. The mean ODs at each assessment time for isolate 201 in the presence of three concentrations of fluxapyroxad LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Flux. 25	102.28
Flux. 5	102.38
Flux. 1	105.07
Control	96.56
LSD5%	10.560
LSD1%	14.018
LSD0.1%	18.180

Treatment	"Growth"
Flux. 25	80.06
Flux. 5	75.39
Flux. 1	74.81
Control	68.72
LSD5%	10.03
LSD1%	13.36
LSD0.1%	17.39

Table 3.178. and 3.179. The mean "growth" of isolate 201 in treatments with 25, 5, and 1 ppm of fluxapyroxad over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

3.2.4.11. Isolate 250

The cross-screening test with the isolate 250 and the fungicide boscalid was run four times. In the first three experiments, the growth curve of the control in all tests was similar showing an initial increase and after reaching a peak between 15 and 25 hours the OD value started a short steep decline within the second 24 hours followed by either a gentle decrease or leveling off until the test was stopped.

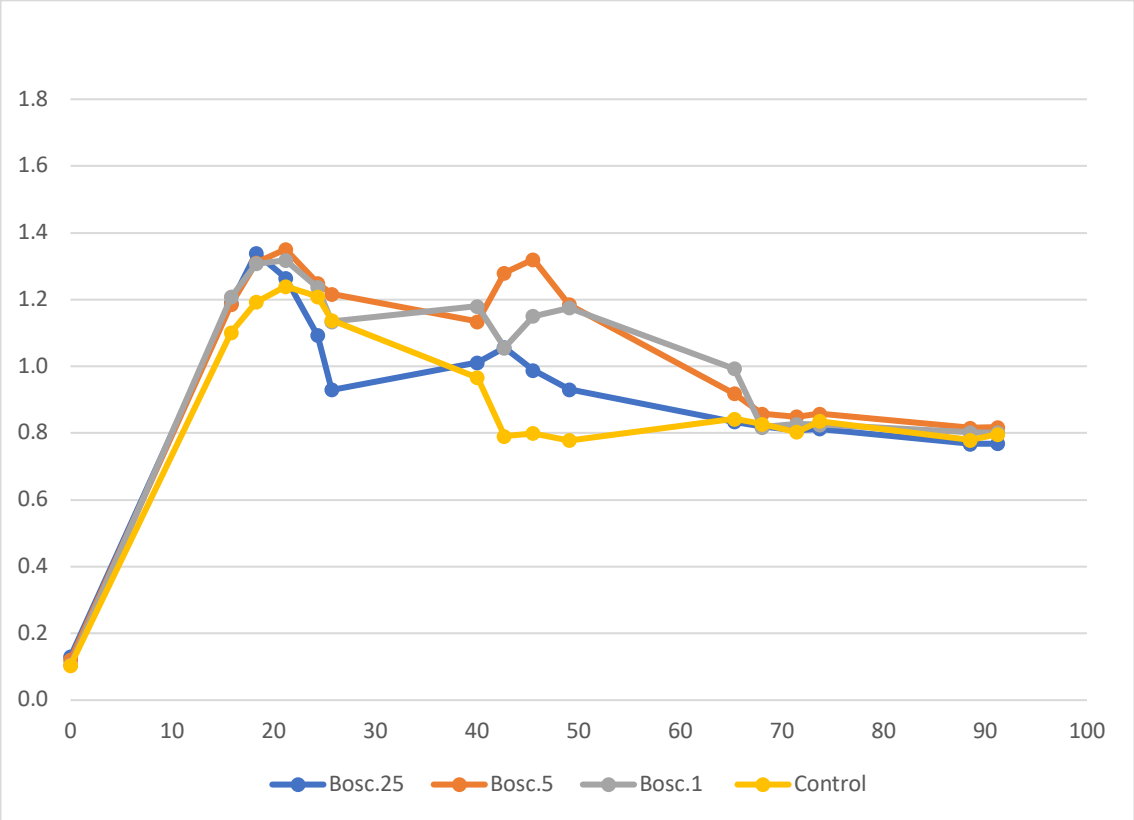


Figure 3.86. The OD curve of isolate 250 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

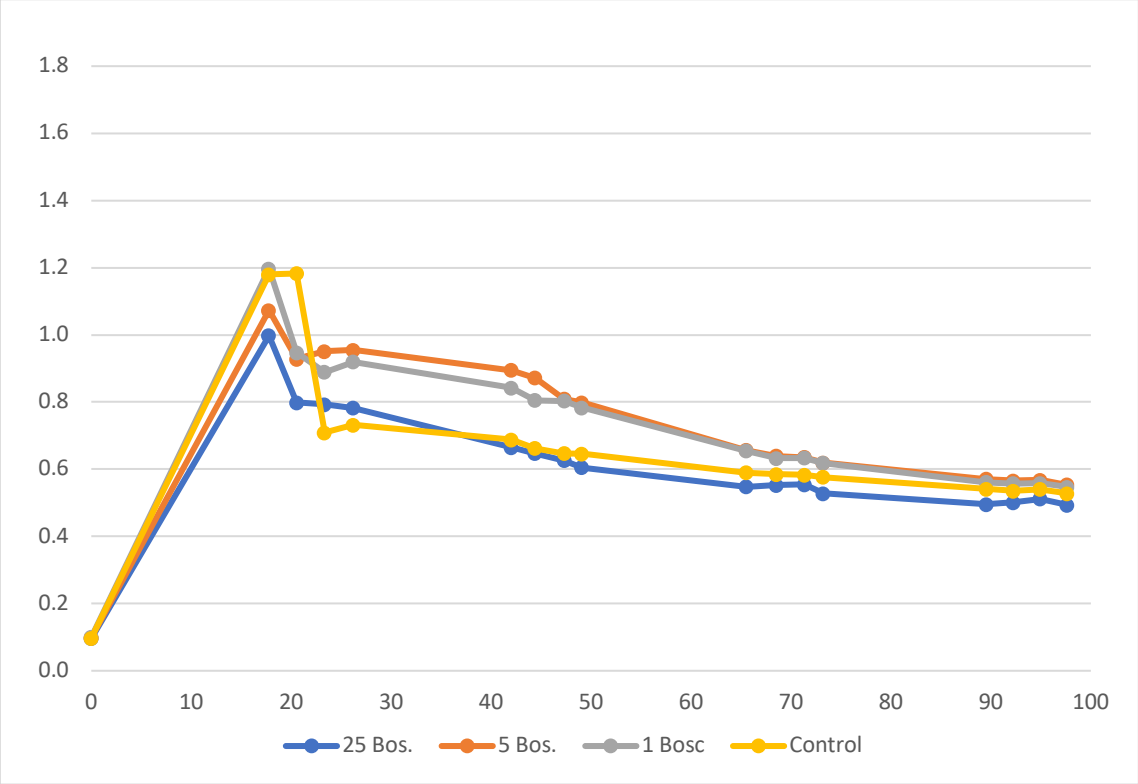


Figure 3.87. The OD curve of isolate 250 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

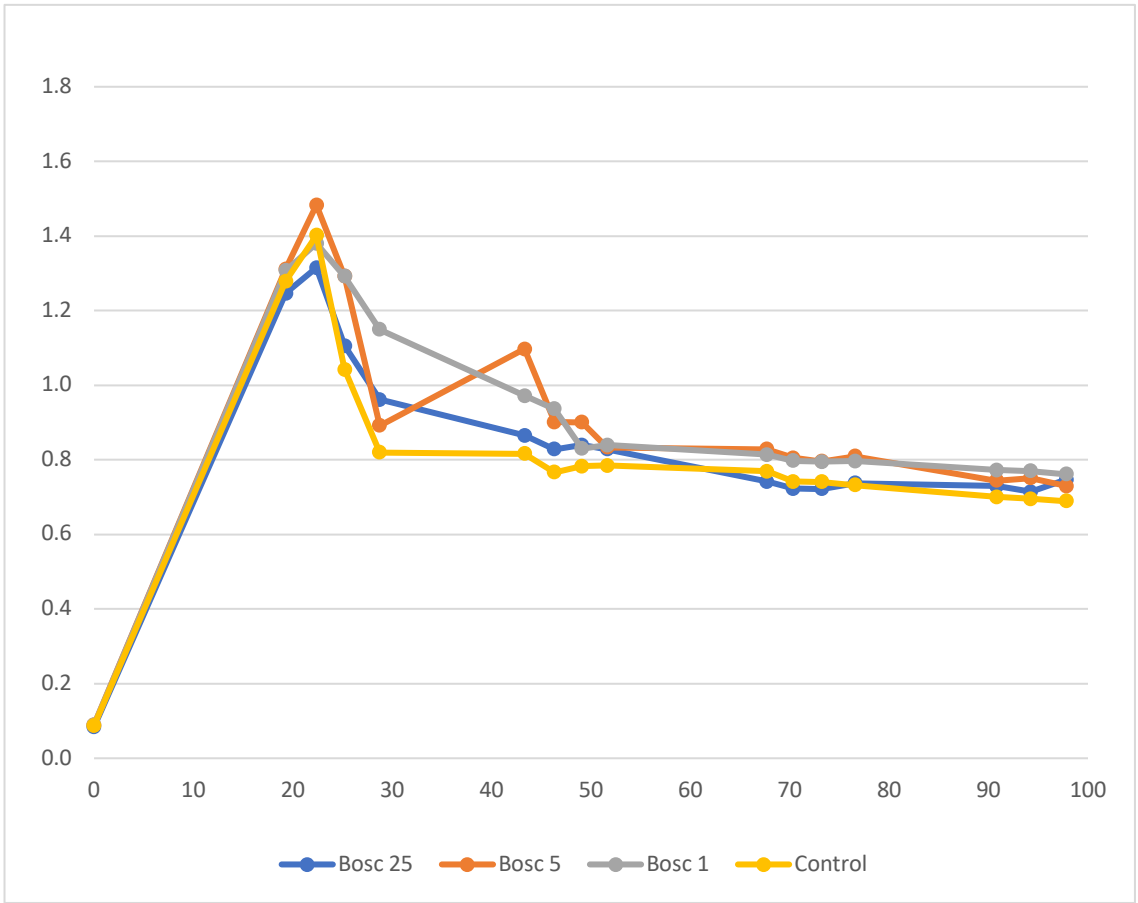


Figure 3.88. The OD curve of isolate 250 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

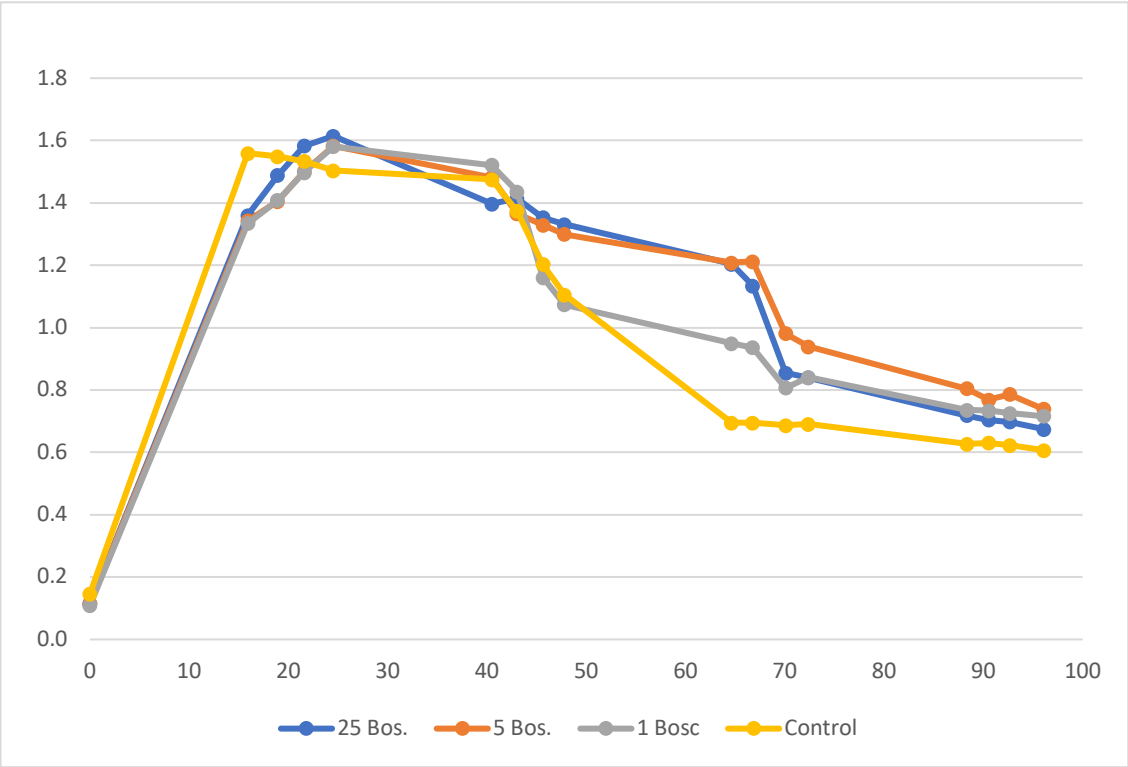


Figure 3.89. The OD curve of isolate 250 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Bosc.25	Bosc.5	Bosc.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.130	0.121	0.106	0.104	1.0E-11	0.013	0.018	0.023
15.95	1.190	1.186	1.208	1.102	8.5E-45	0.044	0.058	0.076
18.97	1.339	1.310	1.308	1.192	1.5E-45	0.046	0.061	0.079
21.60	1.265	1.351	1.318	1.240	2.7E-28	0.092	0.123	0.160
24.60	1.092	1.249	1.237	1.209	2.0E-08	0.238	0.317	0.413
40.50	0.930	1.217	1.134	1.138	2.0E-06	0.255	0.339	0.442
43.05	1.012	1.134	1.180	0.967	8.0E-04	0.249	0.331	0.431
45.72	1.057	1.279	1.055	0.791	5.2E-04	0.241	0.320	0.417
47.82	0.989	1.320	1.151	0.800	1.0E-05	0.223	0.297	0.387
64.67	0.931	1.184	1.175	0.778	2.9E-05	0.216	0.288	0.375
66.80	0.834	0.919	0.993	0.842	5.2E-04	0.136	0.181	0.235
70.08	0.819	0.858	0.818	0.827	1.3E-04	0.119	0.158	0.206
72.38	0.809	0.849	0.828	0.803	4.2E-05	0.116	0.155	0.202
88.40	0.813	0.857	0.825	0.836	5.5E-05	0.116	0.155	0.202
90.53	0.768	0.816	0.803	0.779	1.5E-06	0.106	0.141	0.184
92.67	0.769	0.817	0.801	0.797	2.0E-06	0.109	0.145	0.189

Table 3.180. The mean ODs at each assessment time for isolate 250 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In the first trial with treatments the OD values increased significantly more than those of the control. With the 25 ppm treatment, a peak OD value was recorded earlier (18.22 hours) than the control and the 1 and 5 ppm treatments (21.18 hours), followed by a steeper decline than

that of the control (Fig. 3.86). With the 1 and 5 ppm the decline was slower than the one with the control reflected in significant differences at most timings until the ODs started to plateau out (Table 3.180). It led to significantly greater average "growth" of treatments with the 1 and 5 ppm at the 5% level (Table 3.184).

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.096	0.099	0.100	0.097	2.3E-29	0.003	0.004	0.006
17.77	0.997	1.074	1.197	1.179	3.0E-02	0.204	0.271	0.351
20.57	0.799	0.929	0.948	1.183	2.6E-03	0.204	0.271	0.352
23.33	0.793	0.951	0.889	0.709	7.3E-08	0.069	0.091	0.119
26.22	0.783	0.955	0.919	0.732	2.2E-09	0.055	0.074	0.095
42.00	0.666	0.895	0.842	0.688	1.7E-03	0.092	0.123	0.159
44.40	0.647	0.872	0.805	0.662	4.3E-03	0.095	0.126	0.163
47.33	0.626	0.809	0.804	0.647	9.7E-04	0.080	0.106	0.138
49.08	0.605	0.799	0.783	0.647	9.3E-04	0.076	0.101	0.131
65.55	0.548	0.657	0.655	0.590	2.7E-03	0.050	0.066	0.086
68.55	0.553	0.639	0.633	0.585	9.9E-03	0.047	0.062	0.081
71.35	0.554	0.636	0.634	0.584	2.5E-02	0.049	0.064	0.084
73.23	0.528	0.620	0.618	0.576	2.2E-03	0.044	0.059	0.076
89.55	0.496	0.571	0.561	0.541	1.3E-03	0.041	0.054	0.070
92.25	0.501	0.566	0.557	0.536	3.1E-03	0.043	0.057	0.074
94.95	0.512	0.568	0.559	0.541	2.3E-02	0.052	0.068	0.087
97.62	0.493	0.554	0.547	0.528	1.4E-03	0.041	0.054	0.069

Table 3.181. The mean ODs at each assessment time for isolate 250 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.084	0.090	0.089	0.088	3.5E-03	0.002	0.003	0.004
19.25	1.246	1.311	1.307	1.279	1.2E-27	0.094	0.125	0.162
22.37	1.315	1.483	1.380	1.401	3.3E-13	0.168	0.223	0.291
25.17	1.106	1.292	1.292	1.042	2.8E-10	0.173	0.230	0.300
28.68	0.961	0.892	1.149	0.820	1.8E-06	0.177	0.236	0.307
43.32	0.865	1.097	0.972	0.816	1.8E-05	0.180	0.240	0.312
46.27	0.828	0.901	0.936	0.766	1.1E-07	0.131	0.174	0.227
49.03	0.839	0.900	0.830	0.783	2.2E-09	0.111	0.148	0.193
67.62	0.828	0.833	0.840	0.785	4.3E-10	0.099	0.132	0.172
70.32	0.742	0.828	0.814	0.769	2.9E-11	0.094	0.126	0.163
73.20	0.723	0.805	0.797	0.742	1.6E-11	0.090	0.120	0.156
76.52	0.722	0.796	0.795	0.741	1.7E-11	0.091	0.121	0.158
90.78	0.738	0.809	0.796	0.732	2.7E-11	0.091	0.122	0.159
94.18	0.729	0.744	0.773	0.701	5.6E-10	0.093	0.123	0.160
97.77	0.714	0.752	0.770	0.695	1.2E-09	0.092	0.123	0.160
97.60	0.747	0.730	0.762	0.689	1.9E-07	0.102	0.135	0.176

Table 3.182. The mean ODs at each assessment time for isolate 250 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Bosc. 25	Bosc. 5	Bosc.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.114	0.114	0.108	0.146	1.5E-16	0.009	0.012	0.016
15.90	1.361	1.343	1.335	1.559	5.6E-20	0.048	0.063	0.082
18.90	1.488	1.406	1.409	1.549	1.6E-11	0.056	0.074	0.096
21.58	1.583	1.501	1.498	1.533	2.7E-08	0.054	0.072	0.093
24.52	1.615	1.583	1.581	1.503	3.7E-06	0.062	0.083	0.107
40.48	1.397	1.482	1.521	1.475	7.0E-10	0.135	0.179	0.232
43.03	1.414	1.365	1.436	1.374	1.2E-04	0.188	0.250	0.324
45.63	1.353	1.329	1.161	1.202	9.1E-05	0.246	0.326	0.423
47.80	1.332	1.300	1.073	1.105	1.0E-06	0.211	0.280	0.363
64.63	1.204	1.208	0.949	0.695	3.6E-12	0.186	0.246	0.319
66.75	1.134	1.213	0.936	0.695	1.6E-12	0.186	0.246	0.319
70.10	0.855	0.981	0.807	0.687	5.8E-11	0.103	0.136	0.177
72.37	0.840	0.940	0.841	0.691	5.4E-06	0.102	0.136	0.176
88.33	0.719	0.805	0.736	0.627	5.3E-07	0.066	0.087	0.113
90.53	0.704	0.769	0.734	0.630	3.9E-06	0.067	0.089	0.116
92.67	0.698	0.786	0.726	0.623	7.2E-09	0.051	0.067	0.087
96.08	0.674	0.739	0.716	0.607	3.0E-12	0.037	0.050	0.064

Table 3.183. The mean ODs at each assessment time for isolate 250 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Bosc.25	68.49
Bosc.5	79.17
Bosc.1	79.05
Control	68.33
LSD5%	9.169
LSD1%	12.205
LSD 0.1%	15.896

Treatment	"Growth"
Bosc.25	49.40
Bosc.5	60.68
Bosc.1	60.41
Control	53.86
LSD5%	5.50
LSD1%	7.30
LSD 0.1%	9.47

Treatment	"Growth"
Bosc.25	70.52
Bosc.5	75.80
Bosc.1	76.78
Control	58.91
LSD5%	9.77
LSD1%	13.00
LSD 0.1%	16.93

Treatment	"Growth"
Bosc. 25	94.87
Bosc. 5	97.04
Bosc. 1	89.94
Control	81.93
LSD5%	6.94
LSD1%	9.21
LSD0.1%	11.94

Table 3.184. and 3.185. and 3. 186 and 3.187. The mean "growth" of isolate 250 in treatments with 25, 5, and 1 ppm of boscalid over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

In the second trial (Fig. 3.87), although the initial increase with the control was greater than that with boscalid treatments, after the peak recorded at 20.57 hours the decline with the control was steeper than the one with the 1 and 5 ppm treatments reflected in significant differences

between the treatments 1 and 5 ppm and the control between 26 and 73 hours (Table 3.181). The 25 ppm treatment following the peak value remained very similar to the control. Considering the "growth", the isolate had again significantly greater "growth" in treatments with the 1 and 5 ppm (Table 3.184).

In the third run (Fig. 3.88), after reaching a peak at 22.40, the OD values started to decline, however with the 1 and 5 ppm treatments, the OD values were consistently higher than those of the control, although these differences were only statistically significant at specific timings between 25 and 49 hours (Table 3.182). With the 25 ppm, the numerically higher values were recorded only at specific timings. Nevertheless, the "growth" of all three boscalid treatments was significantly higher than the control (Table 3.186).

In the last run after reaching a peak (with the control at 15.90 and treatments at 24.52 hours), the OD values started a gentle decrease until 40.48 hours where the decline became steeper (Fig. 3.89). From then on, with the control, OD values were lower those of with treatments until the test was stopped reflected in very significant differences mostly at the 1% and 0.1% level (Table 3.183). This resulted in the significantly greater "growth" values of the treatments 5 and 25 at 0.1% and 1 ppm at the 5% level (Table 3.187).

Considering all four trials, the statistically significant higher average "growth" of treatments 1 and 5ppm in all four experiments and 25 ppm in the last two suggests that the presence of the fungicide boscalid enhanced the "growth" of the isolate 250.

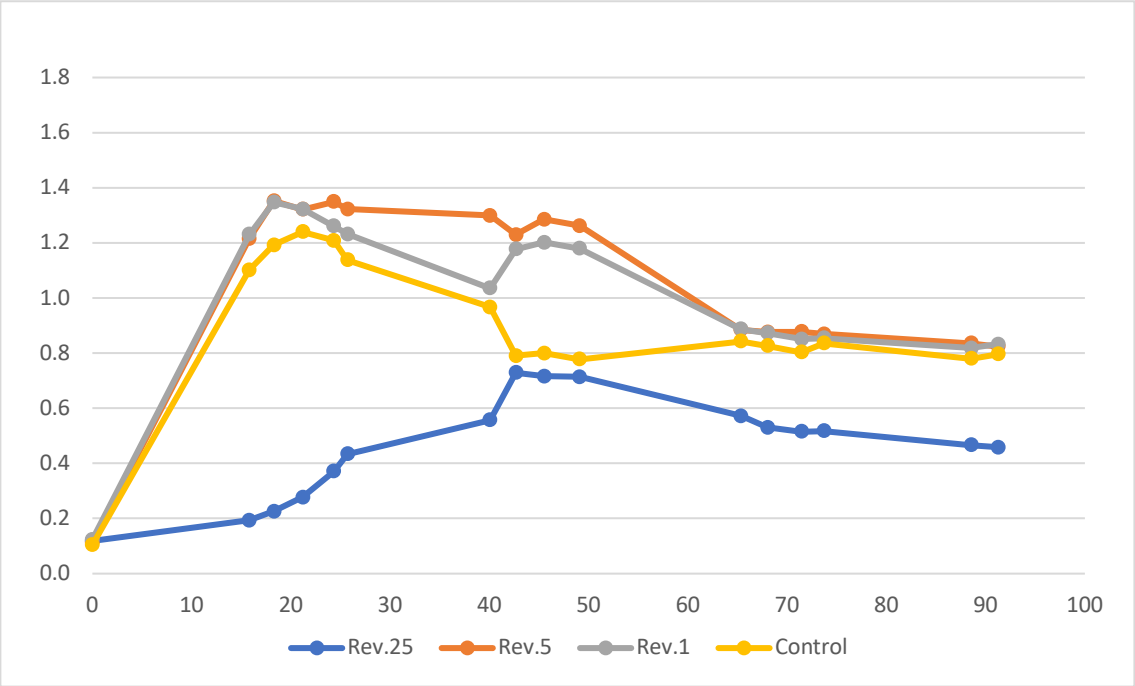


Figure 3.90. The OD curve of isolate 250 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

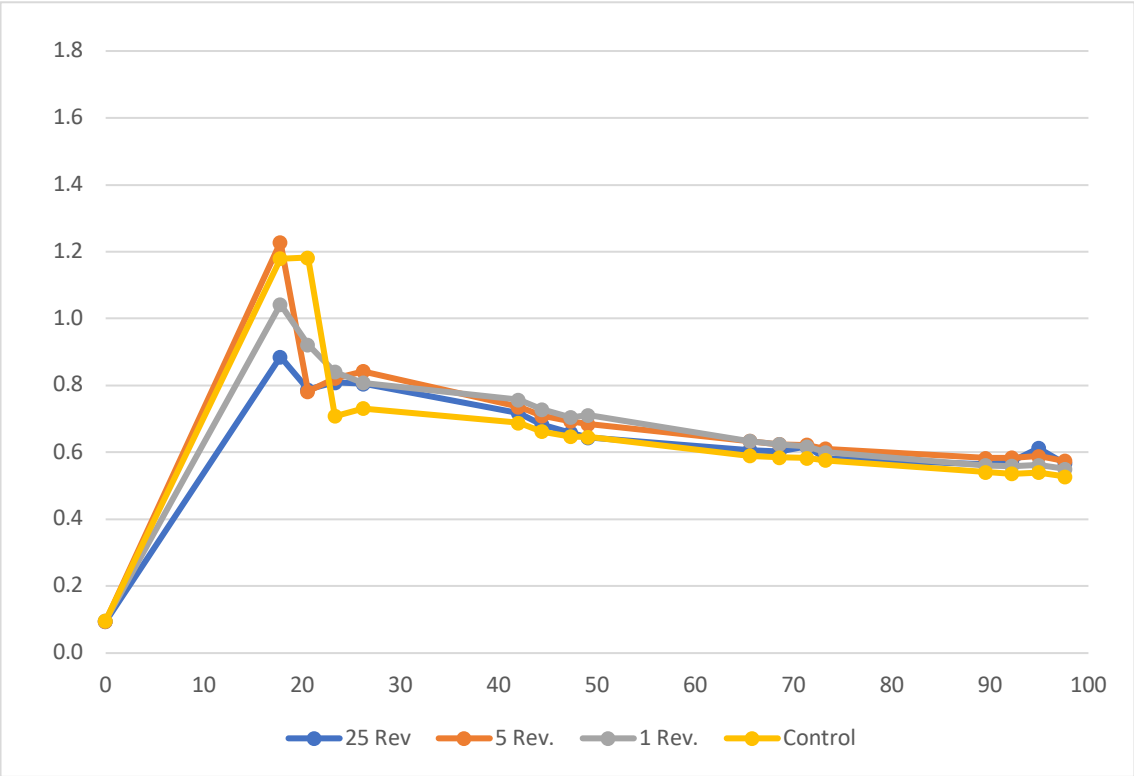


Figure 3.91. The OD curve of isolate 250 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

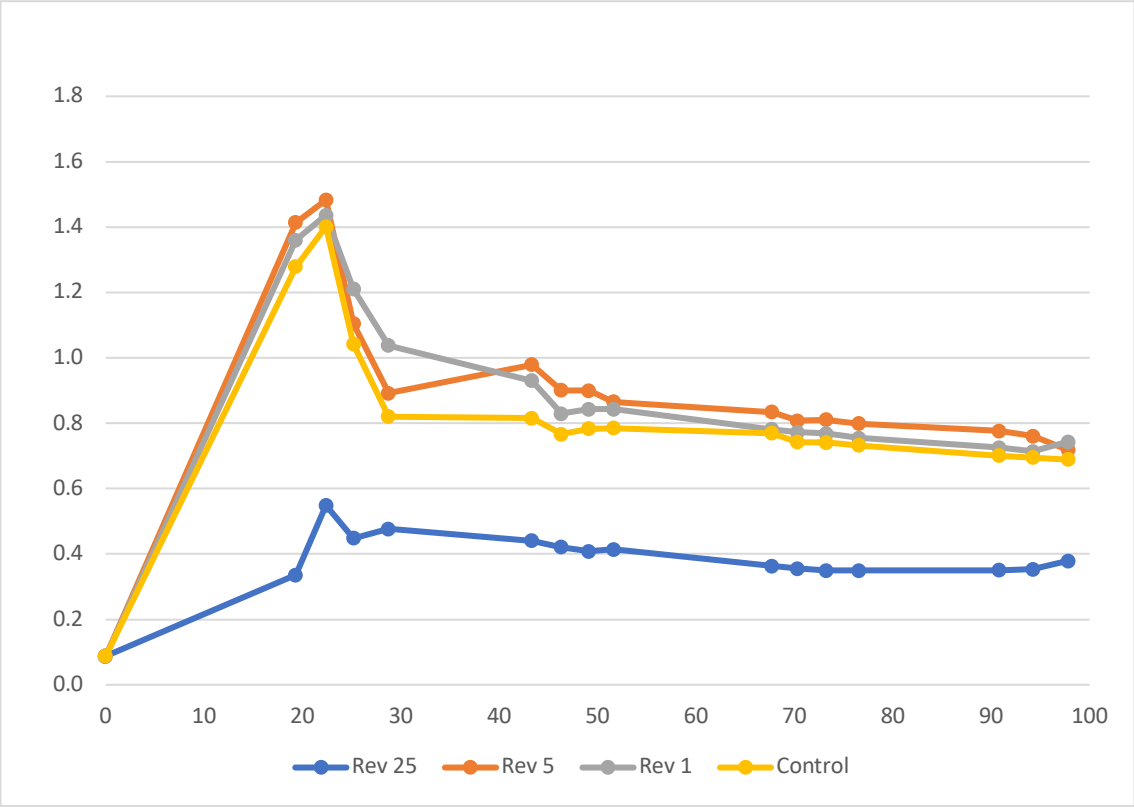


Figure 3.92. The OD curve of isolate 250 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

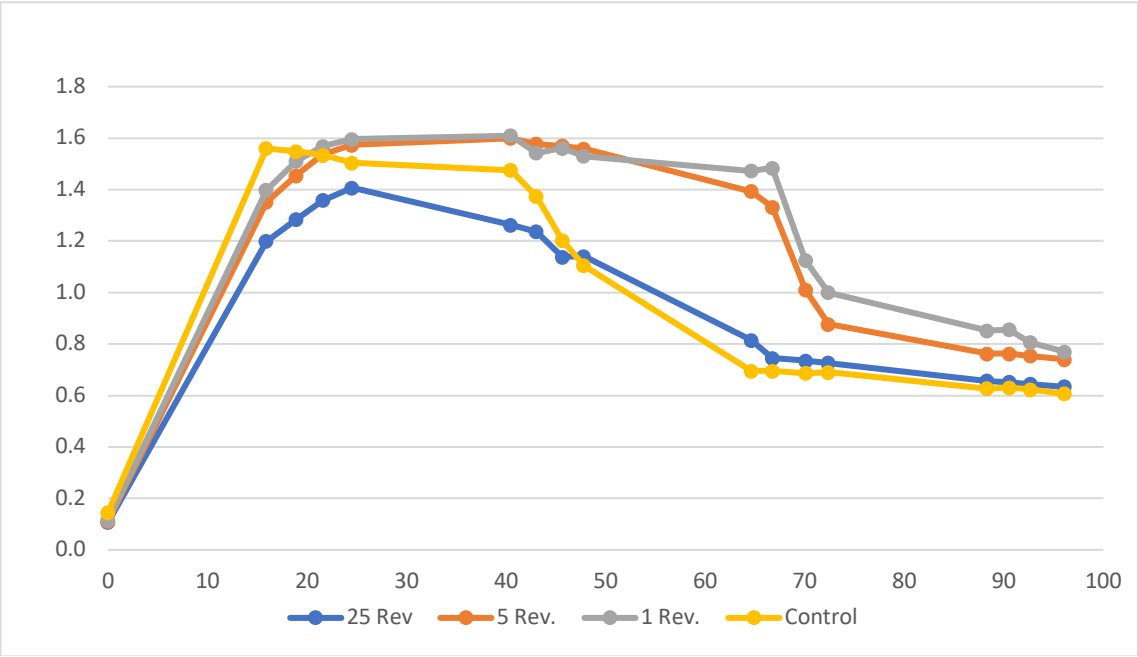


Figure 3.93. The OD curve of isolate 250 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

The cross-screening test with isolate 250 and the fungicide Revysol® was run on four occasions. In first three trials, the OD value with treatments and the control reached a peak between 17 and 23 hours followed by a decline, although in the first run, the fall was more gentile than the other two runs.

Ass. (h)	Rev.25	Rev.5	Rev.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.119	0.114	0.124	0.104	1.0E-11	0.013	0.018	0.023
15.95	0.193	1.215	1.231	1.102	8.5E-45	0.044	0.058	0.076
18.97	0.226	1.352	1.347	1.192	1.5E-45	0.046	0.061	0.079
21.60	0.277	1.321	1.322	1.240	2.7E-28	0.092	0.123	0.160
24.60	0.371	1.349	1.261	1.209	2.0E-08	0.238	0.317	0.413
40.50	0.434	1.322	1.231	1.138	2.0E-06	0.255	0.339	0.442
43.05	0.557	1.299	1.036	0.967	8.0E-04	0.249	0.331	0.431
45.72	0.729	1.230	1.177	0.791	5.2E-04	0.241	0.320	0.417
47.82	0.716	1.286	1.202	0.800	1.0E-05	0.223	0.297	0.387
64.67	0.713	1.262	1.179	0.778	2.9E-05	0.216	0.288	0.375
66.80	0.572	0.884	0.887	0.842	5.2E-04	0.136	0.181	0.235
70.08	0.530	0.877	0.873	0.827	1.3E-04	0.119	0.158	0.206
72.38	0.515	0.878	0.851	0.803	4.2E-05	0.116	0.155	0.202
88.40	0.517	0.869	0.854	0.836	5.5E-05	0.116	0.155	0.202
90.53	0.467	0.836	0.819	0.779	1.5E-06	0.106	0.141	0.184
92.67	0.459	0.825	0.832	0.797	2.0E-06	0.109	0.145	0.189

Table 3.188. The mean ODs at each assessment time for isolate 250 in the presence of three concentrations of Revysol® LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.094	0.095	0.097	0.097	2.3E-29	0.003	0.004	0.006
17.77	0.886	1.229	1.043	1.179	3.0E-02	0.204	0.271	0.351
20.57	0.788	0.783	0.922	1.183	2.6E-03	0.204	0.271	0.352
23.33	0.809	0.821	0.841	0.709	7.3E-08	0.069	0.091	0.119
26.22	0.805	0.842	0.809	0.732	2.2E-09	0.055	0.074	0.095
42.00	0.718	0.737	0.758	0.688	1.7E-03	0.092	0.123	0.159
44.40	0.683	0.710	0.728	0.662	4.3E-03	0.095	0.126	0.163
47.33	0.659	0.693	0.705	0.647	9.7E-04	0.080	0.106	0.138
49.08	0.644	0.684	0.712	0.647	9.3E-04	0.076	0.101	0.131
65.55	0.607	0.634	0.634	0.590	2.7E-03	0.050	0.066	0.086
68.55	0.603	0.624	0.624	0.585	9.9E-03	0.047	0.062	0.081
71.35	0.618	0.623	0.617	0.584	2.5E-02	0.049	0.064	0.084
73.23	0.581	0.611	0.601	0.576	2.2E-03	0.044	0.059	0.076
89.55	0.565	0.584	0.561	0.541	1.3E-03	0.041	0.054	0.070
92.25	0.574	0.584	0.560	0.536	3.1E-03	0.043	0.057	0.074
94.95	0.613	0.589	0.562	0.541	2.3E-02	0.052	0.068	0.087
97.62	0.567	0.575	0.551	0.528	1.4E-03	0.041	0.054	0.069

Table 3.189. The mean ODs at each assessment time for isolate 250 in the presence of three concentrations of Revysol® LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.088	0.086	0.087	0.088	3.5E-03	0.002	0.003	0.004
19.25	0.336	1.414	1.360	1.279	1.2E-27	0.094	0.125	0.162
22.37	0.549	1.483	1.437	1.401	3.3E-13	0.168	0.223	0.291
25.17	0.448	1.105	1.210	1.042	2.8E-10	0.173	0.230	0.300
28.68	0.477	0.892	1.037	0.820	1.8E-06	0.177	0.236	0.307
43.32	0.440	0.979	0.930	0.816	1.8E-05	0.180	0.240	0.312
46.27	0.421	0.901	0.829	0.766	1.1E-07	0.131	0.174	0.227
49.03	0.408	0.900	0.843	0.783	2.2E-09	0.111	0.148	0.193
67.62	0.414	0.866	0.843	0.785	4.3E-10	0.099	0.132	0.172
70.32	0.363	0.834	0.782	0.769	2.9E-11	0.094	0.126	0.163
73.20	0.355	0.807	0.772	0.742	1.6E-11	0.090	0.120	0.156
76.52	0.350	0.810	0.769	0.741	1.7E-11	0.091	0.121	0.158
90.78	0.350	0.799	0.755	0.732	2.7E-11	0.091	0.122	0.159
94.18	0.350	0.777	0.725	0.701	5.6E-10	0.093	0.123	0.160
97.77	0.354	0.761	0.714	0.695	1.2E-09	0.092	0.123	0.160
97.60	0.379	0.718	0.744	0.689	1.9E-07	0.102	0.135	0.176

Table 3.190. The mean ODs at each assessment time for isolate 250 in the presence of three concentrations of Revysol® LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In the first run (Fig. 3.90), with treatments 1 and 5 ppm Revysol®, the OD values were consistently higher than the control until the test was stopped reflected in statistically significant differences between the control and the 1 and 5 ppm treatments at some timings (Table 3.188). The “growth” of the isolate 250 was significantly greater with the 1 and 5 ppm Revysol®

treatments compared to the control. In contrast, the OD values with the 25 ppm treatment showed a markedly lower initial increase and throughout the experiment thereafter were markedly (significant at the 0.1% level) lower than those of the control resulting in not surprisingly a markedly lower "growth" value (Table 3.192).

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.107	0.111	0.115	0.146	1.5E-16	0.009	0.012	0.016
15.90	1.199	1.350	1.398	1.559	5.6E-20	0.048	0.063	0.082
18.90	1.284	1.454	1.510	1.549	1.6E-11	0.056	0.074	0.096
21.58	1.358	1.537	1.569	1.533	2.7E-08	0.054	0.072	0.093
24.52	1.407	1.574	1.596	1.503	3.7E-06	0.062	0.083	0.107
40.48	1.263	1.599	1.609	1.475	7.0E-10	0.135	0.179	0.232
43.03	1.236	1.578	1.542	1.374	1.2E-04	0.188	0.250	0.324
45.63	1.138	1.571	1.561	1.202	9.1E-05	0.246	0.326	0.423
47.80	1.141	1.559	1.530	1.105	1.0E-06	0.211	0.280	0.363
64.63	0.815	1.393	1.473	0.695	3.6E-12	0.186	0.246	0.319
66.75	0.745	1.333	1.484	0.695	1.6E-12	0.186	0.246	0.319
70.10	0.735	1.011	1.124	0.687	5.8E-11	0.103	0.136	0.177
72.37	0.726	0.877	1.000	0.691	5.4E-06	0.102	0.136	0.176
88.33	0.657	0.763	0.852	0.627	5.3E-07	0.066	0.087	0.113
90.53	0.651	0.763	0.856	0.630	3.9E-06	0.067	0.089	0.116
92.67	0.645	0.754	0.806	0.623	7.2E-09	0.051	0.067	0.087
96.08	0.634	0.740	0.770	0.607	3.0E-12	0.037	0.050	0.064

Table 3.191. The mean ODs at each assessment time for isolate 250 in the presence of three concentrations of Revysol® LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Rev.25	31.27
Rev.5	83.08
Rev.1	77.70
Control	68.33
LSD5%	9.169
LSD1%	12.205
LSD 0.1%	15.896

Treatment	"Growth"
Rev.25	52.21
Rev.5	57.45
Rev.1	55.51
Control	53.86
LSD5%	5.50
LSD1%	7.30
LSD 0.1%	9.47

Treatment	"Growth"
Rev.25	26.96
Rev.5	76.22
Rev.1	74.38
Control	58.91
LSD5%	9.77
LSD1%	13.00
LSD 0.1%	16.93

Treatment	"Growth"
Rev. 25	79.80
Rev. 5	103.36
Rev. 1	107.41
Control	81.93
LSD5%	6.94
LSD1%	9.21
LSD0.1%	11.94

Table 3.192. and 3.193. and 3.194. and 3.195. The mean "growth" of isolate 250 in treatments with 25, 5, and 1 ppm of Revysol® over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

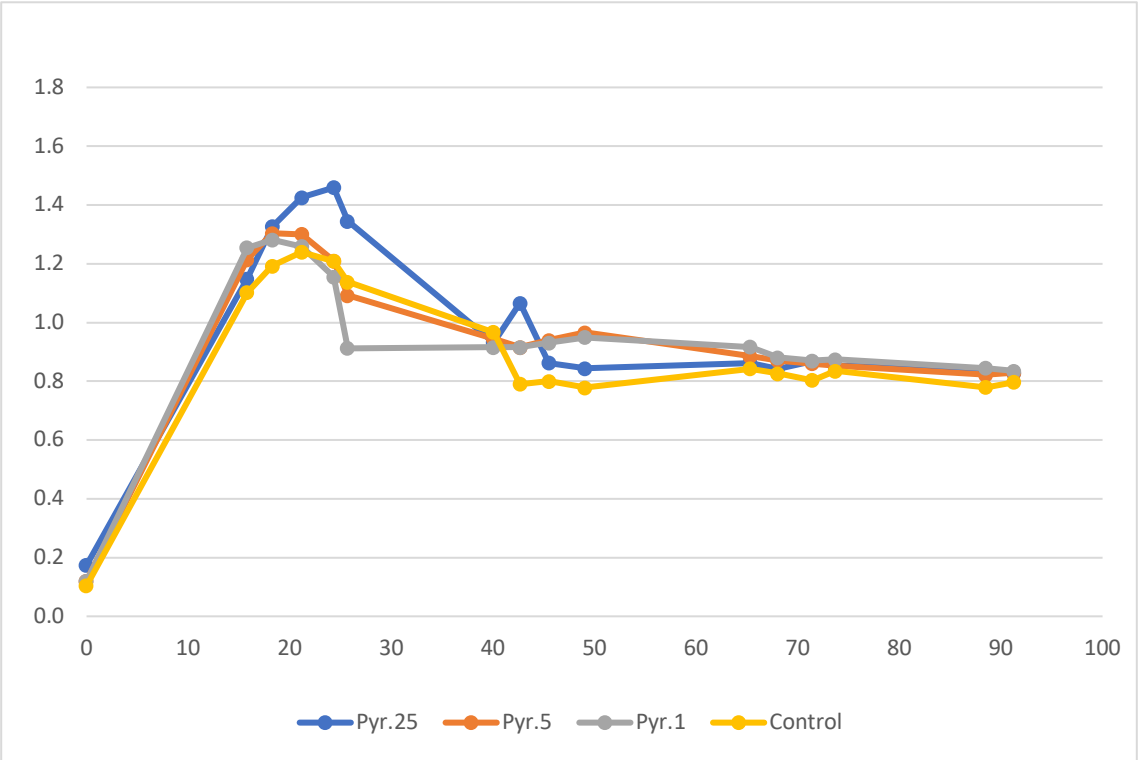


Figure 3.94. The OD curve of isolate 250 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

In the second (Fig. 3.91, Table 3.189) and third runs (Fig. 3.92, Table 3.190), after an initial increase and reaching peak OD values at 17.77 and 22.40 hours respectively the OD values started a steep decline for 6 hours or so and then decreased more gently towards the end of experiment. In the second trial, where there was a tendency for the OD values of the Revysol® treatments to be slightly higher than the control after the steep decline had finished (23.3 hours) and in some cases statistically significant differences were observed.

In the third trial run (Fig. 3.92), the OD values of the 1 and 5 ppm treatments were numerically higher than those of the control over the experimental period with the occasional statistically significant difference (Table 3.190).

The isolate 250 with treatments with 1 and 5 ppm Revysol® showed significantly greater "growth" compared to the control in the third run (Table 3.194) and numerically greater "growth" was observed with particularly 5 ppm in the second (Table 3.193). Similar to the first run (Table 3.192), in the third run the OD values of the 25 ppm were much lower than those of the control and 1 and 5 ppm treatments, resulting in a very significant lower "growth" at the 0.1 % level. In the second run the OD values and the "growth" of the 25ppm treatment differed little from the control.

In the last run (Fig. 3.93), although the control reached a peak value before the 1 and 5 ppm treatments, after reaching an early peak at 15.90 hours the OD value started to decline more

quickly reflected in very significant differences between these treatments and the control at the 0.1 % level from 47.80 hours on (Table 3.191). Moreover the "growth" the isolate with the 1 and 5 ppm treatments showed very significant increases (at the 0.1% level) compared to the control (Table 3.195).

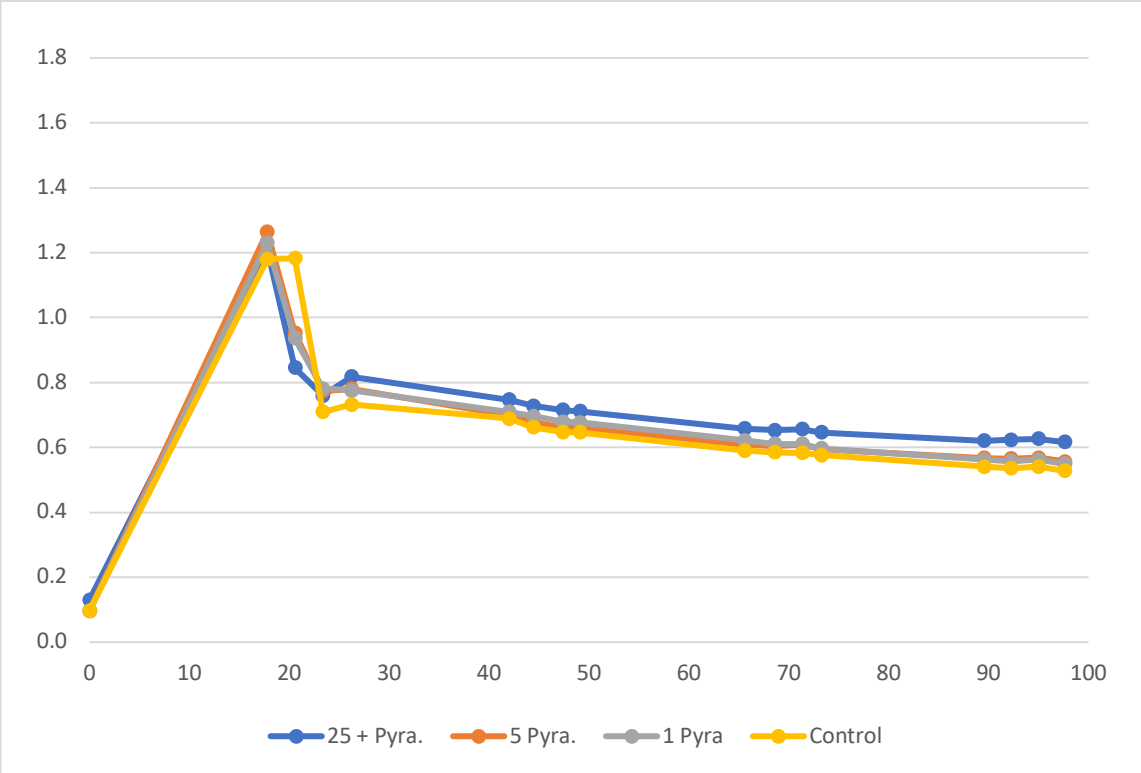


Figure 3.95. The OD curve of isolate 250 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

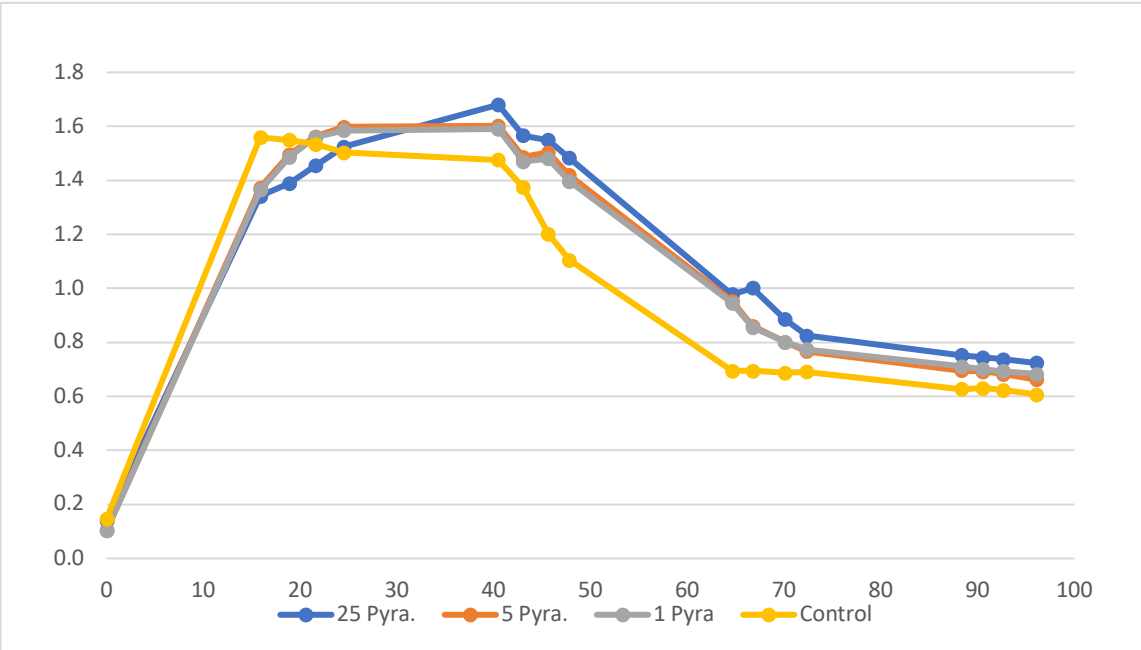


Figure 3.96. The OD curve of isolate 250 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

All runs then suggested that the "growth" of the isolate 250 was enhanced in the presence of 1 and 5 ppm Revysol®. The "growth" observed with these treatments was significantly greater than that of the control, except in the second trial where the higher "growth" was only numerically. Nevertheless, in contrast, the 25 ppm Revysol® treatment consistently resulted in lower "growth" values indicating an inhibitory effect.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.174	0.121	0.119	0.104	1.0E-11	0.013	0.018	0.023
15.95	1.149	1.214	1.254	1.102	8.5E-45	0.044	0.058	0.076
18.97	1.327	1.304	1.282	1.192	1.5E-45	0.046	0.061	0.079
21.60	1.426	1.300	1.259	1.240	2.7E-28	0.092	0.123	0.160
24.60	1.459	1.210	1.156	1.209	2.0E-08	0.238	0.317	0.413
40.50	1.345	1.092	0.912	1.138	2.0E-06	0.255	0.339	0.442
43.05	0.932	0.945	0.916	0.967	8.0E-04	0.249	0.331	0.431
45.72	1.066	0.916	0.915	0.791	5.2E-04	0.241	0.320	0.417
47.82	0.862	0.940	0.931	0.800	1.0E-05	0.223	0.297	0.387
64.67	0.844	0.965	0.949	0.778	2.9E-05	0.216	0.288	0.375
66.80	0.862	0.885	0.917	0.842	5.2E-04	0.136	0.181	0.235
70.08	0.842	0.872	0.881	0.827	1.3E-04	0.119	0.158	0.206
72.38	0.868	0.860	0.870	0.803	4.2E-05	0.116	0.155	0.202
88.40	0.853	0.854	0.874	0.836	5.5E-05	0.116	0.155	0.202
90.53	0.842	0.822	0.845	0.779	1.5E-06	0.106	0.141	0.184
92.67	0.828	0.828	0.835	0.797	2.0E-06	0.109	0.145	0.189

Table 3.196. The mean ODs at each assessment time for isolate 250 in the presence of three concentrations of pyraclostrobin LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.130	0.097	0.097	0.097	2.3E-29	0.003	0.004	0.006
17.77	1.211	1.264	1.230	1.179	3.0E-02	0.204	0.271	0.351
20.57	0.845	0.953	0.936	1.183	2.6E-03	0.204	0.271	0.352
23.33	0.760	0.773	0.781	0.709	7.3E-08	0.069	0.091	0.119
26.22	0.818	0.780	0.775	0.732	2.2E-09	0.055	0.074	0.095
42.00	0.747	0.698	0.710	0.688	1.7E-03	0.092	0.123	0.159
44.40	0.728	0.676	0.697	0.662	4.3E-03	0.095	0.126	0.163
47.33	0.716	0.662	0.679	0.647	9.7E-04	0.080	0.106	0.138
49.08	0.712	0.660	0.677	0.647	9.3E-04	0.076	0.101	0.131
65.55	0.658	0.609	0.622	0.590	2.7E-03	0.050	0.066	0.086
68.55	0.653	0.604	0.611	0.585	9.9E-03	0.047	0.062	0.081
71.35	0.656	0.608	0.610	0.584	2.5E-02	0.049	0.064	0.084
73.23	0.646	0.594	0.597	0.576	2.2E-03	0.044	0.059	0.076
89.55	0.621	0.568	0.562	0.541	1.3E-03	0.041	0.054	0.070
92.25	0.623	0.566	0.558	0.536	3.1E-03	0.043	0.057	0.074
94.95	0.626	0.569	0.562	0.541	2.3E-02	0.052	0.068	0.087
97.62	0.616	0.556	0.550	0.528	1.4E-03	0.041	0.054	0.069

Table 3.197. The mean ODs at each assessment time for isolate 250 in the presence of three concentrations of pyraclostrobin LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.140	0.103	0.104	0.146	1.5E-16	0.009	0.012	0.016
15.90	1.342	1.373	1.365	1.559	5.6E-20	0.048	0.063	0.082
18.90	1.389	1.496	1.486	1.549	1.6E-11	0.056	0.074	0.096
21.58	1.454	1.563	1.559	1.533	2.7E-08	0.054	0.072	0.093
24.52	1.524	1.598	1.584	1.503	3.7E-06	0.062	0.083	0.107
40.48	1.680	1.602	1.591	1.475	7.0E-10	0.135	0.179	0.232
43.03	1.566	1.486	1.470	1.374	1.2E-04	0.188	0.250	0.324
45.63	1.549	1.504	1.481	1.202	9.1E-05	0.246	0.326	0.423
47.80	1.483	1.419	1.397	1.105	1.0E-06	0.211	0.280	0.363
64.63	0.977	0.953	0.946	0.695	3.6E-12	0.186	0.246	0.319
66.75	1.001	0.861	0.856	0.695	1.6E-12	0.186	0.246	0.319
70.10	0.886	0.801	0.801	0.687	5.8E-11	0.103	0.136	0.177
72.37	0.825	0.767	0.774	0.691	5.4E-06	0.102	0.136	0.176
88.33	0.752	0.695	0.712	0.627	5.3E-07	0.066	0.087	0.113
90.53	0.744	0.692	0.701	0.630	3.9E-06	0.067	0.089	0.116
92.67	0.737	0.683	0.694	0.623	7.2E-09	0.051	0.067	0.087
96.08	0.725	0.663	0.682	0.607	3.0E-12	0.037	0.050	0.064

Table 3.198. The mean ODs at each assessment time for isolate 250 in the presence of three concentrations of pyraclostrobin LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The cross-screening test with the isolate 250 and the fungicide pyraclostrobin was run three times. In the first run, after an initial increase the OD value of each group reached a peak between 18 and 25 hours with the highest recorded peak with 25 ppm (1.4) at 24.33 hours and the lowest with control at 21.18 hours reflected in significant differences with 1 and 5 ppm during the first 20 hours and with the 25 ppm until 24.60 hours (Table 3.196).

After the peak, the OD values initially declined steeply followed by a gentile decline before leveling off towards the end of experiment (Fig. 3.94). The “growth”, of the isolate in the presence of the 1 and 5 ppm pyraclostrobin treatments was numerically slightly higher than the control (Table 3.199).

In the second trial (Fig. 3.95), with both the pyraclostrobin treatments and the control the OD values showed a similar tendency. After an initial increase a peak was reached at 17.77 hours followed by a gentile decline from 26.22 hours onwards. However, from 26.22 hours with the 25 ppm the OD value was greater than the control and from 65.6 hours, the difference was statistically significant until the test was stopped (Table 3.197). Despite this however, the “growth” of all treatments, including the 25ppm treatment are only very slightly above that of the control and in all cases statistical significance is not reached. It should be noted that the initial value of the 25ppm treatment is higher than the other treatments and the control and in

calculating the "growth" this initial value is subtracted from the values obtained at the other assessment timings. Accordingly, the significant increases in the OD values seen in the latter half of the experiment do not result in an increase in the "growth" parameter (Table 3.200).
In the third trial, with the control the OD value was consistently higher those of the treatments prior to reaching the maximum observed OD value at 15.90 hours. Afterwards, the OD value of the control started to decline slightly while the treatments continued to increase somewhat, in particular the 25 ppm, which showed the highest recorded value (1.7) at 40.48 hours (Table 3.198). Thereafter, there was a steep fall with the pyraclostrobin treatments, and the control followed by a gentler decrease towards the end of experiment (Fig. 3.97). The earlier decline with the control resulted in significant differences between the control and treatments from 40 hours onwards resulting in significant increases in "growth" for all three treatments compared to the control (Table 3.201). Thus, in all three trials there is some evidence for enhanced "growth" of the isolate 250 in the presence of the fungicide pyraclostrobin.

Treatment	"Growth"
Pyra. 25	68.70
Pyra. 5	71.98
Pyra. 1	71.08
Control	68.33
LSD5%	9.169
LSD1%	12.205
LSD 0.1%	15.896

Treatment	"Growth"
Pyra. 25	55.70
Pyra. 5	55.90
Pyra. 1	55.95
Control	53.86
LSD5%	5.50
LSD1%	7.30
LSD 0.1%	9.47

Treatment	"Growth"
Pyra. 25	93.86
Pyra. 5	94.81
Pyra. 1	94.29
Control	81.93
LSD5%	6.94
LSD1%	9.21
LSD 0.1%	11.94

Table 3.199. and 3.200. and 3.201. The mean "growth" of isolate 250 in treatments with 25, 5, and 1 ppm of pyraclostrobin over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

The cross-screening test with the isolate 250 and the fungicide fluxapyroxad was run on three occasions. In all three tests, the tendency of OD value was identical. There was an initial rapid increase in the OD until peak values were recorded between 18 and 25 hours followed by a steep decline, which eventually became more gradual towards the end of the experiment. In the first two trials (Fig. 3.97, Table 3.202 and Fig. 3.98, Table 3.203 respectively), the control OD values were generally lower than those of the treatments at most timings. Unsurprisingly then the "growth" of the treatments was higher than that of control, in particular the 1 ppm treatment in the first run (Table 3.205) and all three rates in the second run the differences were statistically significant (Table 3.206).

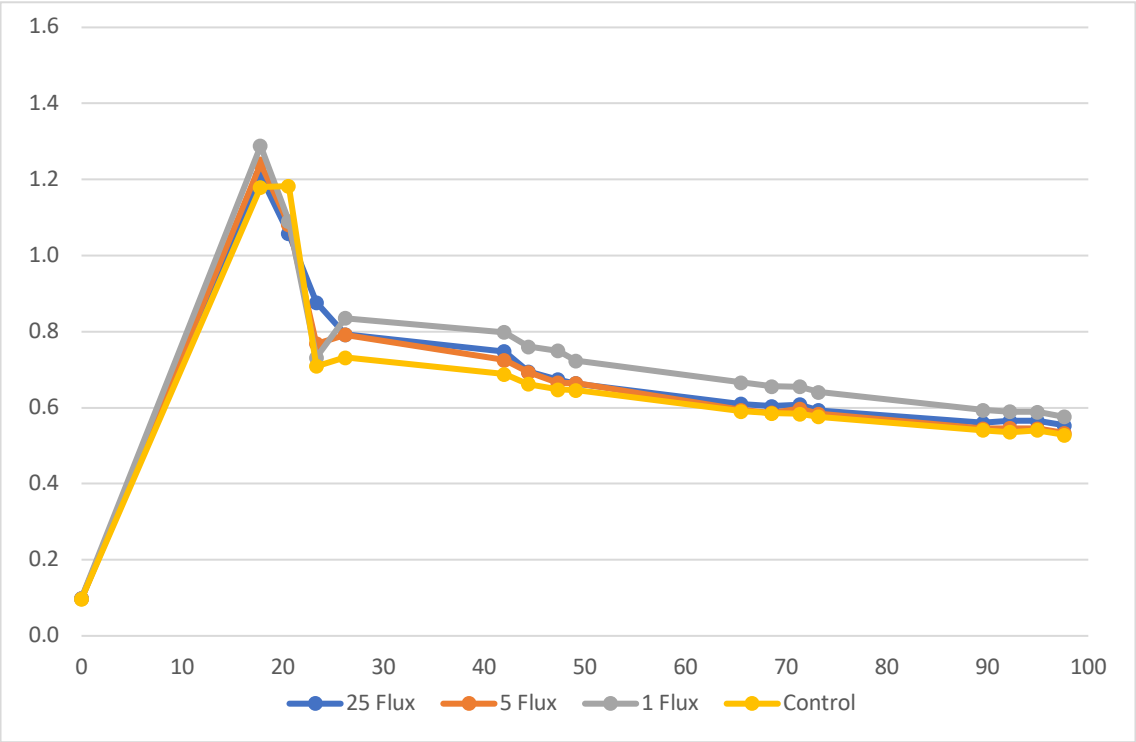


Figure 3.97. The OD curve of isolate 250 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

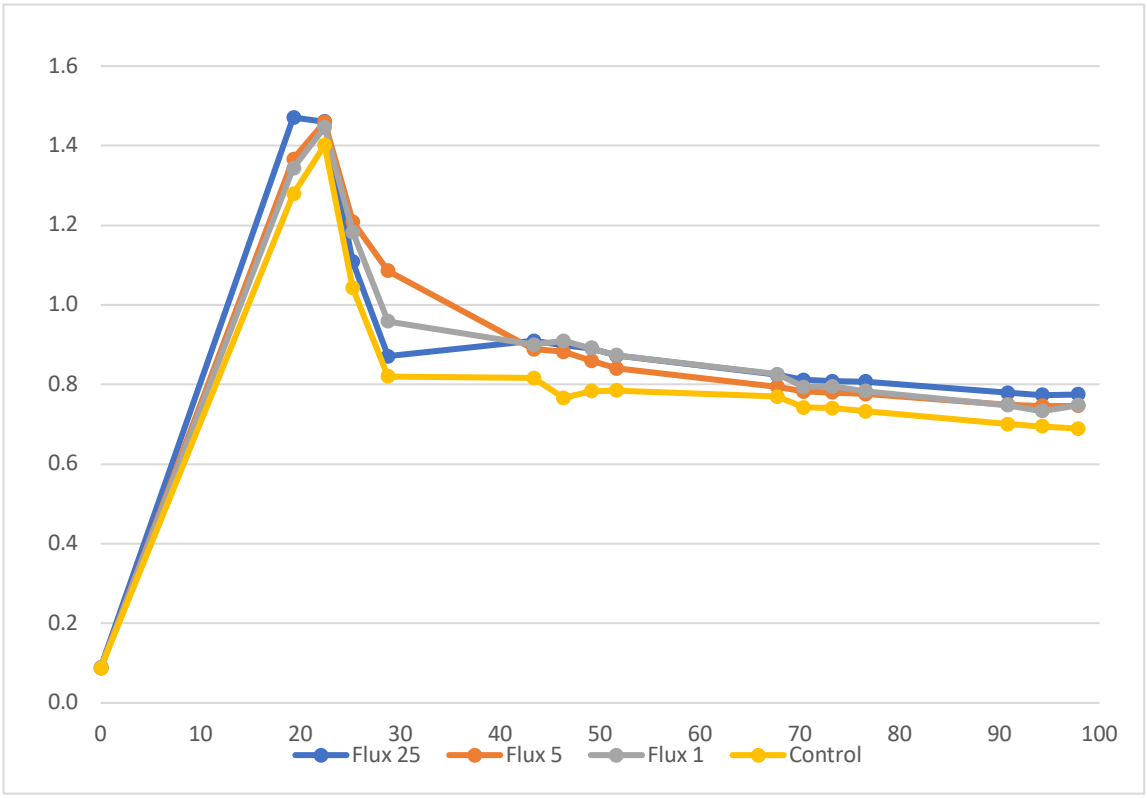


Figure 3.98. The OD curve of isolate 250 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

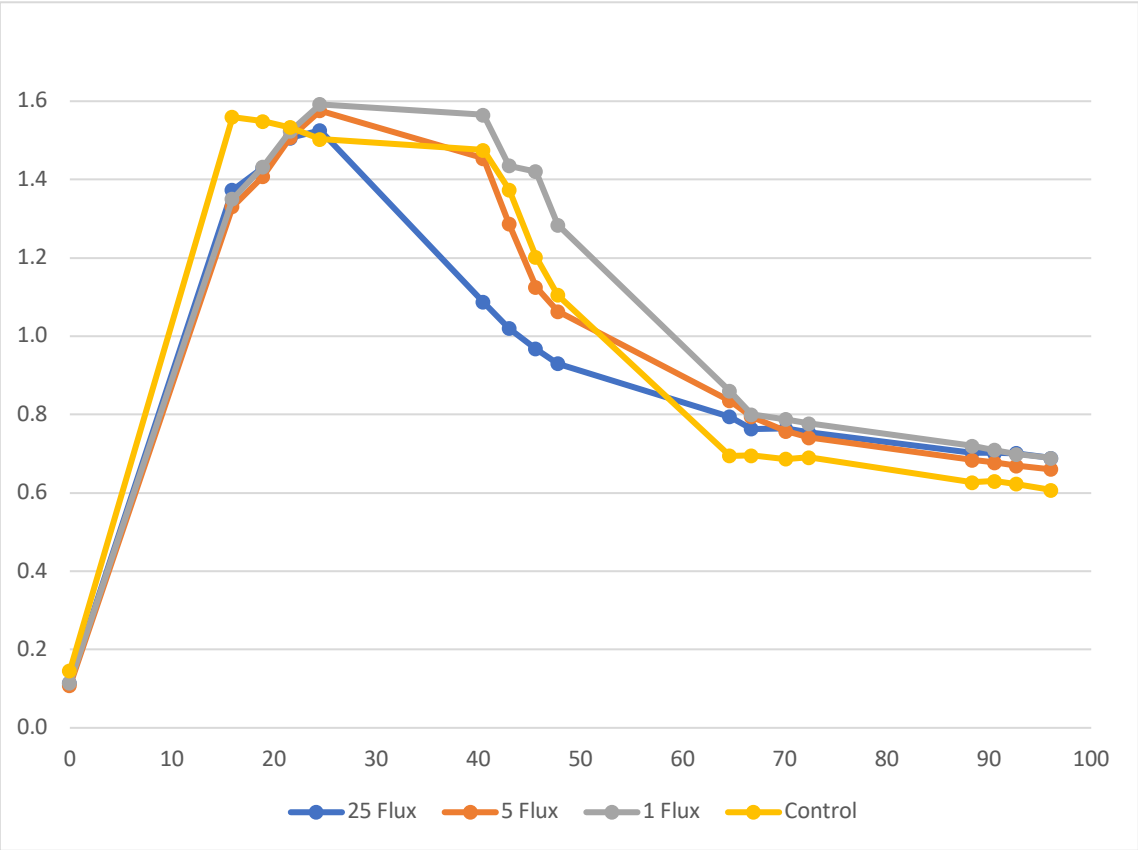


Figure 3.99. The OD curve of isolate 250 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.098	0.098	0.099	0.097	2.3E-29	0.003	0.004	0.006
17.77	1.209	1.241	1.288	1.179	3.0E-02	0.204	0.271	0.351
20.57	1.058	1.082	1.090	1.183	2.6E-03	0.204	0.271	0.352
23.33	0.877	0.768	0.732	0.709	7.3E-08	0.069	0.091	0.119
26.22	0.792	0.792	0.836	0.732	2.2E-09	0.055	0.074	0.095
42.00	0.748	0.725	0.799	0.688	1.7E-03	0.092	0.123	0.159
44.40	0.695	0.693	0.761	0.662	4.3E-03	0.095	0.126	0.163
47.33	0.674	0.665	0.750	0.647	9.7E-04	0.080	0.106	0.138
49.08	0.664	0.665	0.723	0.647	9.3E-04	0.076	0.101	0.131
65.55	0.610	0.594	0.667	0.590	2.7E-03	0.050	0.066	0.086
68.55	0.604	0.586	0.656	0.585	9.9E-03	0.047	0.062	0.081
71.35	0.608	0.598	0.655	0.584	2.5E-02	0.049	0.064	0.084
73.23	0.593	0.584	0.641	0.576	2.2E-03	0.044	0.059	0.076
89.55	0.560	0.545	0.594	0.541	1.3E-03	0.041	0.054	0.070
92.25	0.566	0.546	0.590	0.536	3.1E-03	0.043	0.057	0.074
94.95	0.565	0.545	0.589	0.541	2.3E-02	0.052	0.068	0.087
97.62	0.553	0.534	0.577	0.528	1.4E-03	0.041	0.054	0.069

Table 3.202. The mean ODs at each assessment time for isolate 250 in the presence of three concentrations of fluxapyroxad LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.089	0.087	0.088	0.088	3.5E-03	0.002	0.003	0.004
19.25	1.471	1.366	1.344	1.279	1.2E-27	0.094	0.125	0.162
22.37	1.461	1.459	1.446	1.401	3.3E-13	0.168	0.223	0.291
25.17	1.109	1.209	1.183	1.042	2.8E-10	0.173	0.230	0.300
28.68	0.871	1.086	0.959	0.820	1.8E-06	0.177	0.236	0.307
43.32	0.910	0.889	0.900	0.816	1.8E-05	0.180	0.240	0.312
46.27	0.899	0.882	0.910	0.766	1.1E-07	0.131	0.174	0.227
49.03	0.891	0.859	0.891	0.783	2.2E-09	0.111	0.148	0.193
67.62	0.872	0.841	0.873	0.785	4.3E-10	0.099	0.132	0.172
70.32	0.825	0.795	0.826	0.769	2.9E-11	0.094	0.126	0.163
73.20	0.811	0.782	0.794	0.742	1.6E-11	0.090	0.120	0.156
76.52	0.808	0.780	0.796	0.741	1.7E-11	0.091	0.121	0.158
90.78	0.807	0.776	0.783	0.732	2.7E-11	0.091	0.122	0.159
94.18	0.779	0.750	0.748	0.701	5.6E-10	0.093	0.123	0.160
97.77	0.773	0.746	0.733	0.695	1.2E-09	0.092	0.123	0.160
97.60	0.776	0.746	0.748	0.689	1.9E-07	0.102	0.135	0.176

Table 3.203. The mean ODs at each assessment time for isolate 250 in the presence of three concentrations of fluxapyroxad LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.114	0.107	0.115	0.146	1.5E-16	0.009	0.012	0.016
15.90	1.373	1.331	1.351	1.559	5.6E-20	0.048	0.063	0.082
18.90	1.430	1.407	1.432	1.549	1.6E-11	0.056	0.074	0.096
21.58	1.506	1.507	1.524	1.533	2.7E-08	0.054	0.072	0.093
24.52	1.525	1.576	1.592	1.503	3.7E-06	0.062	0.083	0.107
40.48	1.087	1.454	1.565	1.475	7.0E-10	0.135	0.179	0.232
43.03	1.020	1.287	1.435	1.374	1.2E-04	0.188	0.250	0.324
45.63	0.968	1.126	1.420	1.202	9.1E-05	0.246	0.326	0.423
47.80	0.930	1.063	1.284	1.105	1.0E-06	0.211	0.280	0.363
64.63	0.795	0.836	0.860	0.695	3.6E-12	0.186	0.246	0.319
66.75	0.764	0.794	0.800	0.695	1.6E-12	0.186	0.246	0.319
70.10	0.764	0.757	0.788	0.687	5.8E-11	0.103	0.136	0.177
72.37	0.754	0.741	0.777	0.691	5.4E-06	0.102	0.136	0.176
88.33	0.703	0.684	0.720	0.627	5.3E-07	0.066	0.087	0.113
90.53	0.702	0.677	0.709	0.630	3.9E-06	0.067	0.089	0.116
92.67	0.701	0.669	0.698	0.623	7.2E-09	0.051	0.067	0.087
96.08	0.689	0.660	0.689	0.607	3.0E-12	0.037	0.050	0.064

Table 3.204. The mean ODs at each assessment time for isolate 250 in the presence of three concentrations of fluxapyroxad LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In the last trial (Fig. 3.99), the control consistently showed OD values which were numerically greater than fluxapyroxad treatments until reaching its peak value. From then on however, the higher ODs with the 1 ppm treatment throughout the remainder of the experiment and the 5

ppm treatment at specific timings were observed compared to the control resulting in a statistically significant increase in “growth” with the 1ppm fluxapyroxad treatment and a numerically increased “growth” for the 5ppm treatment (Table 3.204, 3.207). In this case however no increase in “growth” was observed for the 25ppm treatment. Nevertheless, the observation that in all three tests, the “growth” with all three rates of fluxapyroxad was higher than the control points in the direction of the enhanced “growth” of the strain 250 in the presence of fluxapyroxad.

Treatment	“Growth”
Flux. 25	56.45
Flux. 5	55.73
Flux. 1	60.33
Control	53.86
LSD5%	5.50
LSD1%	7.30
LSD0.1%	9.47

Treatment	“Growth”
Flux. 25	75.98
Flux. 5	75.50
Flux. 1	74.80
Control	58.91
LSD5%	9.77
LSD1%	13.00
LSD0.1%	16.93

Treatment	“Growth”
Flux. 25	79.11
Flux. 5	85.43
Flux. 1	90.64
Control	81.93
LSD5%	6.94
LSD1%	9.21
LSD0.1%	11.94

Table 3.205. and 3.206. and 3.207. The mean “growth” of isolate 250 in treatments with 25, 5, and 1 ppm of fluxapyroxad over the four-day experimental period. Significant increases in “growth” compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

3.2.4.12. Isolate 280

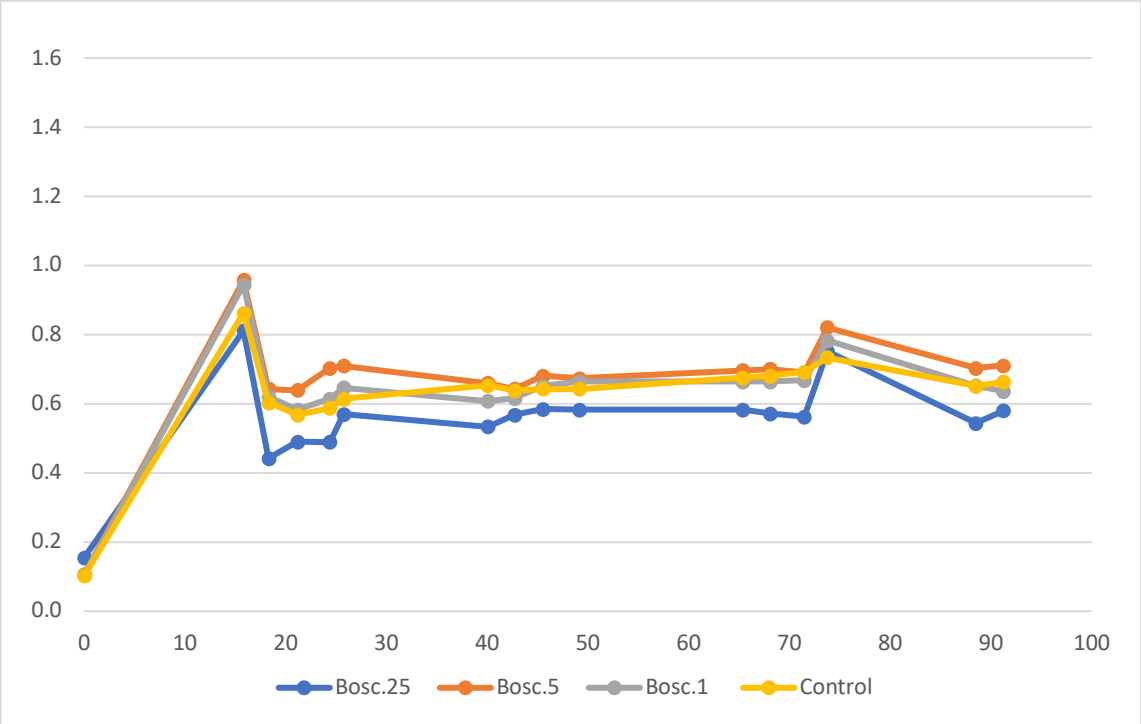


Figure 3.100. The OD curve of isolate 280 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

The cross-screening test with the isolate 280 and the fungicide boscalid was run on three occasions. In the first and third trials (Fig. 3.100, Table 3.208 and Fig. 3.102, Table 3.210), there was a similar tendency for OD values to have an initial growth until reaching the maximum observed OD values at 15.83 and 20.50 hours in the first and the third run respectively. Then, a steep decline followed until 18.30 hours in the first and 23.32 hours in the third. Thereafter, OD values leveled off for the remainder of the test in the third run and until 71.47 hours in the first run where there was a gentile increase until the next assessment followed by plateauing off.

In the second trial, there was no peak OD value recorded and since the first measurement after the initial reading immediately after setting up the test was at 19.15 hours, it's assumed that the peak value might occur between time zero and 19.15 hours. Like the other two trials, the OD values plateaued out after the initial increase in the OD values until the test was stopped (Fig. 3.101).

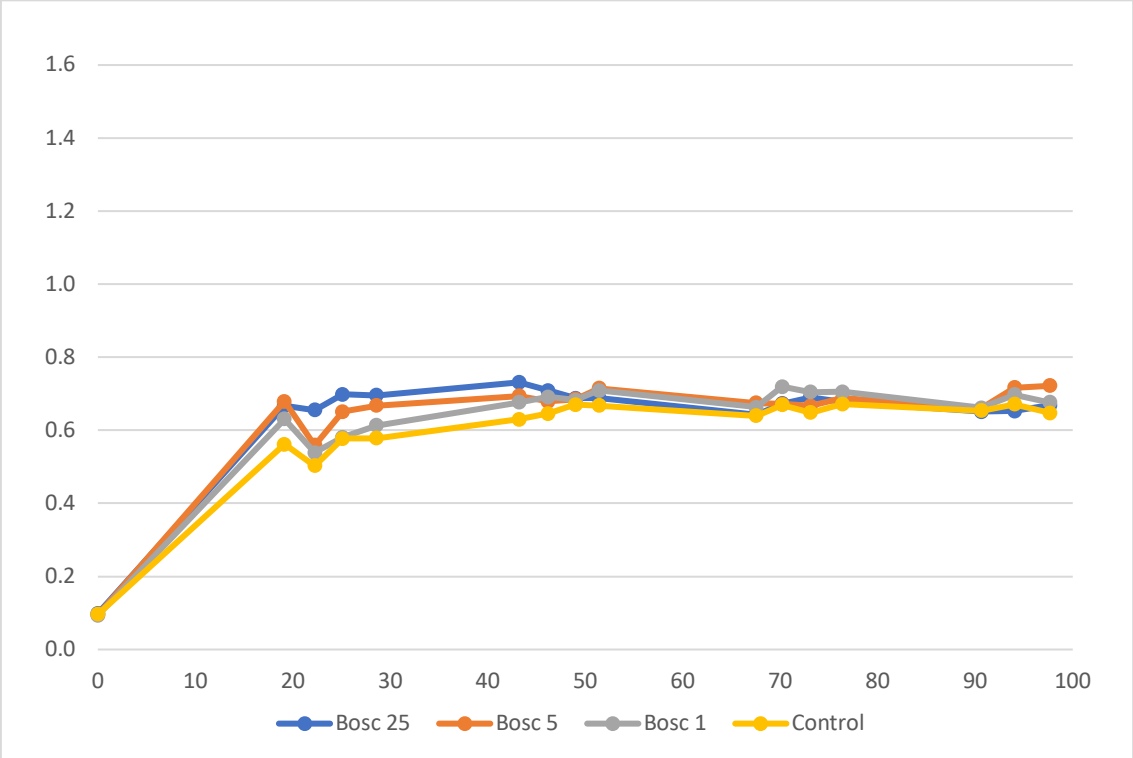


Figure 3.101. The OD curve of isolate 280 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

In the first and third trial, the 1 and 5 ppm had higher OD values compared to the control throughout the whole experiment. Although the statistical significances were only reached at some assessment timings within the first 25 hours in the first run (Table 3.208) and within the first 42 hours in the third trial (Table 3.210). In contrast, the OD values with the 25 ppm

treatment were lower than the control, and these differences were statistically significant at most timings in the first trial and from 65 hours onwards in the third trial.

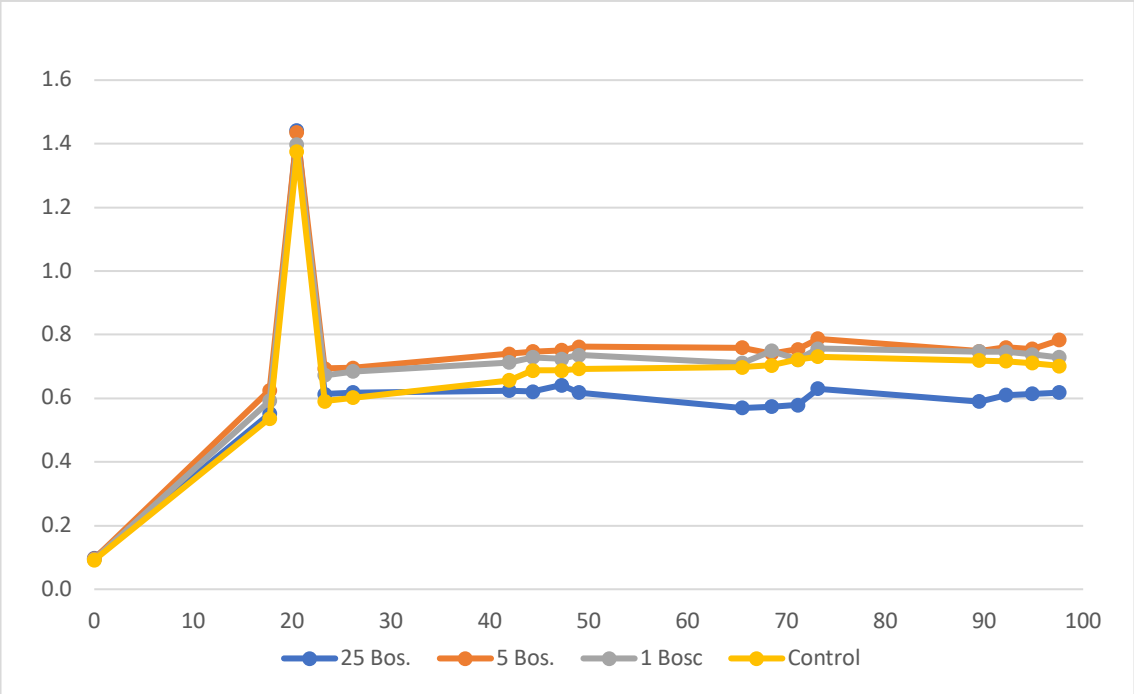


Figure 3.102. The OD curve of isolate 280 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Bosc.25	Bosc.5	Bosc.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.155	0.106	0.104	0.103	3.3E-30	0.007	0.009	0.012
15.92	0.812	0.957	0.944	0.863	9.3E-34	0.055	0.073	0.095
18.38	0.442	0.643	0.620	0.603	2.9E-23	0.088	0.117	0.152
21.27	0.491	0.639	0.582	0.568	6.8E-13	0.095	0.126	0.164
24.45	0.489	0.703	0.614	0.587	4.6E-28	0.044	0.059	0.076
25.78	0.570	0.709	0.646	0.615	1.2E-24	0.053	0.070	0.091
40.13	0.534	0.660	0.608	0.653	6.7E-14	0.066	0.087	0.114
45.62	0.568	0.643	0.616	0.639	1.9E-11	0.066	0.088	0.115
49.17	0.585	0.681	0.652	0.643	1.9E-10	0.075	0.100	0.131
65.40	0.582	0.673	0.664	0.643	9.0E-17	0.071	0.094	0.122
68.10	0.582	0.697	0.664	0.675	1.1E-26	0.053	0.071	0.092
71.53	0.571	0.700	0.664	0.681	1.9E-30	0.045	0.059	0.077
73.78	0.563	0.690	0.668	0.692	1.2E-29	0.047	0.062	0.081
88.62	0.753	0.821	0.783	0.733	4.2E-15	0.103	0.137	0.179
91.35	0.544	0.703	0.652	0.652	4.8E-23	0.059	0.079	0.103
96.18	0.580	0.711	0.636	0.664	1.1E-22	0.058	0.078	0.101

Table 3.208. The mean ODs at each assessment time for isolate 280 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In the second trial, the OD values with all boscalid treatments were higher than the control at most timings and statistically significant with the 25 ppm up to 43 hours (Table 3.209).
Reflecting these results, the "growth" of the treatments with 25 ppm in the first and third trial was significantly lower than the control (Table 3.211 and 3.213 respectively). The treatments with 1 and 5 ppm had a higher "growth", however the significant level was reached only with the 5 ppm in the first run. Although it should be noted that the 5ppm treatment in the third trial bordered on statistical significance. But in the second trial (Table 3.212) all treatments had significantly higher "growth" than the control (Table 3.213). The three experiments suggest that the growth of the isolate 280 is enhanced in the presence of the fungicide boscalid at 5 ppm.

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.099	0.095	0.094	0.096	1.1E-01	0.003	0.004	0.006
19.25	0.667	0.679	0.631	0.561	4.6E-10	0.104	0.138	0.180
22.37	0.655	0.559	0.539	0.503	3.2E-05	0.119	0.159	0.207
25.17	0.699	0.651	0.581	0.577	2.9E-11	0.092	0.123	0.160
28.68	0.696	0.668	0.614	0.578	4.2E-12	0.086	0.114	0.149
43.32	0.731	0.694	0.676	0.630	1.8E-09	0.096	0.128	0.167
46.27	0.709	0.679	0.692	0.646	5.8E-10	0.093	0.124	0.161
49.03	0.688	0.685	0.683	0.670	8.1E-10	0.096	0.128	0.166
67.62	0.688	0.715	0.709	0.668	2.8E-12	0.089	0.118	0.154
70.32	0.643	0.674	0.664	0.639	9.1E-11	0.093	0.124	0.161
73.20	0.673	0.672	0.720	0.670	9.0E-11	0.097	0.129	0.168
76.52	0.690	0.667	0.704	0.648	1.1E-11	0.094	0.125	0.162
90.78	0.677	0.688	0.706	0.672	1.9E-10	0.106	0.141	0.183
94.18	0.651	0.660	0.662	0.654	2.7E-11	0.100	0.133	0.173
97.77	0.653	0.717	0.698	0.671	1.7E-10	0.106	0.141	0.183
97.60	0.668	0.722	0.677	0.648	1.2E-11	0.097	0.129	0.168

Table 3.209. The mean ODs at each assessment time for isolate 280 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The cross-screening test with the isolate 280 and the fungicide Revysol® was run three times. The OD values with 25 ppm increased slightly relative to the initial value at the start of the experiment in all three tests. This led to statistically significant differences compared to the control.
In the first and third trials (Fig. 3.103 and 3.105 respectively), the OD values showed an initial increase until reaching a peak value at 15.83 hours in the first and 20.50 hours in the second run. A steep decline followed and then from 24.37 hours in the first and 23.32 hours in the third trial the OD started to level off until the end of experiment.

In the first run the OD values with the Revysol® treatments remained very similar to the one with the control (Table 3.214, 3.217). But in the third run, with the control OD values were lower than those with the 1 and 5 ppm Revysol® treatments over the course of the experiment with significant differences occurring between 23.3 and 47.3 hours (Table 3.216).

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.098	0.096	0.094	0.092	1.4E-16	0.012	0.016	0.021
17.75	0.552	0.626	0.592	0.537	7.4E-11	0.084	0.111	0.144
20.50	1.443	1.436	1.398	1.375	2.1E-15	0.143	0.190	0.246
23.32	0.613	0.694	0.673	0.591	2.9E-16	0.071	0.095	0.123
26.20	0.619	0.696	0.685	0.602	5.9E-14	0.077	0.102	0.132
41.95	0.624	0.740	0.713	0.656	9.0E-16	0.072	0.096	0.125
44.35	0.621	0.747	0.729	0.688	8.6E-17	0.074	0.099	0.128
47.27	0.641	0.751	0.724	0.687	1.1E-15	0.078	0.103	0.134
49.02	0.619	0.762	0.736	0.693	2.2E-14	0.083	0.110	0.143
65.52	0.570	0.759	0.711	0.698	5.6E-17	0.079	0.105	0.136
68.52	0.574	0.741	0.749	0.704	6.8E-17	0.081	0.107	0.139
71.17	0.579	0.754	0.722	0.722	1.3E-16	0.082	0.109	0.141
73.18	0.631	0.788	0.756	0.731	1.7E-16	0.086	0.115	0.149
89.50	0.590	0.748	0.748	0.719	3.0E-21	0.074	0.098	0.127
92.20	0.610	0.760	0.746	0.716	1.6E-18	0.081	0.108	0.140
94.85	0.615	0.755	0.738	0.710	2.2E-19	0.079	0.105	0.136
97.58	0.618	0.783	0.729	0.702	2.3E-18	0.083	0.110	0.142

Table 3.210. The mean ODs at each assessment time for isolate 280 in the presence of three concentrations of boscalid LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Bosc. 25	37.30
Bosc. 5	51.81
Bosc. 1	48.81
Control	47.72
LSD5%	2.325
LSD1%	3.095
LSD 0.1%	4.032

Treatment	"Growth"
Bosc. 25	51.08
Bosc. 5	51.40
Bosc. 1	50.15
Control	35.33
LSD5%	8.78
LSD1%	11.69
LSD 0.1%	15.22

Treatment	"Growth"
Bosc. 25	46.98
Bosc. 5	58.47
Bosc. 1	56.49
Control	53.00
LSD5%	5.67
LSD1%	7.52
LSD 0.1%	9.76

Table 3.211. and 3.212. and 3.213. The mean "growth" of isolate 280 in treatments with 25, 5, and 1 ppm of boscalid over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

In the second trial, the higher OD values with 1 and 5 ppm in most timing points compared to the one with the control were reflected in the significant "growth" of the 1 ppm at 0.1% level and 5 ppm at 1% level (Table 3.215, 3.218).

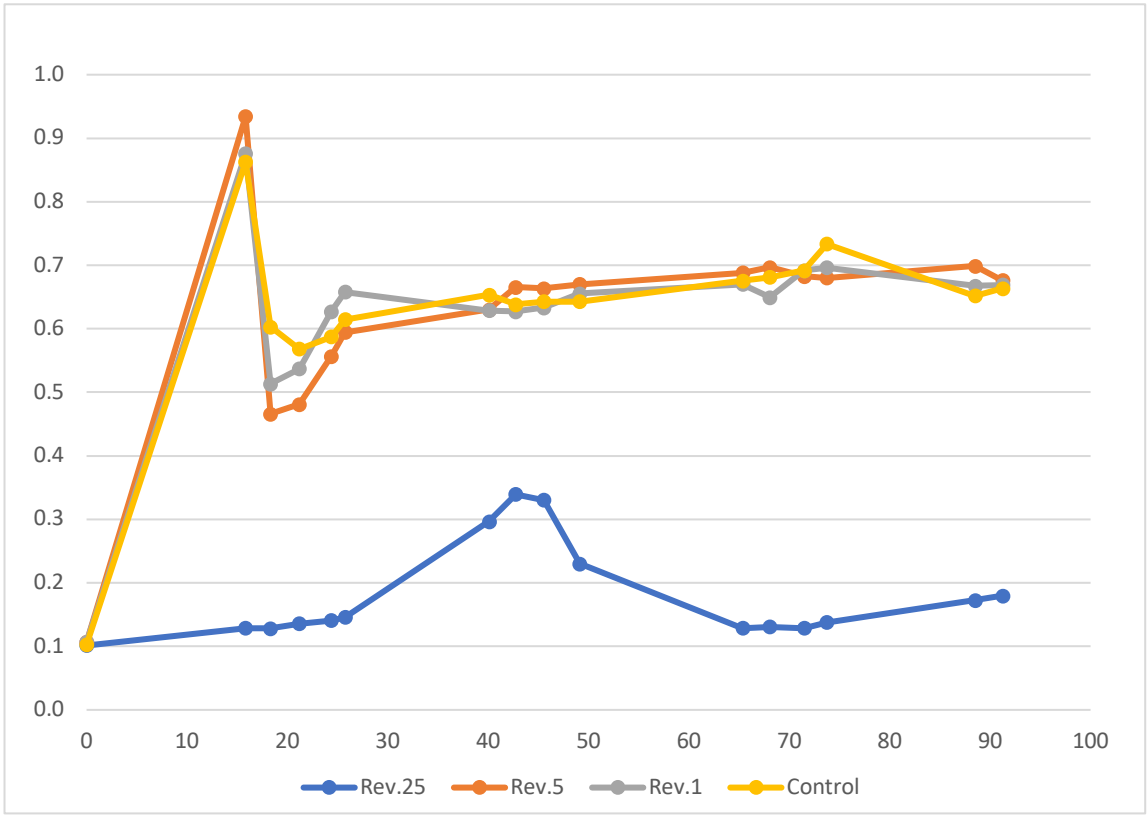


Figure 3.103. The OD curve of isolate 280 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

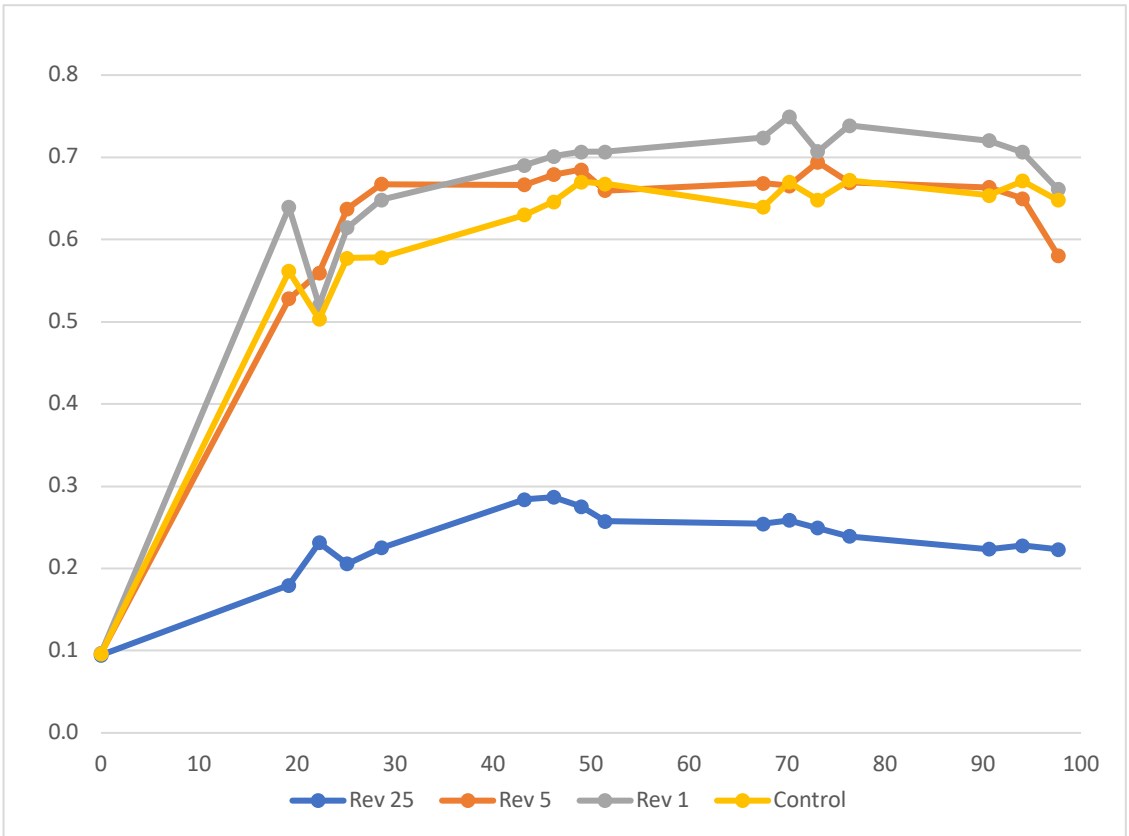


Figure 3.104. The OD curve of isolate 280 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

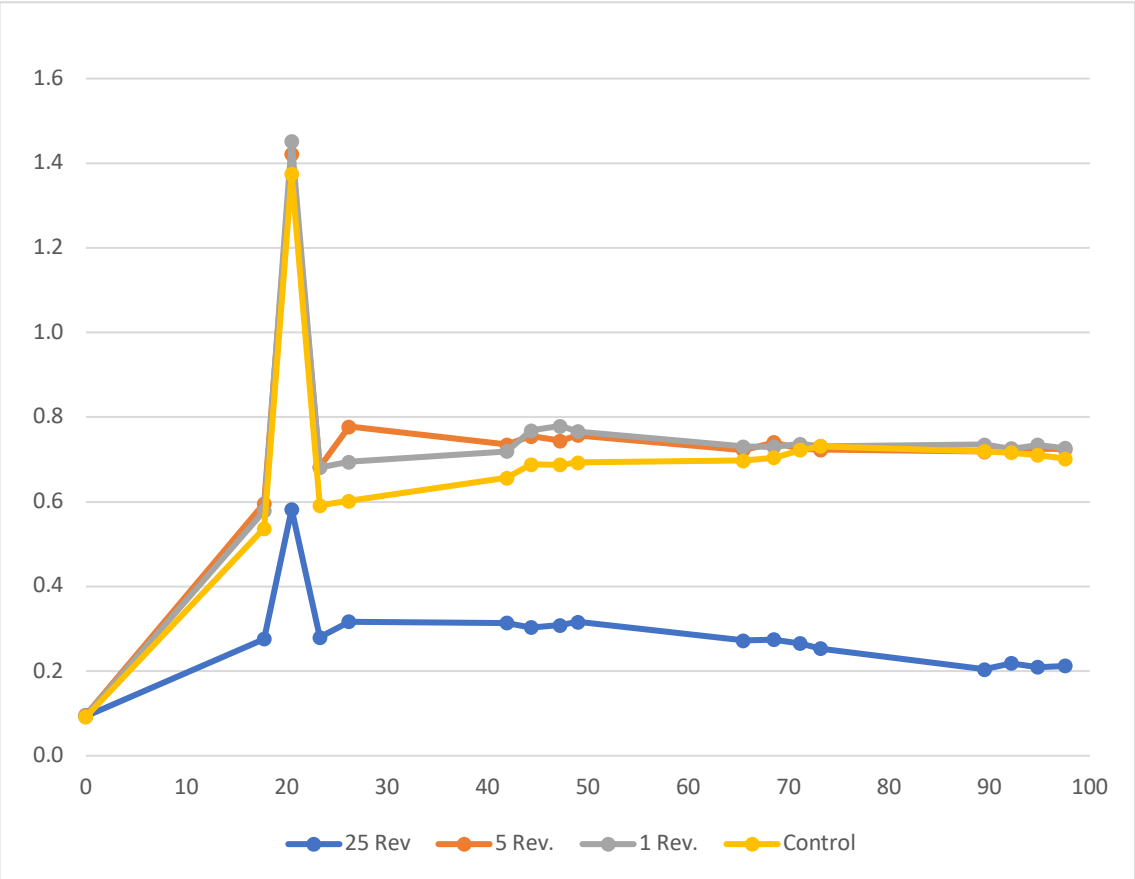


Figure 3.105. The OD curve of isolate 280 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Rev.25	Rev.5	Rev.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.101	0.107	0.107	0.103	3.3E-30	0.007	0.009	0.012
15.92	0.129	0.934	0.876	0.863	9.3E-34	0.055	0.073	0.095
18.38	0.128	0.466	0.513	0.603	2.9E-23	0.088	0.117	0.152
21.27	0.136	0.481	0.537	0.568	6.8E-13	0.095	0.126	0.164
24.45	0.141	0.557	0.627	0.587	4.6E-28	0.044	0.059	0.076
25.78	0.146	0.594	0.658	0.615	1.2E-24	0.053	0.070	0.091
40.13	0.297	0.630	0.629	0.653	6.7E-14	0.066	0.087	0.114
45.62	0.339	0.666	0.627	0.639	1.9E-11	0.066	0.088	0.115
49.17	0.330	0.664	0.633	0.643	1.9E-10	0.075	0.100	0.131
65.40	0.230	0.670	0.655	0.643	9.0E-17	0.071	0.094	0.122
68.10	0.129	0.688	0.670	0.675	1.1E-26	0.053	0.071	0.092
71.53	0.131	0.697	0.649	0.681	1.9E-30	0.045	0.059	0.077
73.78	0.129	0.683	0.692	0.692	1.2E-29	0.047	0.062	0.081
88.62	0.138	0.680	0.696	0.733	4.2E-15	0.103	0.137	0.179
91.35	0.173	0.699	0.667	0.652	4.8E-23	0.059	0.079	0.103
96.18	0.180	0.676	0.669	0.664	1.1E-22	0.058	0.078	0.101

Table 3.214. The mean ODs at each assessment time for isolate 280 in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.095	0.097	0.097	0.096	1.1E-01	0.003	0.004	0.006
19.25	0.180	0.528	0.639	0.561	4.6E-10	0.104	0.138	0.180
22.37	0.231	0.559	0.520	0.503	3.2E-05	0.119	0.159	0.207
25.17	0.206	0.637	0.615	0.577	2.9E-11	0.092	0.123	0.160
28.68	0.225	0.668	0.648	0.578	4.2E-12	0.086	0.114	0.149
43.32	0.284	0.667	0.691	0.630	1.8E-09	0.096	0.128	0.167
46.27	0.287	0.679	0.701	0.646	5.8E-10	0.093	0.124	0.161
49.03	0.275	0.685	0.707	0.670	8.1E-10	0.096	0.128	0.166
67.62	0.257	0.660	0.707	0.668	2.8E-12	0.089	0.118	0.154
70.32	0.254	0.669	0.724	0.639	9.1E-11	0.093	0.124	0.161
73.20	0.259	0.666	0.750	0.670	9.0E-11	0.097	0.129	0.168
76.52	0.249	0.694	0.707	0.648	1.1E-11	0.094	0.125	0.162
90.78	0.239	0.669	0.739	0.672	1.9E-10	0.106	0.141	0.183
94.18	0.223	0.664	0.721	0.654	2.7E-11	0.100	0.133	0.173
97.77	0.228	0.650	0.706	0.671	1.7E-10	0.106	0.141	0.183
97.60	0.223	0.581	0.661	0.648	1.2E-11	0.097	0.129	0.168

Table 3.215. The mean ODs at each assessment time for isolate 280 in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.093	0.096	0.095	0.092	1.4E-16	0.012	0.016	0.021
17.75	0.276	0.596	0.579	0.537	7.4E-11	0.084	0.111	0.144
20.50	0.583	1.422	1.451	1.375	2.1E-15	0.143	0.190	0.246
23.32	0.280	0.682	0.681	0.591	2.9E-16	0.071	0.095	0.123
26.20	0.317	0.777	0.694	0.602	5.9E-14	0.077	0.102	0.132
41.95	0.314	0.735	0.720	0.656	9.0E-16	0.072	0.096	0.125
44.35	0.304	0.755	0.769	0.688	8.6E-17	0.074	0.099	0.128
47.27	0.309	0.744	0.779	0.687	1.1E-15	0.078	0.103	0.134
49.02	0.316	0.757	0.766	0.693	2.2E-14	0.083	0.110	0.143
65.52	0.272	0.722	0.731	0.698	5.6E-17	0.079	0.105	0.136
68.52	0.275	0.740	0.730	0.704	6.8E-17	0.081	0.107	0.139
71.17	0.266	0.725	0.737	0.722	1.3E-16	0.082	0.109	0.141
73.18	0.253	0.723	0.732	0.731	1.7E-16	0.086	0.115	0.149
89.50	0.204	0.718	0.735	0.719	3.0E-21	0.074	0.098	0.127
92.20	0.218	0.726	0.725	0.716	1.6E-18	0.081	0.108	0.140
94.85	0.210	0.725	0.735	0.710	2.2E-19	0.079	0.105	0.136
97.58	0.212	0.724	0.727	0.702	2.3E-18	0.083	0.110	0.142

Table 3.216. The mean ODs at each assessment time for isolate 280 in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The OD values with the 25 ppm Revysol® treatment were very low throughout the experiment. Unsurprisingly strongly significant lower “growth” of the 25 ppm treatment at the 0.1% level was observed suggesting an inhibitory effect of the Revysol® 25 ppm on the “growth” of the

isolate 280. Although the "growth" values of the 1 and 5 ppm Revysol® treatments were only statistically significant in the second trial (Table 3.218), a higher "growth" bordering on statistical significance of these two rates in the third run suggests enhanced "growth" of the isolate 280 in the presence of Revysol® at the 1 and 5 ppm (Table 3.219).

Treatment	"Growth"
Rev.25	6.65
Rev.5	47.66
Rev.1	47.26
Control	47.72
LSD5%	2.325
LSD1%	3.095
LSD 0.1%	4.032

Treatment	"Growth"
Rev. 25	12.62
Rev. 5	48.13
Rev. 1	52.01
Control	35.33
LSD5%	8.78
LSD1%	11.69
LSD0.1%	15.22

Treatment	"Growth"
Rev.25	16.93
Rev. 5	57.20
Rev. 1	56.85
Control	53.00
LSD5%	5.67
LSD1%	7.52
LSD0.1%	9.76

Table 3.217. and 3.218, and 3.219. The mean "growth" of isolate 280 in treatments with 25, 5, and 1 ppm of Revysol® over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

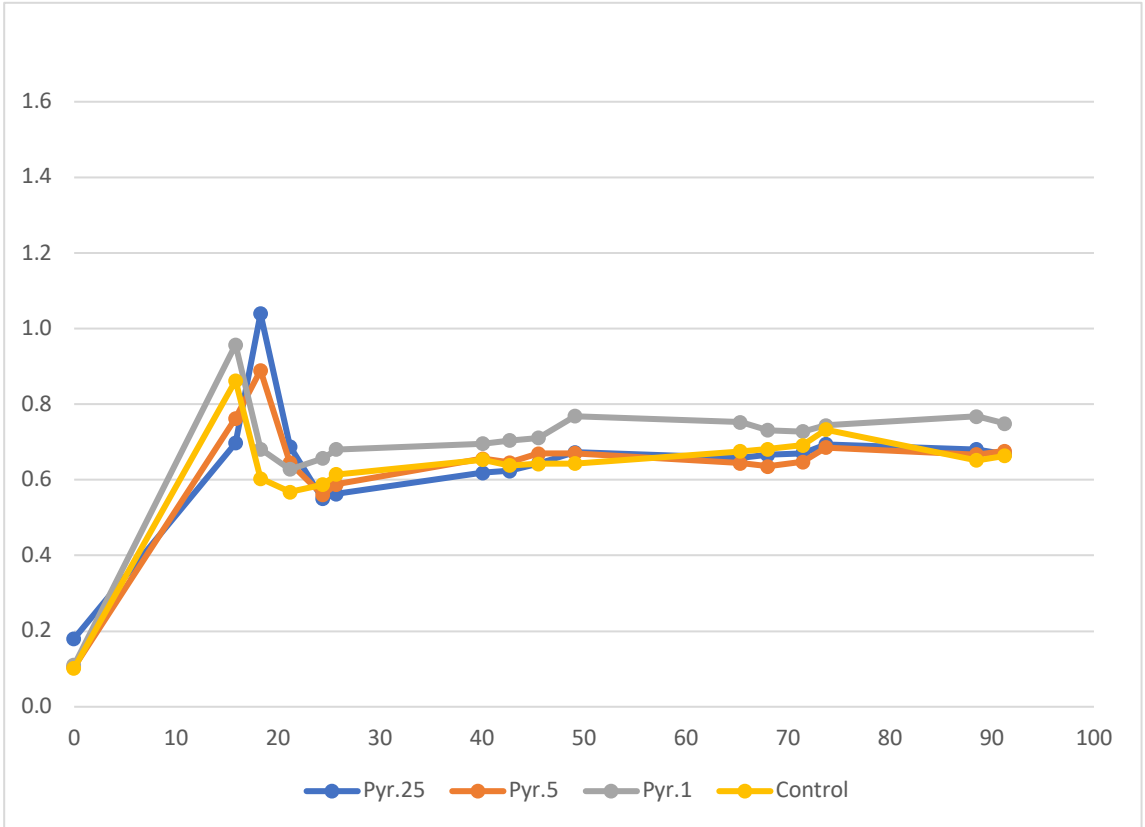


Figure 3.106. The OD curve of isolate 280 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

The cross-crossing test with the isolate 280 and the fungicide pyraclostrobin was run on two occasions. In the first trial, with the control and 1 ppm the peak was reached earlier at 15.83 hours and also the decline began earlier. The highest peak was recorded with the 25 ppm

simultaneously with the 5 ppm at 18.30 hours (1.0), however the decline with these two doses also was greater. Then from 25.73 hours all treatments and the control started to plateau out (Fig. 3.106). With the 1 ppm treatment, the OD values were significantly higher than the control at most timing points (Table 3.220) reflected in the significantly greater "growth" at the 0.1 % level (Table 3.222).

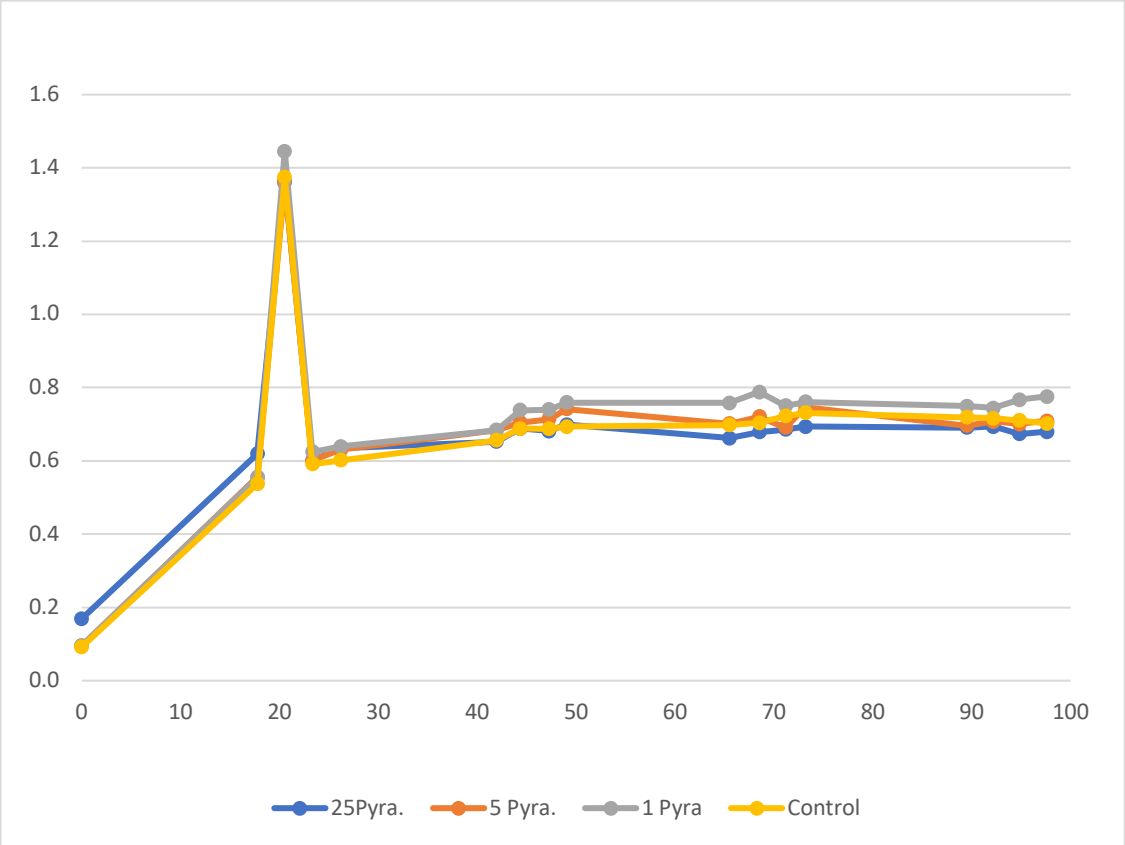


Figure 3.107. The OD curve of isolate 280 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

In the second run a similar tendency in the OD values was observed but with a higher peak recorded with the 1 ppm (1.4) at 20.50 hours (Fig. 3.107). Like in the first run, throughout the whole second trial the OD value with the 1 ppm was higher than the control, but the difference compared to the control was only significant at one assessment timing (68.5 hours) (Table 3.221). Nevertheless, the "growth" with the 1 ppm treatment was again significantly higher than the control on this occasion at the 5% level (Table 3.223).

The observation that in both tests, the "growth" with the 1 ppm rate of pyraclostrobin was significantly higher than the control points in the direction of the enhanced "growth" of the isolate 280 in the presence of 1ppm pyraclostrobin.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.181	0.105	0.110	0.103	3.3E-30	0.007	0.009	0.012
15.92	0.697	0.763	0.958	0.863	9.3E-34	0.055	0.073	0.095
18.38	1.040	0.890	0.681	0.603	2.9E-23	0.088	0.117	0.152
21.27	0.689	0.645	0.628	0.568	6.8E-13	0.095	0.126	0.164
24.45	0.550	0.561	0.657	0.587	4.6E-28	0.044	0.059	0.076
25.78	0.563	0.588	0.681	0.615	1.2E-24	0.053	0.070	0.091
40.13	0.619	0.656	0.696	0.653	6.7E-14	0.066	0.087	0.114
45.62	0.624	0.646	0.704	0.639	1.9E-11	0.066	0.088	0.115
49.17	0.643	0.670	0.711	0.643	1.9E-10	0.075	0.100	0.131
65.40	0.673	0.670	0.769	0.643	9.0E-17	0.071	0.094	0.122
68.10	0.658	0.645	0.753	0.675	1.1E-26	0.053	0.071	0.092
71.53	0.666	0.636	0.731	0.681	1.9E-30	0.045	0.059	0.077
73.78	0.670	0.648	0.728	0.692	1.2E-29	0.047	0.062	0.081
88.62	0.694	0.686	0.744	0.733	4.2E-15	0.103	0.137	0.179
91.35	0.681	0.667	0.768	0.652	4.8E-23	0.059	0.079	0.103
96.18	0.668	0.677	0.749	0.664	1.1E-22	0.058	0.078	0.101

Table 3.220. The mean ODs at each assessment time for isolate 280 in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.169	0.095	0.096	0.092	1.4E-16	0.012	0.016	0.021
17.75	0.620	0.555	0.554	0.537	7.4E-11	0.084	0.111	0.144
20.50	1.363	1.359	1.445	1.375	2.1E-15	0.143	0.190	0.246
23.32	0.601	0.601	0.625	0.591	2.9E-16	0.071	0.095	0.123
26.20	0.634	0.630	0.640	0.602	5.9E-14	0.077	0.102	0.132
41.95	0.652	0.683	0.684	0.656	9.0E-16	0.072	0.096	0.125
44.35	0.688	0.705	0.738	0.688	8.6E-17	0.074	0.099	0.128
47.27	0.682	0.714	0.740	0.687	1.1E-15	0.078	0.103	0.134
49.02	0.699	0.742	0.759	0.693	2.2E-14	0.083	0.110	0.143
65.52	0.662	0.701	0.758	0.698	5.6E-17	0.079	0.105	0.136
68.52	0.679	0.721	0.788	0.704	6.8E-17	0.081	0.107	0.139
71.17	0.686	0.691	0.751	0.722	1.3E-16	0.082	0.109	0.141
73.18	0.694	0.746	0.761	0.731	1.7E-16	0.086	0.115	0.149
89.50	0.691	0.696	0.750	0.719	3.0E-21	0.074	0.098	0.127
92.20	0.693	0.708	0.744	0.716	1.6E-18	0.081	0.108	0.140
94.85	0.674	0.701	0.767	0.710	2.2E-19	0.079	0.105	0.136
97.58	0.680	0.710	0.776	0.702	2.3E-18	0.083	0.110	0.142

Table 3.221. The mean ODs at each assessment time for isolate 280 in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The cross-screening test with the isolate 280 and the fungicide fluxapyroxad was carried out on two occasions. In the first trial, the OD values after an initial increase until 19.15 hours, continued to slightly increase until the end of the trial (Fig. 3.108, Table 3.224). The

consistently higher OD values with the fluxapyroxad treatments compared to the control while not reaching statistical significance, resulted in a very significant higher "growth" of all three fluxapyroxad treatments at the 0.1% level (Table 3.226).

Treatment	"Growth"
Pyra.25	40.51
Pyra.5	47.06
Pyra.1	53.39
Control	47.72
LSD5%	2.325
LSD1%	3.095
LSD 0.1%	4.032

Treatment	"Growth"
Pyra. 25	45.99
Pyra. 5	53.83
Pyra. 1	56.35
Control	53.00
LSD5%	5.67
LSD1%	7.52
LSD0.1%	9.76

Table 3.222. and 3.223. The mean "growth" of isolate 280 in treatments with 25, 5, and 1 ppm of pyraclostrobin over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

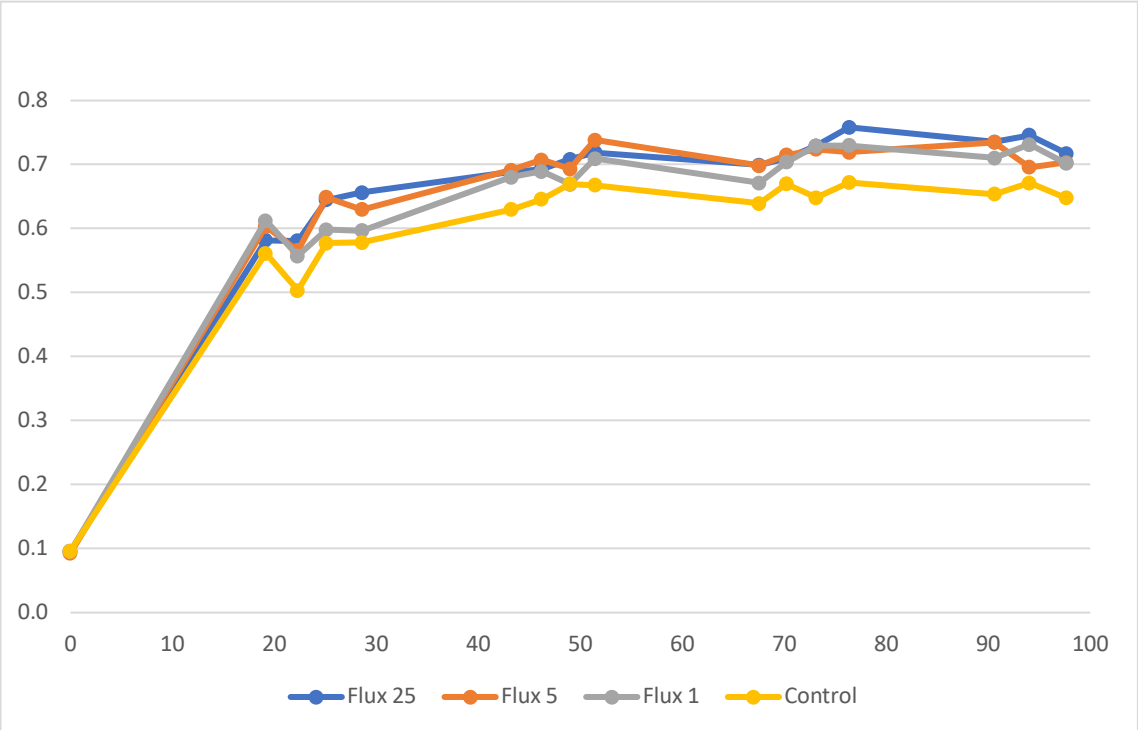


Figure 3.108. The OD curve of isolate 280 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

In the second trial, after the initial increase in OD values, culminated in a peak with the highest value being recorded with 25 ppm at 20.50 hours a steep decline followed. From 23.32 hours onwards, the OD values levelled off until the test was finished (Fig. 3.109). The OD values with the fluxapyroxad treatments were most of the time higher than those with the control especially with 25 ppm where the values were very significant at the 1% and 0.5% level (Table.3.225).

This resulted in statistically significantly greater "growth" values for the 25ppm treatment at the 0.1% level but also numerically higher "growth" of the 1 and 5 ppm treatments compared to the control (Table 3.227). In both runs the higher "growth" of the fluxapyroxad treatments compared to the control suggest an enhanced growth of the strain 280 in the presence of fluxapyroxad.

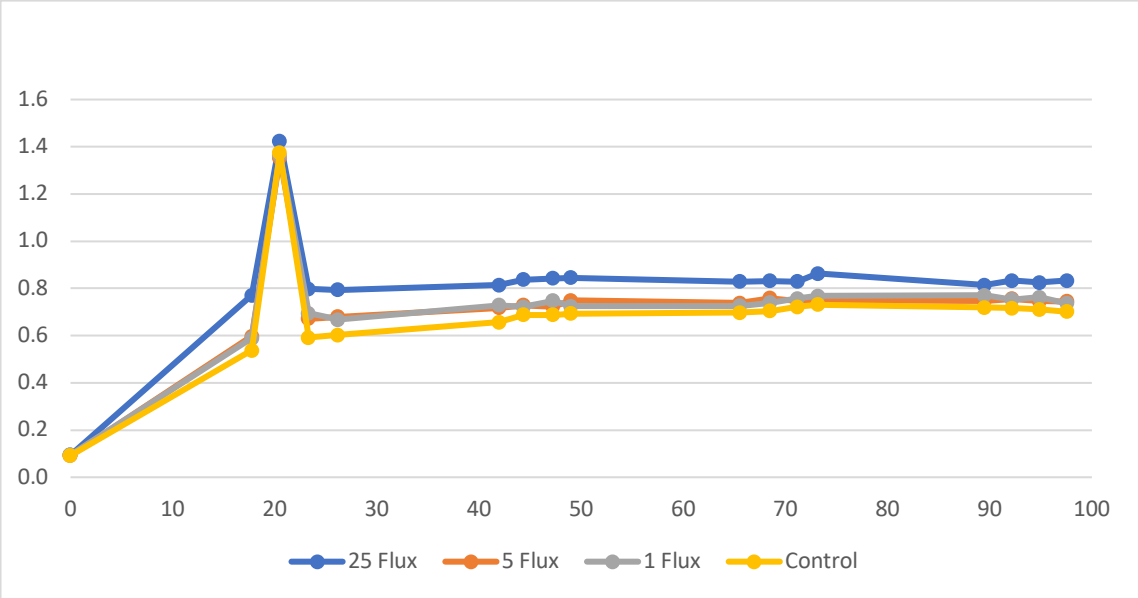


Figure 3.109. The OD curve of isolate 280 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.096	0.093	0.095	0.096	1.1E-01	0.003	0.004	0.006
19.25	0.582	0.603	0.612	0.561	4.6E-10	0.104	0.138	0.180
22.37	0.581	0.566	0.557	0.503	3.2E-05	0.119	0.159	0.207
25.17	0.645	0.649	0.598	0.577	2.9E-11	0.092	0.123	0.160
28.68	0.656	0.630	0.597	0.578	4.2E-12	0.086	0.114	0.149
43.32	0.689	0.691	0.680	0.630	1.8E-09	0.096	0.128	0.167
46.27	0.693	0.707	0.689	0.646	5.8E-10	0.093	0.124	0.161
49.03	0.709	0.693	0.669	0.670	8.1E-10	0.096	0.128	0.166
67.62	0.719	0.739	0.709	0.668	2.8E-12	0.089	0.118	0.154
70.32	0.699	0.698	0.671	0.639	9.1E-11	0.093	0.124	0.161
73.20	0.710	0.715	0.704	0.670	9.0E-11	0.097	0.129	0.168
76.52	0.730	0.724	0.730	0.648	1.1E-11	0.094	0.125	0.162
90.78	0.758	0.720	0.730	0.672	1.9E-10	0.106	0.141	0.183
94.18	0.735	0.735	0.711	0.654	2.7E-11	0.100	0.133	0.173
97.77	0.746	0.696	0.732	0.671	1.7E-10	0.106	0.141	0.183
97.60	0.718	0.703	0.702	0.648	1.2E-11	0.097	0.129	0.168

Table 3.224. The mean ODs at each assessment time for isolate 280 in the presence of three concentrations of fluxapyroxad. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.094	0.092	0.095	0.092	1.4E-16	0.012	0.016	0.021
17.75	0.771	0.597	0.590	0.537	7.4E-11	0.084	0.111	0.144
20.50	1.423	1.354	1.364	1.375	2.1E-15	0.143	0.190	0.246
23.32	0.798	0.671	0.694	0.591	2.9E-16	0.071	0.095	0.123
26.20	0.793	0.680	0.668	0.602	5.9E-14	0.077	0.102	0.132
41.95	0.814	0.717	0.728	0.656	9.0E-16	0.072	0.096	0.125
44.35	0.837	0.728	0.722	0.688	8.6E-17	0.074	0.099	0.128
47.27	0.842	0.723	0.748	0.687	1.1E-15	0.078	0.103	0.134
49.02	0.846	0.748	0.724	0.693	2.2E-14	0.083	0.110	0.143
65.52	0.830	0.738	0.725	0.698	5.6E-17	0.079	0.105	0.136
68.52	0.831	0.758	0.740	0.704	6.8E-17	0.081	0.107	0.139
71.17	0.829	0.747	0.758	0.722	1.3E-16	0.082	0.109	0.141
73.18	0.863	0.751	0.767	0.731	1.7E-16	0.086	0.115	0.149
89.50	0.814	0.746	0.771	0.719	3.0E-21	0.074	0.098	0.127
92.20	0.832	0.756	0.753	0.716	1.6E-18	0.081	0.108	0.140
94.85	0.824	0.746	0.762	0.710	2.2E-19	0.079	0.105	0.136
97.58	0.833	0.745	0.736	0.702	2.3E-18	0.083	0.110	0.142

Table 3.225. The mean ODs at each assessment time for isolate 280 in the presence of three concentrations of fluxapyroxad. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Flux. 25	52.19
Flux. 5	52.05
Flux. 1	50.69
Control	35.33
LSD5%	8.78
LSD1%	11.69
LSD0.1%	15.22

Treatment	"Growth"
Flux. 25	66.11
Flux. 5	57.08
Flux. 1	56.86
Control	53.00
LSD5%	5.67
LSD1%	7.52
LSD0.1%	9.76

Table 3.226. and 3.327. The mean "growth" of isolate 280 in treatments with 25, 5, and 1 ppm of fluxapyroxad over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

3.2.4.13. Isolate 284

The isolate 284 has been tested with the fungicide boscalid in a cross-screening test three times. In all runs the isolate showed an initial increase reaching a maximum value between 15.00 and 17.00 hours.

In the first trial, the maximum observed OD values were reached after 17.00 hours and the 5 ppm boscalid treatment achieved the highest peak (0.7). After the attainment of peak values, there was a steady decline in the OD values for the remainder of the experiment (Fig. 3.110). Although the peak value recorded was not the highest, the 1 ppm treatment declined more

slowly than the other treatments resulting in a very significant differences compared to the control from 40.48 hours onwards (Table 3.228). The 5 and 25 ppm also showed higher OD values than the control throughout the whole experiment and the differences were significant at some timing points. This resulted in significantly greater "growth" of the treatment with 1 and 5 ppm and numerically higher "growth" with 25 ppm (Table 3.231).

In the second run, the peak values were observed earlier at 15.00 hours and again the 5 ppm Revysol® treatment achieved the highest peak (0.9) followed by a steep decline until and a slower decline towards the end of the experiment (Fig. 3.111).

The OD values with the 5 and 25 ppm were higher numerically (not statistically) than the control at most timings. The 1 ppm showed lower ODs from 25 hours or so which became significantly different during the last experimental day (Table 3.229). Reflecting these results, the "growth" of treatments with 5 and 25 were numerically higher and with 1 ppm numerically lower than the control (Table 3.232).

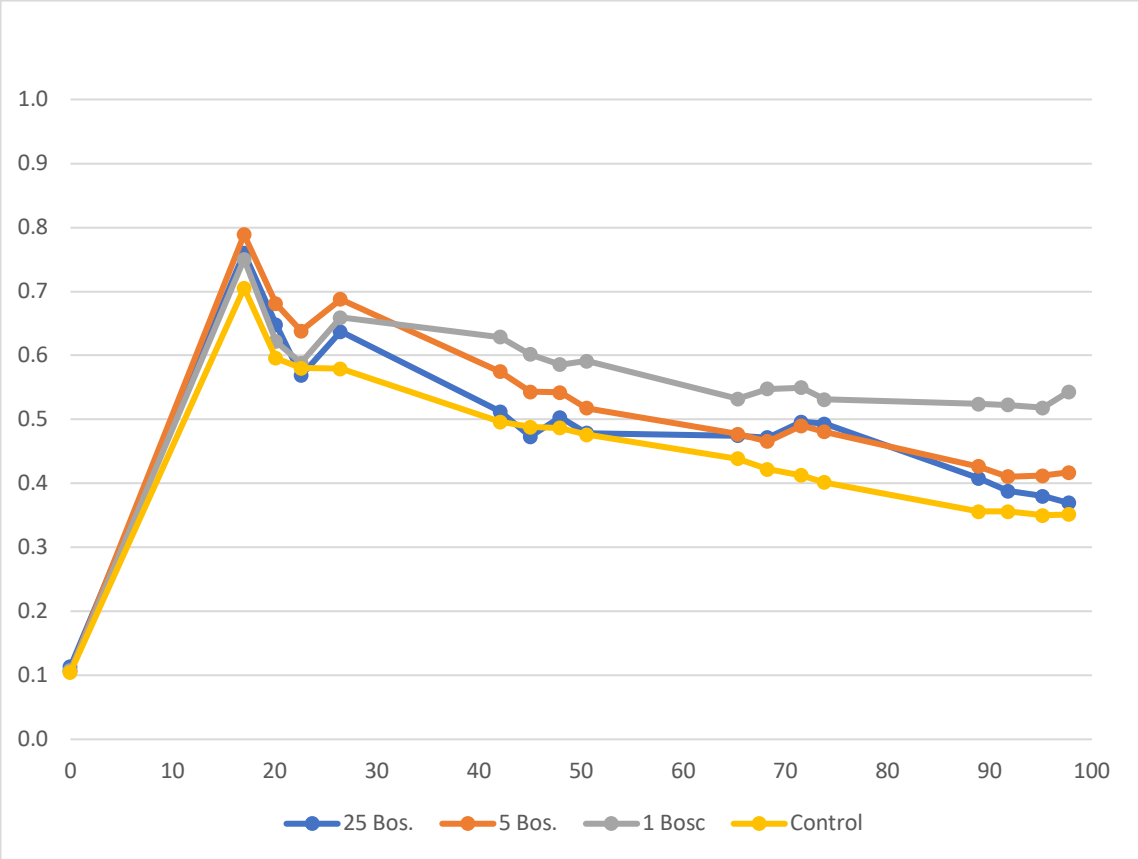


Figure 3.110. The OD curve of isolate 284 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

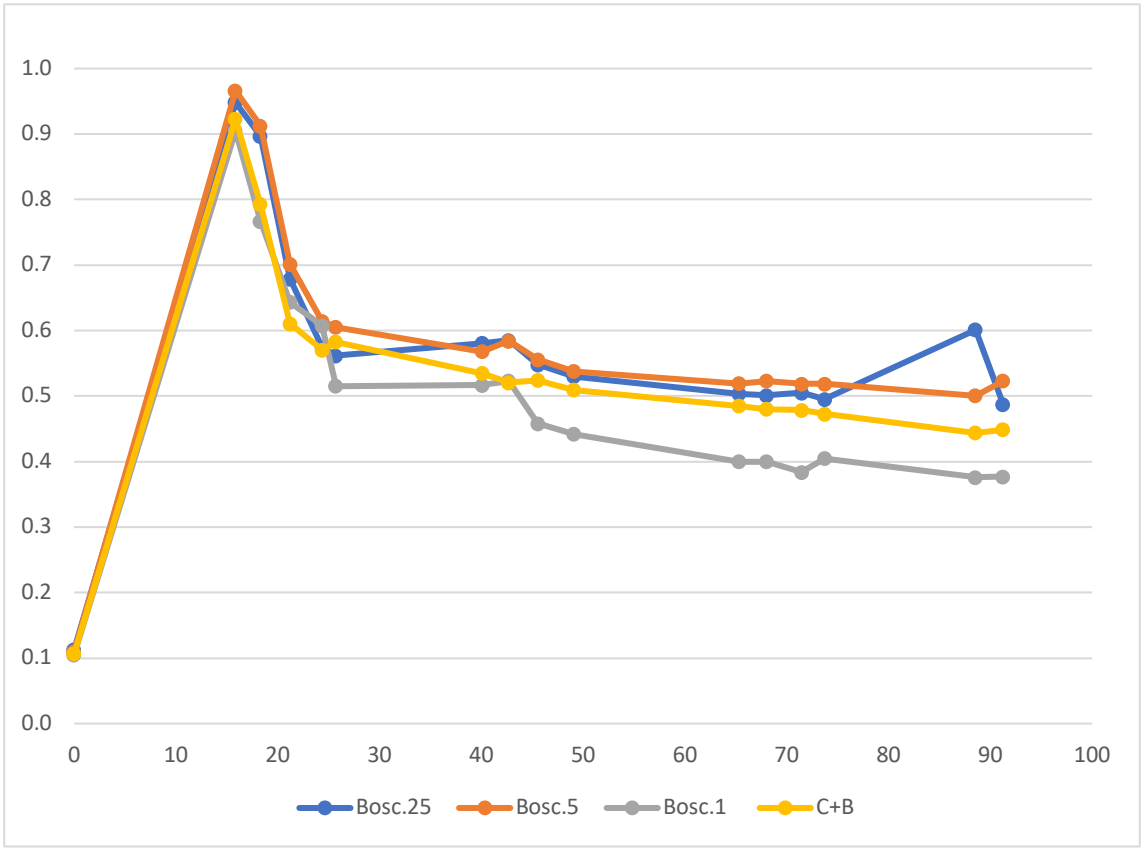


Figure 3.111. The OD curve of isolate 284 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

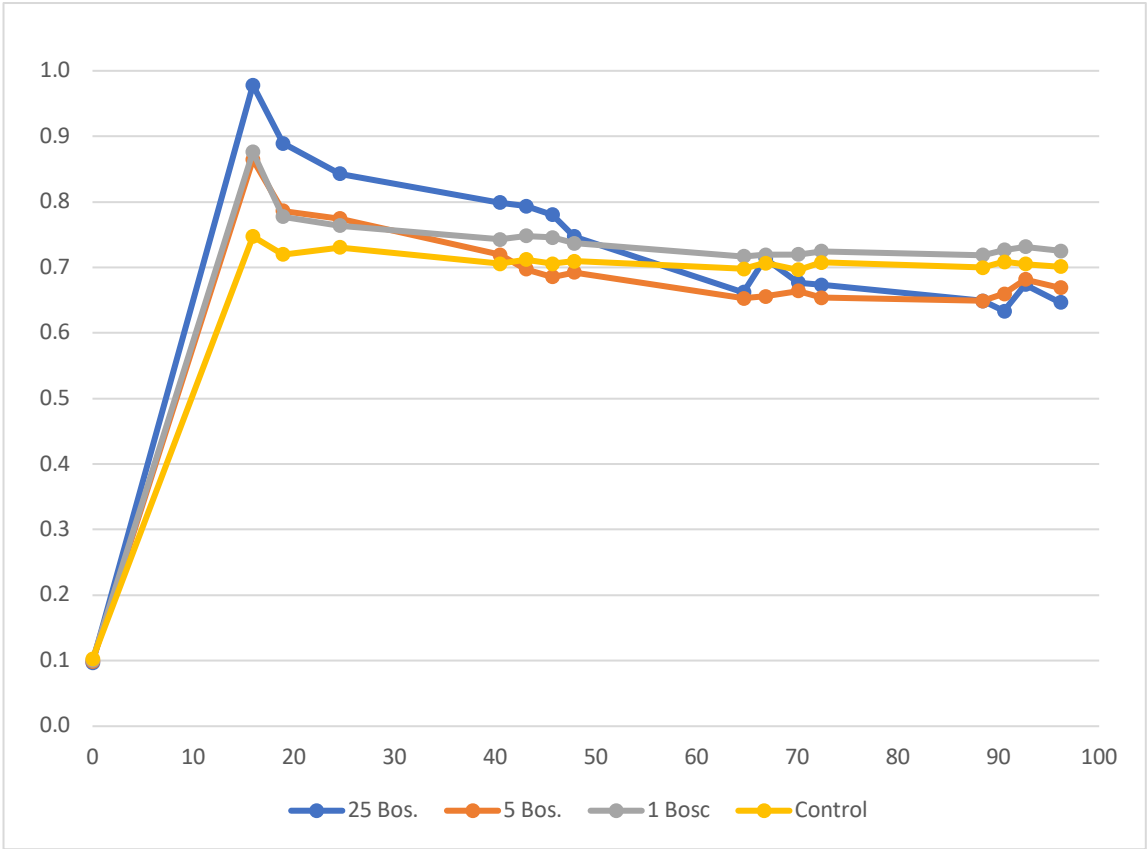


Figure 3.112. The OD curve of isolate 284 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.114	0.107	0.107	0.105	4.1E-11	0.010	0.013	0.017
15.90	0.760	0.789	0.751	0.705	1.2E-19	0.083	0.110	0.143
18.90	0.648	0.681	0.622	0.596	4.6E-18	0.073	0.097	0.125
21.58	0.569	0.638	0.588	0.580	3.4E-19	0.066	0.088	0.114
24.52	0.637	0.688	0.659	0.579	1.4E-20	0.062	0.082	0.106
40.48	0.512	0.575	0.629	0.496	1.4E-18	0.057	0.075	0.098
43.03	0.473	0.543	0.602	0.488	7.3E-18	0.058	0.077	0.100
45.63	0.503	0.542	0.586	0.487	3.2E-19	0.054	0.072	0.093
47.80	0.479	0.518	0.591	0.476	1.4E-20	0.053	0.070	0.091
64.63	0.475	0.477	0.532	0.439	3.6E-11	0.082	0.109	0.141
66.75	0.472	0.466	0.548	0.422	8.2E-12	0.081	0.107	0.139
70.10	0.496	0.490	0.550	0.412	8.2E-13	0.077	0.102	0.132
72.37	0.493	0.481	0.531	0.401	1.1E-12	0.078	0.104	0.135
88.33	0.408	0.427	0.524	0.356	1.9E-14	0.077	0.102	0.132
90.53	0.388	0.411	0.523	0.356	2.3E-14	0.076	0.100	0.130
92.67	0.380	0.412	0.518	0.350	4.2E-14	0.078	0.103	0.134
96.08	0.370	0.417	0.543	0.351	1.2E-13	0.074	0.098	0.127

Table 3.228. The mean ODs at each assessment time for isolate 284 in the presence of three concentrations of boscalid. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Bosc.25	Bosc.5	Bosc.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.113	0.110	0.105	0.107	2.4E-38	0.004	0.005	0.007
15.85	0.948	0.966	0.905	0.923	4.3E-34	0.051	0.068	0.089
18.30	0.897	0.913	0.767	0.793	3.9E-23	0.089	0.118	0.154
21.20	0.679	0.702	0.644	0.611	4.2E-21	0.078	0.104	0.135
24.37	0.574	0.615	0.608	0.571	8.0E-18	0.074	0.099	0.128
25.72	0.562	0.605	0.515	0.583	1.4E-18	0.071	0.095	0.123
40.07	0.581	0.568	0.517	0.535	7.2E-16	0.089	0.118	0.154
42.70	0.585	0.584	0.523	0.521	1.1E-13	0.088	0.117	0.153
45.55	0.548	0.556	0.458	0.524	7.3E-15	0.082	0.110	0.143
49.10	0.530	0.538	0.442	0.509	3.1E-17	0.081	0.107	0.140
65.33	0.504	0.519	0.400	0.485	2.9E-18	0.072	0.095	0.124
68.03	0.501	0.523	0.400	0.480	5.3E-17	0.077	0.102	0.133
71.45	0.505	0.519	0.384	0.479	2.5E-19	0.071	0.094	0.123
73.72	0.495	0.519	0.405	0.473	3.2E-24	0.058	0.077	0.100
88.53	0.601	0.501	0.376	0.444	4.3E-21	0.063	0.083	0.109
91.25	0.487	0.523	0.377	0.449	3.4E-21	0.062	0.083	0.108

Table 3.229. The mean ODs at each assessment time for isolate 284 in the presence of three concentrations of boscalid. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.097	0.099	0.101	0.102	7.0E-17	0.012	0.016	0.020
15.92	0.978	0.865	0.876	0.747	1.8E-23	0.081	0.108	0.139
18.90	0.890	0.786	0.777	0.720	5.8E-20	0.074	0.098	0.128
24.58	0.843	0.774	0.764	0.730	9.4E-15	0.082	0.109	0.142
40.48	0.799	0.719	0.743	0.706	1.1E-14	0.078	0.104	0.135
43.05	0.794	0.697	0.748	0.712	1.0E-15	0.076	0.101	0.131
45.68	0.780	0.686	0.746	0.706	1.0E-16	0.075	0.100	0.129
47.83	0.747	0.693	0.737	0.710	7.0E-18	0.071	0.095	0.123
64.68	0.662	0.653	0.717	0.698	1.7E-23	0.061	0.081	0.104
66.82	0.714	0.656	0.719	0.707	4.0E-20	0.071	0.094	0.122
70.12	0.677	0.664	0.720	0.696	7.0E-24	0.061	0.081	0.105
72.38	0.673	0.654	0.725	0.708	3.4E-23	0.064	0.085	0.110
88.42	0.649	0.649	0.719	0.700	3.3E-22	0.069	0.091	0.118
90.55	0.634	0.660	0.727	0.708	1.8E-25	0.061	0.082	0.106
92.65	0.674	0.682	0.732	0.705	1.7E-22	0.068	0.090	0.117
96.15	0.647	0.669	0.725	0.701	2.9E-23	0.066	0.088	0.114

Table 3.230. The mean ODs at each assessment time for isolate 284 in the presence of three concentrations of boscalid. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Bosc. 25	36.83
Bosc. 5	40.06
Bosc. 1	43.49
Control	34.03
LSD5%	4.50
LSD1%	5.98
LSD0.1%	7.75

Treatment	"Growth"
Bosc.25	40.84
Bosc.5	41.42
Bosc.1	33.94
Control	37.95
LSD5%	4.41
LSD1%	5.87
LSD 0.1%	7.65

Treatment	"Growth"
Bosc. 25	58.58
Bosc. 5	54.04
Bosc. 1	57.39
Control	53.88
LSD5%	5.73
LSD1%	7.60
LSD0.1%	9.86

Table 3.231. and 3.232. and 3.233. The mean "growth" of isolate 284 in treatments with 25, 5, and 1 ppm of boscalid over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

In the third trial, for all treatments a peak value was recorded at 15.92 hours. The 25 ppm achieved the highest peak (0.9) and the control the lowest peak (0.7) reflected in a very significantly greater OD values of the treatments compared to the control (Fig. 3.112). Following these maximum OD values, the ODs started to decline. The two highest rates of boscalid showed the steepest fall while the control and 1 ppm continued to level out from 24.58 hours onwards. Hence, although there was a tendency for the OD values for the 5 and 25 ppm to be higher than the control earlier in the experiment, after 48 hours onwards this was no longer the case (Table 3.230). The "growth" of the boscalid treatments were numerically higher than the control with the 25 ppm treatment bordering on statistical significance (Table 3.233).

The observation that in all three trials, the “growth” of all three rates of boscalid was higher than the control points to enhanced “growth” of the isolate 284 in the presence of the fungicide boscalid.

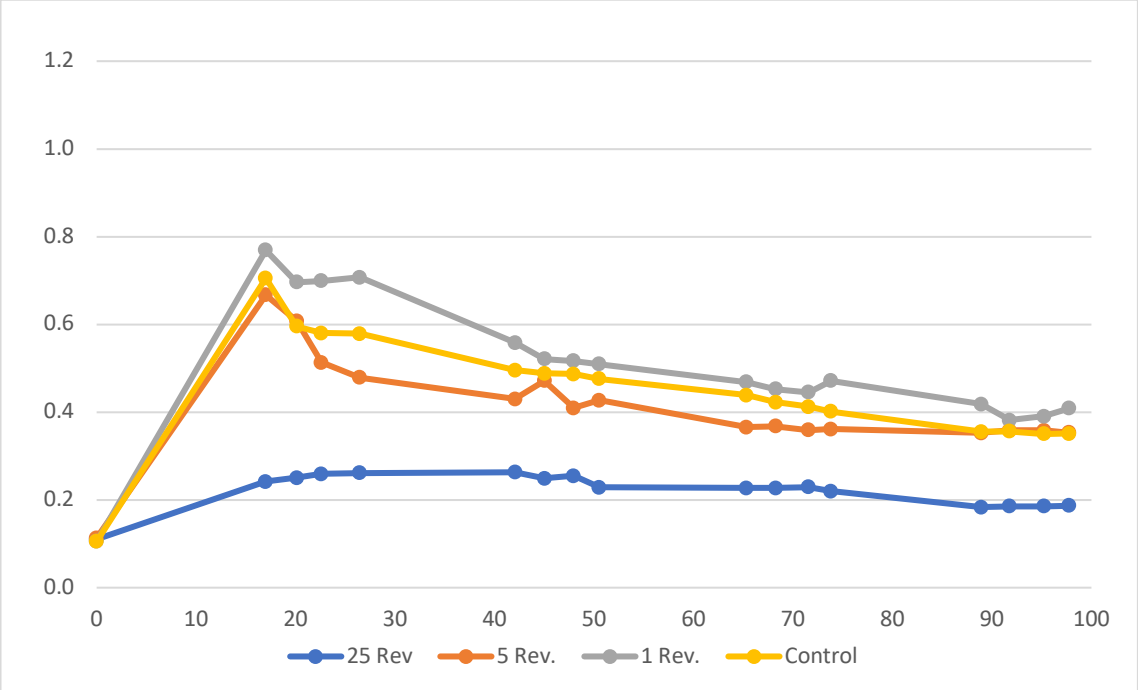


Figure 3.113. The OD curve of isolate 284 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

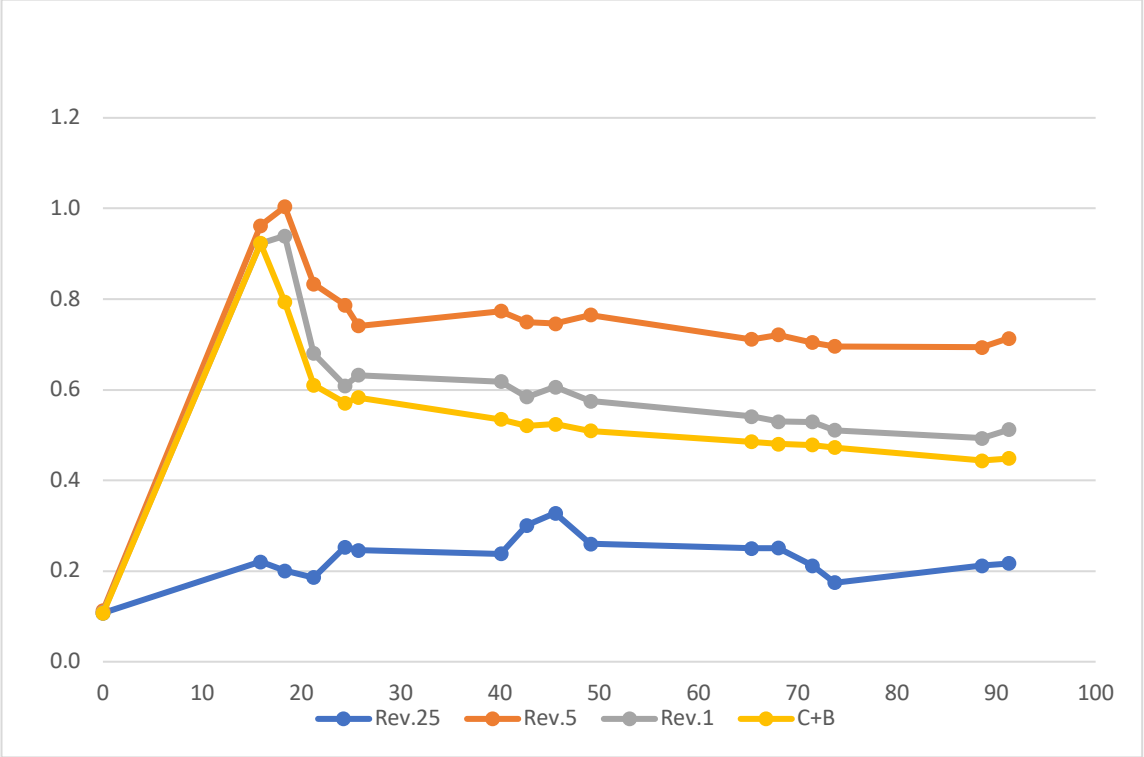


Figure 3.114. The OD curve of isolate 284 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

The cross-screening with the isolate 284 and the fungicide Revysol® was carried out on three occasions. In all three runs, the OD values with 25 ppm increased relatively little compared to the controls leading to highly significant differences.

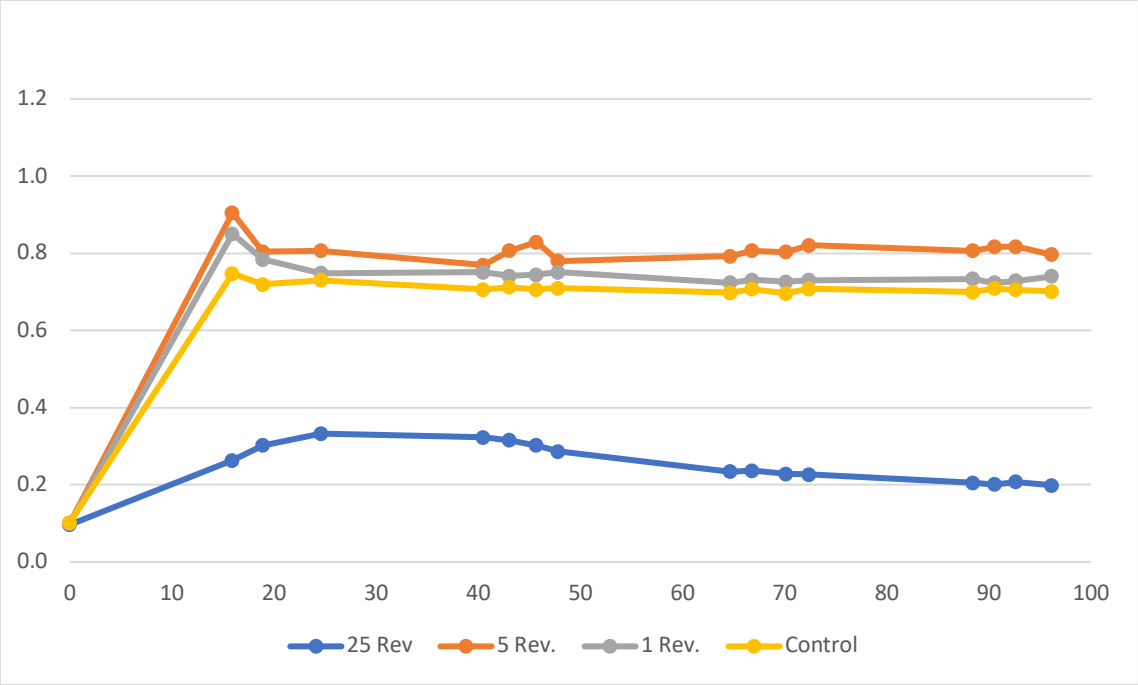


Figure 3.115. The OD curve of isolate 284 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.110	0.113	0.105	0.105	4.1E-11	0.010	0.013	0.017
15.90	0.242	0.667	0.769	0.705	1.2E-19	0.083	0.110	0.143
18.90	0.250	0.608	0.696	0.596	4.6E-18	0.073	0.097	0.125
21.58	0.259	0.513	0.700	0.580	3.4E-19	0.066	0.088	0.114
24.52	0.261	0.479	0.707	0.579	1.4E-20	0.062	0.082	0.106
40.48	0.263	0.430	0.558	0.496	1.4E-18	0.057	0.075	0.098
43.03	0.249	0.472	0.521	0.488	7.3E-18	0.058	0.077	0.100
45.63	0.255	0.409	0.517	0.487	3.2E-19	0.054	0.072	0.093
47.80	0.229	0.427	0.510	0.476	1.4E-20	0.053	0.070	0.091
64.63	0.227	0.366	0.468	0.439	3.6E-11	0.082	0.109	0.141
66.75	0.227	0.368	0.453	0.422	8.2E-12	0.081	0.107	0.139
70.10	0.230	0.359	0.445	0.412	8.2E-13	0.077	0.102	0.132
72.37	0.220	0.362	0.472	0.401	1.1E-12	0.078	0.104	0.135
88.33	0.183	0.352	0.418	0.356	1.9E-14	0.077	0.102	0.132
90.53	0.185	0.359	0.382	0.356	2.3E-14	0.076	0.100	0.130
92.67	0.185	0.358	0.391	0.350	4.2E-14	0.078	0.103	0.134
96.08	0.187	0.354	0.409	0.351	1.2E-13	0.074	0.098	0.127

Table 3.234. The mean ODs at each assessment time for isolate 284 in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Rev.25	Rev.5	Rev.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.108	0.112	0.108	0.107	2.4E-38	0.004	0.005	0.007
15.85	0.220	0.962	0.923	0.923	4.3E-34	0.051	0.068	0.089
18.30	0.200	1.004	0.940	0.793	3.9E-23	0.089	0.118	0.154
21.20	0.186	0.834	0.681	0.611	4.2E-21	0.078	0.104	0.135
24.37	0.253	0.787	0.609	0.571	8.0E-18	0.074	0.099	0.128
25.72	0.246	0.741	0.632	0.583	1.4E-18	0.071	0.095	0.123
40.07	0.238	0.774	0.618	0.535	7.2E-16	0.089	0.118	0.154
42.70	0.301	0.750	0.585	0.521	1.1E-13	0.088	0.117	0.153
45.55	0.328	0.746	0.606	0.524	7.3E-15	0.082	0.110	0.143
49.10	0.260	0.765	0.575	0.509	3.1E-17	0.081	0.107	0.140
65.33	0.250	0.712	0.541	0.485	2.9E-18	0.072	0.095	0.124
68.03	0.251	0.721	0.530	0.480	5.3E-17	0.077	0.102	0.133
71.45	0.212	0.704	0.529	0.479	2.5E-19	0.071	0.094	0.123
73.72	0.175	0.696	0.511	0.473	3.2E-24	0.058	0.077	0.100
88.53	0.212	0.694	0.493	0.444	4.3E-21	0.063	0.083	0.109
91.25	0.217	0.714	0.512	0.449	3.4E-21	0.062	0.083	0.108

Table 3.235. The mean ODs at each assessment time for isolate 284 in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The OD curves showed a rapid rise to a peak value between 16 and 19 hours followed by a decline for the remainder of the first trial but in the other two trials it eventually started to plateau out towards the end. In the first trial the 1 ppm Revysol® treatment had higher OD values than the control, but it only reached a significant level within the first day (Fig. 3.113, Table 3.234). However, the consistently higher OD values with the Revysol® treatments compared to the control resulted in a significant higher "growth" of the treatment with 1 ppm (Table 3.237).

In the second run, the 1 and 5 ppm treatments had higher OD values than the control from the peak onwards (Fig. 3.114, Table 3.235). With the 1 ppm treatment the ODs were mostly numerically higher but with the 5 ppm were very significant so at the 0.1% level (Table 3.238). Similar to the second trial, in the third trial the 1 and 5 ppm also had higher ODs than the control throughout the whole experiment (Fig. 3.115) and again the differences with the 5 ppm treatment were mostly significant resulting in a significant higher "growth" value at the 1% level (Table 3.236, 3.239).

the Observation that in all three tests, the "growth" of the treatment with 25 ppm was significantly lower than the control indicates an inhibitory effect. Moreover, the higher "growth" of the treatments with the 1 and 5 ppm (except the 5 ppm in the first trial) showed the enhanced "growth" of the strain 284 in the presence of the fungicide Revysol® at dose rates 1 and 5 ppm.

Three cross-screening tests with the isolate 284 and the fungicide pyraclostrobin showed similar tendencies; an initial increase followed by a decline after reaching a peak between 16 and 17 hours. Thereafter, the ODs started gradually to level off (Fig. 3.116, 3.117, and 3.118 respectively). The OD values of the 25 and then the 5 ppm treatments were significantly greater than the control mostly at the 0.1% level in the first two runs (Table 3.240 and 3.241 respectively). In addition, in the second trial with the 1 ppm treatment, the ODs were significantly greater than the control. In the third run however the 25ppm treatment declined more quickly and from 40.48 hours the OD values of this treatment were less than those of the control (Table 3.242).

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.097	0.101	0.100	0.102	7.0E-17	0.012	0.016	0.020
15.92	0.264	0.905	0.851	0.747	1.8E-23	0.081	0.108	0.139
18.90	0.302	0.804	0.784	0.720	5.8E-20	0.074	0.098	0.128
24.58	0.333	0.807	0.748	0.730	9.4E-15	0.082	0.109	0.142
40.48	0.323	0.769	0.751	0.706	1.1E-14	0.078	0.104	0.135
43.05	0.315	0.807	0.741	0.712	1.0E-15	0.076	0.101	0.131
45.68	0.302	0.829	0.745	0.706	1.0E-16	0.075	0.100	0.129
47.83	0.286	0.780	0.751	0.710	7.0E-18	0.071	0.095	0.123
64.68	0.234	0.793	0.724	0.698	1.7E-23	0.061	0.081	0.104
66.82	0.236	0.806	0.731	0.707	4.0E-20	0.071	0.094	0.122
70.12	0.229	0.803	0.726	0.696	7.0E-24	0.061	0.081	0.105
72.38	0.227	0.820	0.731	0.708	3.4E-23	0.064	0.085	0.110
88.42	0.205	0.807	0.734	0.700	3.3E-22	0.069	0.091	0.118
90.55	0.201	0.817	0.724	0.708	1.8E-25	0.061	0.082	0.106
92.65	0.208	0.817	0.729	0.705	1.7E-22	0.068	0.090	0.117
96.15	0.198	0.797	0.740	0.701	2.9E-23	0.066	0.088	0.114

Table 3.236. The mean ODs at each assessment time for isolate 284 in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Rev. 25	10.81
Rev. 5	29.15
Rev. 1	39.49
Control	34.03
LSD5%	4.50
LSD1%	5.98
LSD0.1%	7.75

Treatment	"Growth"
Rev. 25	10.51
Rev. 5	54.77
Rev. 1	42.47
Control	37.95
LSD5%	4.412
LSD1%	5.873
LSD 0.1%	7.649

Treatment	"Growth"
Rev. 25	14.89
Rev. 5	62.63
Rev. 1	57.57
Control	53.88
LSD5%	5.73
LSD1%	7.60
LSD0.1%	9.86

Table 3.237. and 3.238. and 3.239. The mean "growth" of isolate 284 in treatments with 25, 5, and 1 ppm of Revysol® over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

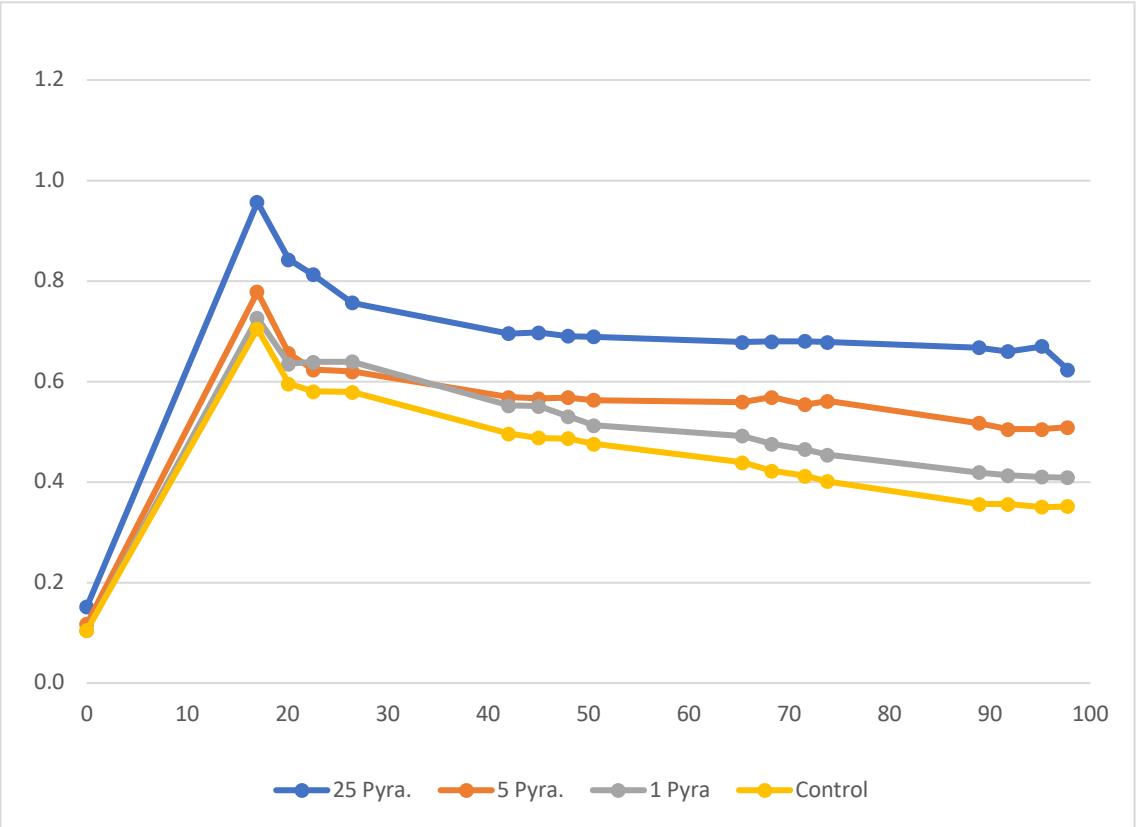


Figure 3.116. The OD curve of isolate 284 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

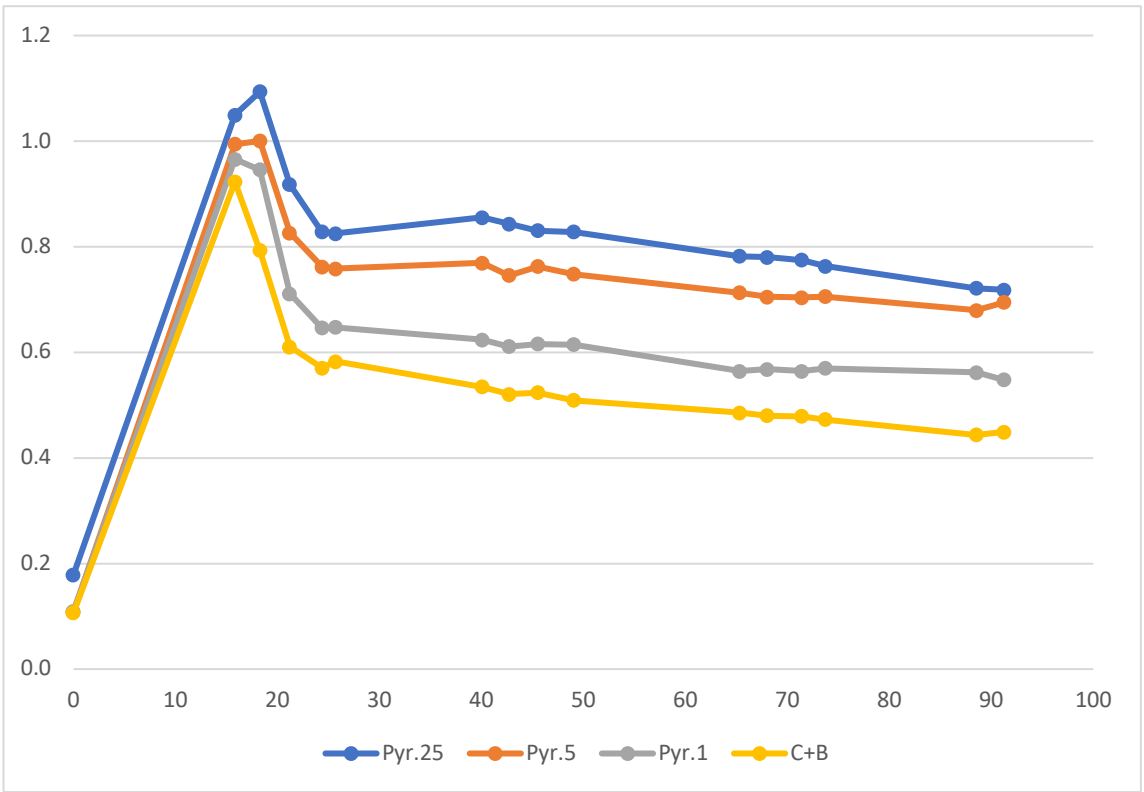


Figure 3.117. The OD curve of isolate 284 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

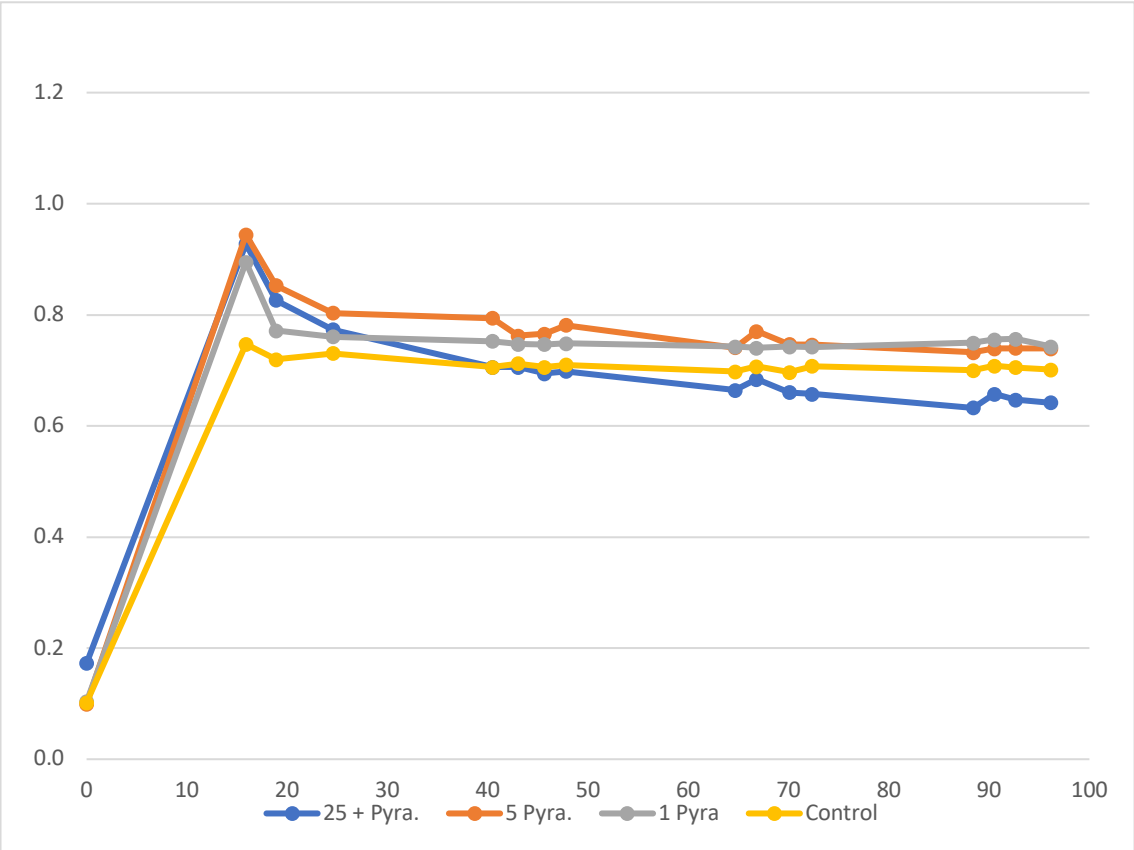


Figure 3.118. The OD curve of isolate 284 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.152	0.118	0.105	0.105	4.1E-11	0.010	0.013	0.017
15.90	0.957	0.778	0.727	0.705	1.2E-19	0.083	0.110	0.143
18.90	0.843	0.656	0.635	0.596	4.6E-18	0.073	0.097	0.125
21.58	0.813	0.624	0.639	0.580	3.4E-19	0.066	0.088	0.114
24.52	0.757	0.620	0.640	0.579	1.4E-20	0.062	0.082	0.106
40.48	0.695	0.569	0.553	0.496	1.4E-18	0.057	0.075	0.098
43.03	0.698	0.566	0.551	0.488	7.3E-18	0.058	0.077	0.100
45.63	0.691	0.568	0.530	0.487	3.2E-19	0.054	0.072	0.093
47.80	0.690	0.563	0.513	0.476	1.4E-20	0.053	0.070	0.091
64.63	0.679	0.559	0.492	0.439	3.6E-11	0.082	0.109	0.141
66.75	0.680	0.569	0.476	0.422	8.2E-12	0.081	0.107	0.139
70.10	0.680	0.554	0.465	0.412	8.2E-13	0.077	0.102	0.132
72.37	0.678	0.561	0.454	0.401	1.1E-12	0.078	0.104	0.135
88.33	0.667	0.517	0.419	0.356	1.9E-14	0.077	0.102	0.132
90.53	0.660	0.505	0.414	0.356	2.3E-14	0.076	0.100	0.130
92.67	0.670	0.505	0.410	0.350	4.2E-14	0.078	0.103	0.134
96.08	0.624	0.509	0.409	0.351	1.2E-13	0.074	0.098	0.127

Table 3.240. The mean ODs at each assessment time for isolate 284 in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.179	0.109	0.110	0.107	2.4E-38	0.004	0.005	0.007
15.85	1.049	0.994	0.966	0.923	4.3E-34	0.051	0.068	0.089
18.30	1.094	1.001	0.946	0.793	3.9E-23	0.089	0.118	0.154
21.20	0.918	0.826	0.711	0.611	4.2E-21	0.078	0.104	0.135
24.37	0.828	0.762	0.646	0.571	8.0E-18	0.074	0.099	0.128
25.72	0.825	0.758	0.648	0.583	1.4E-18	0.071	0.095	0.123
40.07	0.856	0.769	0.624	0.535	7.2E-16	0.089	0.118	0.154
42.70	0.843	0.745	0.611	0.521	1.1E-13	0.088	0.117	0.153
45.55	0.831	0.763	0.616	0.524	7.3E-15	0.082	0.110	0.143
49.10	0.828	0.748	0.615	0.509	3.1E-17	0.081	0.107	0.140
65.33	0.782	0.713	0.564	0.485	2.9E-18	0.072	0.095	0.124
68.03	0.780	0.705	0.568	0.480	5.3E-17	0.077	0.102	0.133
71.45	0.775	0.704	0.565	0.479	2.5E-19	0.071	0.094	0.123
73.72	0.763	0.706	0.570	0.473	3.2E-24	0.058	0.077	0.100
88.53	0.721	0.679	0.562	0.444	4.3E-21	0.063	0.083	0.109
91.25	0.718	0.695	0.548	0.449	3.4E-21	0.062	0.083	0.108

Table 3.241. The mean ODs at each assessment time for isolate 284 in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.173	0.100	0.103	0.102	7.0E-17	0.012	0.016	0.020
15.92	0.928	0.944	0.895	0.747	1.8E-23	0.081	0.108	0.139
18.90	0.827	0.853	0.772	0.720	5.8E-20	0.074	0.098	0.128
24.58	0.773	0.803	0.761	0.730	9.4E-15	0.082	0.109	0.142
40.48	0.706	0.794	0.753	0.706	1.1E-14	0.078	0.104	0.135
43.05	0.706	0.762	0.747	0.712	1.0E-15	0.076	0.101	0.131
45.68	0.694	0.766	0.747	0.706	1.0E-16	0.075	0.100	0.129
47.83	0.698	0.782	0.749	0.710	7.0E-18	0.071	0.095	0.123
64.68	0.665	0.741	0.743	0.698	1.7E-23	0.061	0.081	0.104
66.82	0.684	0.770	0.740	0.707	4.0E-20	0.071	0.094	0.122
70.12	0.660	0.747	0.743	0.696	7.0E-24	0.061	0.081	0.105
72.38	0.658	0.746	0.742	0.708	3.4E-23	0.064	0.085	0.110
88.42	0.633	0.733	0.750	0.700	3.3E-22	0.069	0.091	0.118
90.55	0.658	0.740	0.756	0.708	1.8E-25	0.061	0.082	0.106
92.65	0.647	0.740	0.757	0.705	1.7E-22	0.068	0.090	0.117
96.15	0.642	0.740	0.743	0.701	2.9E-23	0.066	0.088	0.114

Table 3.242. The mean ODs at each assessment time for isolate 284 in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In general, all the "growth" values of the treatments were greater than those of the control with the one exception of the 25ppm treatment in the third run (Table 3.245). Apart from this one exception, the "growth" of the 5 and 25 ppm treatments were significantly greater than that of the control and in the first two experiments (Table 3.243 and 3.244 respectively), these were

statistically significant at the 0.1% level. This clearly suggests a promotive effect of the fungicide pyraclostrobin on the "growth of the isolate 284.

Treatment	"Growth"
Pyra. 25	51.56
Pyra. 5	42.11
Pyra. 1	38.50
Control	34.03
LSD5%	4.50
LSD1%	5.98
LSD0.1%	7.75

Treatment	"Growth"
Pyra.25	55.00
Pyra.5	55.16
Pyra.1	45.17
Control	37.95
LSD5%	4.412
LSD1%	5.873
LSD 0.1%	7.649

Treatment	"Growth"
Pyra. 25	48.19
Pyra. 5	60.70
Pyra. 1	58.44
Control	53.88
LSD5%	5.73
LSD1%	7.60
LSD0.1%	9.86

Table 3.243. and 3.244. and 3.245. The mean "growth" of isolate 284 in treatments with 25, 5, and 1 ppm of pyraclostrobin over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

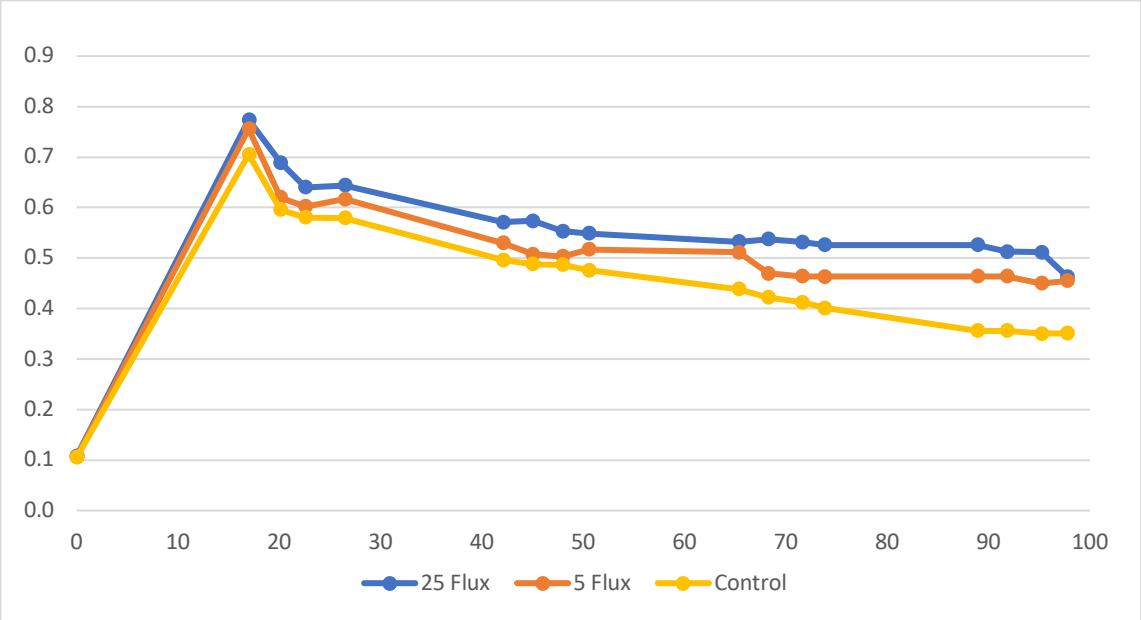


Figure 3.119. The OD curve of isolate 284 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

The cross-screening with the isolate 284 and the fungicide fluxapyroxad was run twice. In the first trial, after the initial increase, the OD values reached a peak followed by a decline until the test was completed (Fig. 3.119). The highest peak was recorded with the 1 ppm treatment (0.8). Not only with the 1 ppm but also with the 25 ppm treatment, the OD values were very significantly greater than the control at most timings reflected in the very significant greater "growth" of treatments at the 0.1 % level with the 1 and 25 ppm treatments and at the 5% level with the 5 ppm treatment (Table 3.246, 3.248).

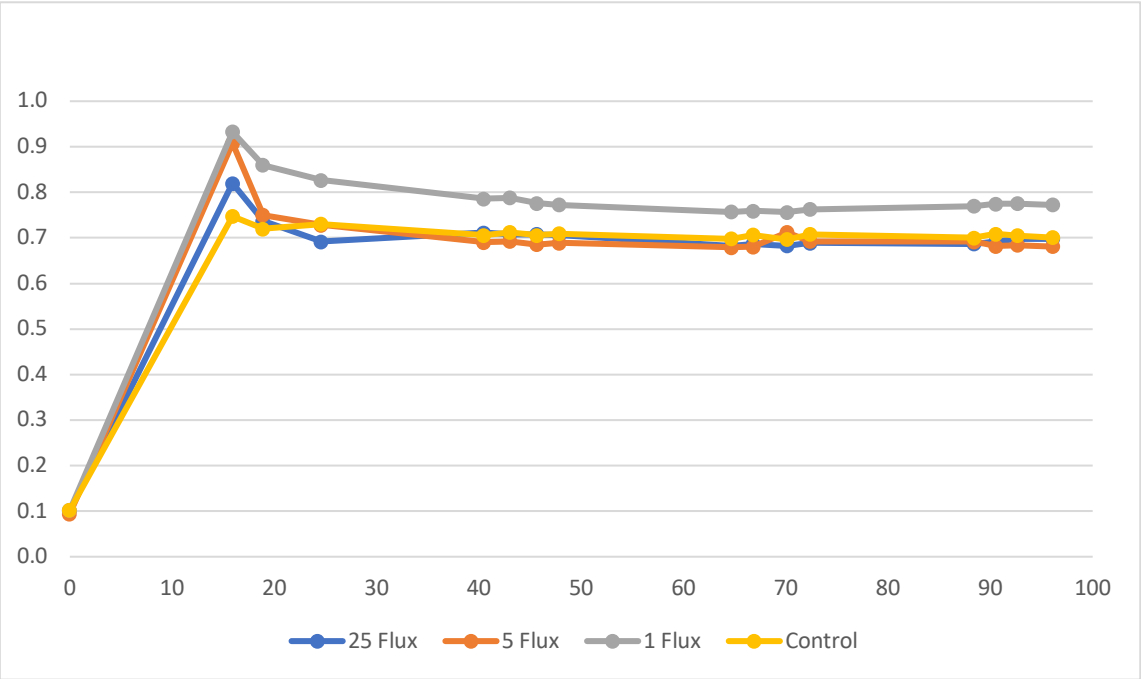


Figure 3.120. The OD curve of isolate 284 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.108	0.106	0.110	0.105	4.1E-11	0.010	0.013	0.017
15.90	0.774	0.756	0.813	0.705	1.2E-19	0.083	0.110	0.143
18.90	0.689	0.620	0.710	0.596	4.6E-18	0.073	0.097	0.125
21.58	0.640	0.602	0.686	0.580	3.4E-19	0.066	0.088	0.114
24.52	0.644	0.617	0.682	0.579	1.4E-20	0.062	0.082	0.106
40.48	0.571	0.530	0.616	0.496	1.4E-18	0.057	0.075	0.098
43.03	0.574	0.508	0.602	0.488	7.3E-18	0.058	0.077	0.100
45.63	0.553	0.503	0.597	0.487	3.2E-19	0.054	0.072	0.093
47.80	0.549	0.517	0.579	0.476	1.4E-20	0.053	0.070	0.091
64.63	0.532	0.511	0.543	0.439	3.6E-11	0.082	0.109	0.141
66.75	0.537	0.470	0.535	0.422	8.2E-12	0.081	0.107	0.139
70.10	0.532	0.464	0.528	0.412	8.2E-13	0.077	0.102	0.132
72.37	0.526	0.463	0.528	0.401	1.1E-12	0.078	0.104	0.135
88.33	0.526	0.464	0.513	0.356	1.9E-14	0.077	0.102	0.132
90.53	0.513	0.464	0.508	0.356	2.3E-14	0.076	0.100	0.130
92.67	0.512	0.450	0.506	0.350	4.2E-14	0.078	0.103	0.134
96.08	0.463	0.455	0.485	0.351	1.2E-13	0.074	0.098	0.127

Table 3.246. The mean ODs at each assessment time for isolate 284 in the presence of three concentrations of fluxapyroxad. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In the second trial though, after a short decline the OD values started to level off towards the end of experiment (Fig. 3.120). As in the first run, in the second trial the 1 ppm treatment showed the highest peak (0.9). With the 1 ppm treatment the OD value was significantly greater

than the control within the first 43 hours and also from 88 hours onwards (Table 3.247) This was reflected in the significantly greater "growth" of the 1 ppm treatment. With the 5 and 25 ppm treatments the OD value was similar to the one with control after the decline onwards (Table 3.249).

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.101	0.095	0.102	0.102	7.0E-17	0.012	0.016	0.020
15.92	0.820	0.908	0.933	0.747	1.8E-23	0.081	0.108	0.139
18.90	0.737	0.750	0.860	0.720	5.8E-20	0.074	0.098	0.128
24.58	0.692	0.728	0.827	0.730	9.4E-15	0.082	0.109	0.142
40.48	0.712	0.690	0.786	0.706	1.1E-14	0.078	0.104	0.135
43.05	0.708	0.693	0.788	0.712	1.0E-15	0.076	0.101	0.131
45.68	0.708	0.686	0.776	0.706	1.0E-16	0.075	0.100	0.129
47.83	0.707	0.689	0.773	0.710	7.0E-18	0.071	0.095	0.123
64.68	0.683	0.679	0.757	0.698	1.7E-23	0.061	0.081	0.104
66.82	0.687	0.681	0.759	0.707	4.0E-20	0.071	0.094	0.122
70.12	0.682	0.712	0.756	0.696	7.0E-24	0.061	0.081	0.105
72.38	0.689	0.693	0.763	0.708	3.4E-23	0.064	0.085	0.110
88.42	0.687	0.692	0.770	0.700	3.3E-22	0.069	0.091	0.118
90.55	0.692	0.682	0.775	0.708	1.8E-25	0.061	0.082	0.106
92.65	0.698	0.684	0.776	0.705	1.7E-22	0.068	0.090	0.117
96.15	0.698	0.682	0.773	0.701	2.9E-23	0.066	0.088	0.114

Table 3.247. The mean ODs at each assessment time for isolate 284 in the presence of three concentrations of fluxapyroxad. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Flux. 25	42.55
Flux. 5	38.93
Flux. 1	44.15
Control	34.03
LSD5%	4.50
LSD1%	5.98
LSD0.1%	7.75

Treatment	"Growth"
Flux. 25	53.79
Flux. 5	55.14
Flux. 1	61.44
Control	53.88
LSD5%	5.73
LSD1%	7.60
LSD0.1%	9.86

Table 3.248. and 3.249. The mean "growth" of isolate 284 in treatments with 25, 5, and 1 ppm of fluxapyroxad over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

Nevertheless, the significantly increased "growth" of all fluxapyroxad treatments in the first run and the significantly increased "growth" value of the 1 ppm treatment in the second run at the 5% level suggests a positive effect of the fungicide fluxapyroxad on the "growth" of the isolate 284.

3.2.4.14. Isolate 285

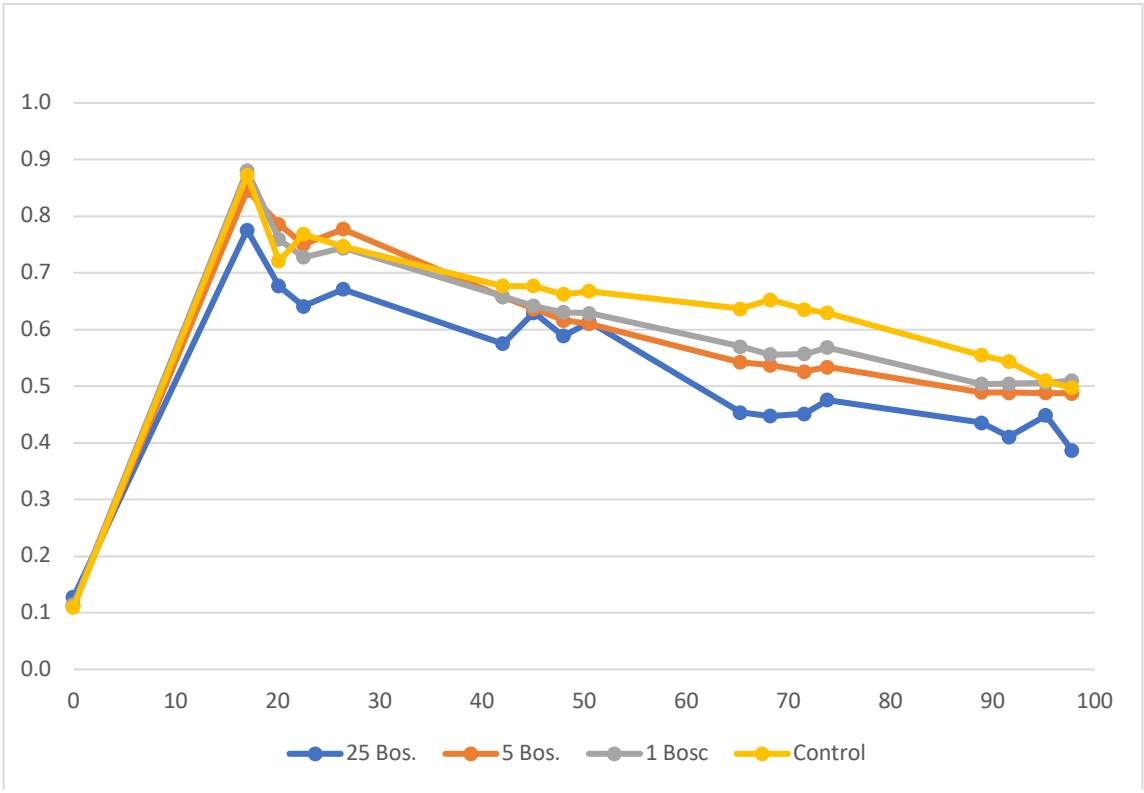


Figure 3.121. The OD curve of isolate 285 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

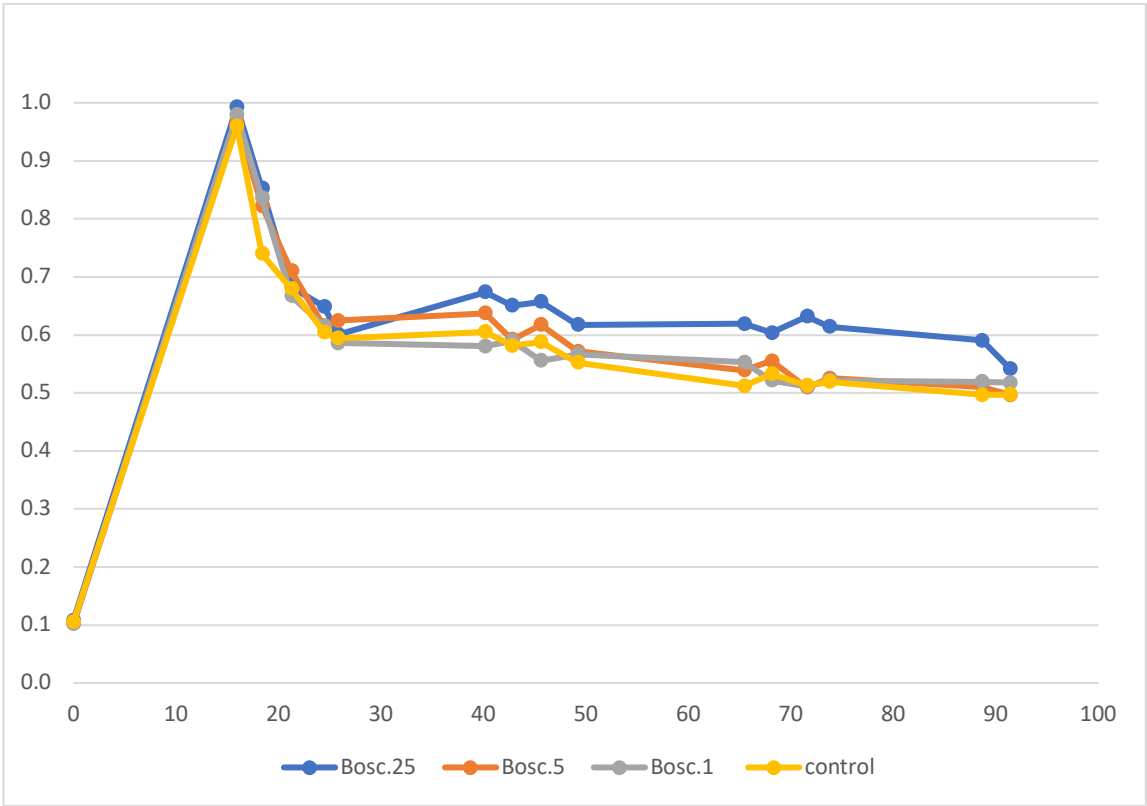


Figure 3.122. The OD curve of isolate 285 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

The cross-screening test between the isolate 285 and the fungicide boscalid was carried out twice. In the first run, after the initial increase the maximum observed OD values were achieved at 17.02 hours followed by a decline towards the end (Fig. 3.121). The OD values with the 25 ppm were significantly lower than the control at most timings reflected in a very significant lower "growth" of the 25 ppm treatment (Table 3. 250). The OD values with the 1 and 5 ppm were at a similar level to the control until 42.05 hours after which the OD value with the control continued to be greater than 1 and 5 ppm until the test was finished. Reflecting these results, the "growth" of all three treatments was lower than the control numerically (Table 3.252).

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.127	0.113	0.114	0.110	1.2E-26	0.007	0.009	0.011
15.90	0.776	0.846	0.881	0.873	4.3E-21	0.096	0.128	0.166
18.90	0.677	0.786	0.759	0.721	4.0E-18	0.083	0.110	0.143
21.58	0.641	0.750	0.728	0.768	1.5E-21	0.069	0.092	0.119
24.52	0.671	0.777	0.744	0.747	1.3E-19	0.070	0.093	0.120
40.48	0.575	0.658	0.658	0.677	4.5E-17	0.059	0.078	0.102
43.03	0.630	0.636	0.642	0.677	5.7E-15	0.064	0.085	0.110
45.63	0.588	0.617	0.630	0.662	1.3E-16	0.065	0.086	0.112
47.80	0.588	0.617	0.630	0.662	2.5E-14	0.076	0.101	0.131
64.63	0.614	0.610	0.629	0.668	1.9E-19	0.068	0.090	0.116
66.75	0.454	0.543	0.570	0.637	8.1E-22	0.068	0.090	0.117
70.10	0.448	0.537	0.556	0.652	6.4E-24	0.064	0.084	0.109
72.37	0.451	0.526	0.557	0.635	1.0E-19	0.078	0.104	0.135
88.33	0.476	0.534	0.568	0.629	2.1E-15	0.090	0.120	0.155
90.53	0.435	0.489	0.504	0.555	3.0E-16	0.089	0.118	0.153
92.67	0.410	0.489	0.504	0.543	3.0E-16	0.092	0.122	0.158
96.08	0.387	0.487	0.510	0.497	8.5E-16	0.196	0.256	0.330

Table 3.249. The mean ODs at each assessment time for isolate 285 in the presence of three concentrations of boscalid. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Similar to the first run, in the second run the peak was achieved after an initial increase at 15.09 hours followed by a decline. However, the OD values level led off from 25.82 hours onwards. (Fig. 3.122). The OD value with the control was similar to or lower (numerically) than the at most timings (Table. 3.251). The exception of this situation was the 25 ppm that showed greater OD values than the control from 40 hours onwards although the ODs were only numerically different. The "growth" of all the treatments were higher than the control but in no case significantly so (Table. 3.253). Although 25 ppm was approaching significance. However, in the first experiment the reverse was true, with all the treatments being numerically less than the

control. Accordingly, it is difficult to recognize a consistent effect of boscalid on the isolate 285 over the course of both experiments.

Ass. (h)	Bosc.25	Bosc.5	Bosc.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.108	0.102	0.102	0.106	2.1E-36	0.004	0.005	0.007
15.92	0.993	0.969	0.979	0.960	8.3E-33	0.058	0.077	0.101
18.38	0.852	0.822	0.836	0.740	1.0E-24	0.086	0.114	0.149
21.28	0.683	0.710	0.667	0.679	9.6E-22	0.069	0.092	0.120
24.45	0.649	0.615	0.615	0.605	1.8E-18	0.074	0.099	0.129
25.82	0.601	0.624	0.586	0.594	5.2E-19	0.073	0.097	0.126
40.17	0.674	0.638	0.580	0.605	4.6E-16	0.100	0.133	0.173
42.80	0.651	0.592	0.590	0.582	2.8E-14	0.107	0.142	0.185
45.62	0.657	0.617	0.556	0.588	1.2E-14	0.101	0.135	0.176
49.20	0.618	0.572	0.567	0.552	7.3E-13	0.108	0.144	0.188
65.43	0.619	0.539	0.553	0.512	5.2E-12	0.120	0.159	0.207
68.13	0.604	0.555	0.522	0.533	3.8E-14	0.108	0.143	0.186
71.57	0.632	0.509	0.511	0.513	1.6E-13	0.117	0.156	0.203
73.78	0.614	0.525	0.521	0.519	1.8E-14	0.106	0.141	0.184
88.67	0.590	0.511	0.519	0.497	1.2E-14	0.096	0.128	0.166
91.38	0.542	0.497	0.518	0.497	1.0E-12	0.106	0.141	0.184

Table 3.250. The mean ODs at each assessment time for isolate 285 in the presence of three concentrations of boscalid. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Bosc. 25	38.86
Bosc. 5	46.12
Bosc. 1	47.04
Control	50.37
LSD5%	4.87
LSD1%	6.46
LSD0.1%	8.38

Treatment	"Growth"
Bosc.25	47.03
Bosc.5	43.64
Bosc.1	42.67
Control	41.48
LSD5%	7.058
LSD1%	9.395
LSD0.1%	12.237

Table 3.251. and 3.252. The mean "growth" of isolate 285 in treatments with 25, 5, and 1 ppm of boscalid over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

The cross-screening test with the isolate 285 and the fungicide Revysol® was conducted on two occasions. In both trials, the OD value with the 25 ppm increased only slightly beyond the initial value at the start of experiment leading to statistically significant reductions compared to the control throughout the experiments.

In the first trial, after an initial increase the OD values reached a peak at 17.02 hours followed by a decline towards the end of experiment (Fig. 3.123). The OD value with the control and the 5 ppm treatment were at a similar level throughout the whole experiment. But with the 1 ppm,

the OD value started a steeper decline than the control from 26.40 hours onwards reflected in statistically significant lower OD values at most timing points (Table 3.254) and a resulting significantly lower “growth” value (Table 3.256).

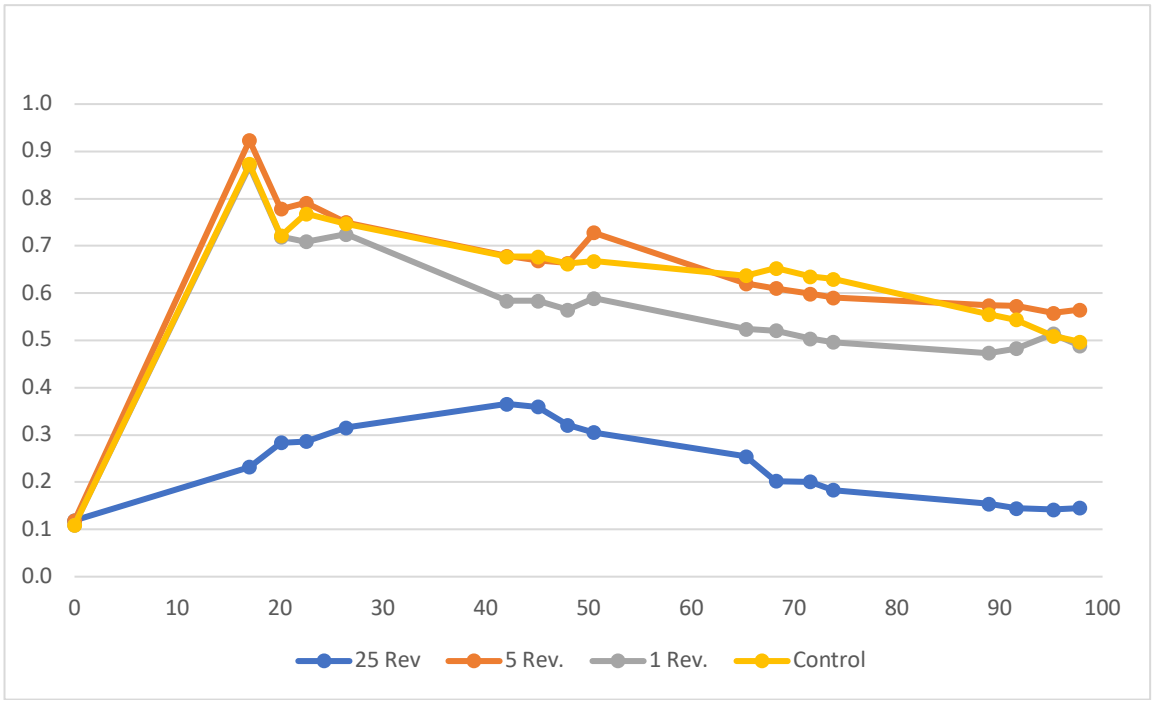


Figure 3.123. The OD curve of isolate 285 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

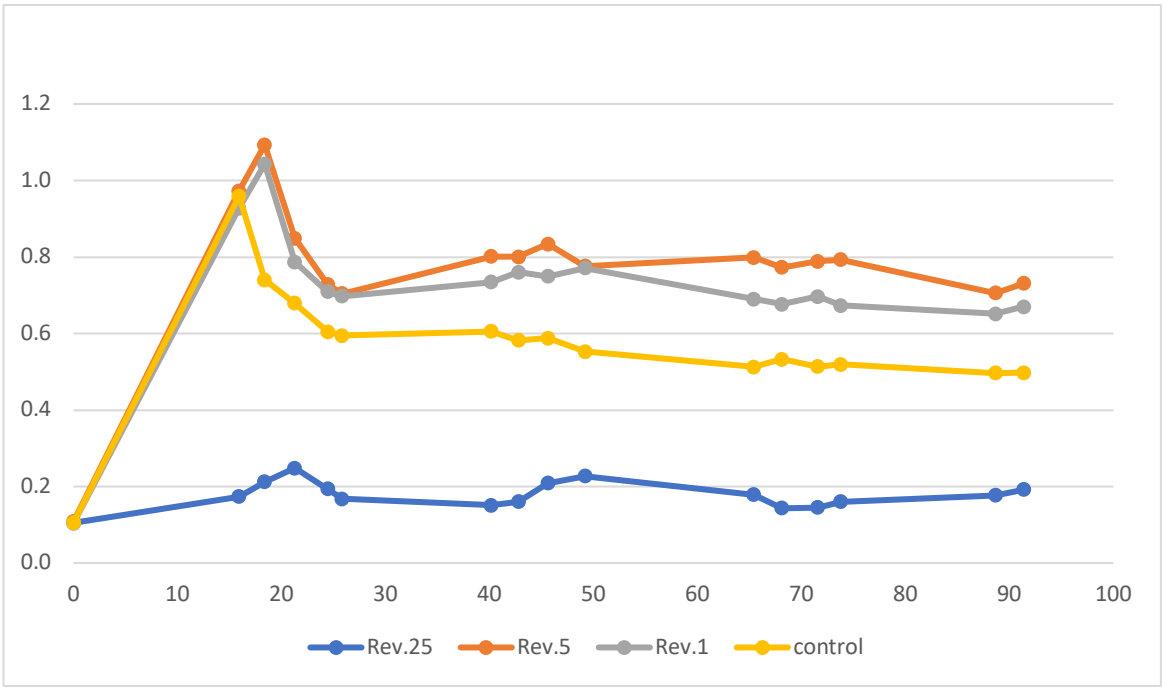


Figure 3.124. The OD curve of isolate 285 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.119	0.118	0.110	0.110	1.2E-26	0.007	0.009	0.011
15.90	0.232	0.923	0.869	0.873	4.3E-21	0.096	0.128	0.166
18.90	0.284	0.778	0.719	0.721	4.0E-18	0.083	0.110	0.143
21.58	0.287	0.791	0.709	0.768	1.5E-21	0.069	0.092	0.119
24.52	0.315	0.750	0.725	0.747	1.3E-19	0.070	0.093	0.120
40.48	0.366	0.678	0.583	0.677	4.5E-17	0.059	0.078	0.102
43.03	0.360	0.669	0.584	0.677	5.7E-15	0.064	0.085	0.110
45.63	0.321	0.664	0.565	0.662	1.3E-16	0.065	0.086	0.112
47.80	0.321	0.664	0.565	0.662	2.5E-14	0.076	0.101	0.131
64.63	0.305	0.728	0.589	0.668	1.9E-19	0.068	0.090	0.116
66.75	0.255	0.620	0.524	0.637	8.1E-22	0.068	0.090	0.117
70.10	0.202	0.610	0.521	0.652	6.4E-24	0.064	0.084	0.109
72.37	0.201	0.598	0.504	0.635	1.0E-19	0.078	0.104	0.135
88.33	0.183	0.591	0.496	0.629	2.1E-15	0.090	0.120	0.155
90.53	0.154	0.574	0.473	0.555	3.0E-16	0.089	0.118	0.153
92.67	0.145	0.573	0.483	0.543	3.0E-16	0.092	0.122	0.158
96.08	0.145	0.565	0.489	0.497	8.5E-16	0.196	0.256	0.330

Table 3.254. The mean ODs at each assessment time for isolate 285 in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Rev.25	Rev.5	Rev.1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.105	0.109	0.104	0.106	2.1E-36	0.004	0.005	0.007
15.92	0.174	0.973	0.928	0.960	8.3E-33	0.058	0.077	0.101
18.38	0.212	1.093	1.043	0.740	1.0E-24	0.086	0.114	0.149
21.28	0.249	0.849	0.787	0.679	9.6E-22	0.069	0.092	0.120
24.45	0.194	0.729	0.709	0.605	1.8E-18	0.074	0.099	0.129
25.82	0.168	0.704	0.698	0.594	5.2E-19	0.073	0.097	0.126
40.17	0.151	0.801	0.735	0.605	4.6E-16	0.100	0.133	0.173
42.80	0.161	0.800	0.761	0.582	2.8E-14	0.107	0.142	0.185
45.62	0.209	0.834	0.749	0.588	1.2E-14	0.101	0.135	0.176
49.20	0.227	0.776	0.771	0.552	7.3E-13	0.108	0.144	0.188
65.43	0.178	0.799	0.690	0.512	5.2E-12	0.120	0.159	0.207
68.13	0.144	0.773	0.676	0.533	3.8E-14	0.108	0.143	0.186
71.57	0.145	0.788	0.697	0.513	1.6E-13	0.117	0.156	0.203
73.78	0.160	0.793	0.673	0.519	1.8E-14	0.106	0.141	0.184
88.67	0.177	0.706	0.652	0.497	1.2E-14	0.096	0.128	0.166
91.38	0.192	0.731	0.670	0.497	1.0E-12	0.106	0.141	0.184

Table 3.255. The mean ODs at each assessment time for isolate 285 in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In contrast, in the second trial. a steep decline followed the peaks at 15.93 (control) and 18.38 (1 and 5 ppm treatments) and then from 25.82 hours the ODs started to level off until the test was stopped (Fig. 3.124). The 1 and 5 ppm had a greater initial growth and reached a higher

peak at 18.38 hours than the control at 15.93, reflected in significant ODs from the peak point (18.38) until the end of experiment (Table 3.255). Reflecting these results, the "growth" of the treatment with the Revysol® 1 and 5 ppm treatments was significantly higher than the control at the 1% and 0.1% level respectively (Table 3.257).

Over the two experiments a consistent and highly significant reduction in "growth" was obtained with the 25ppm treatment. However, for the 1 and 5 ppm treatments somewhat contradictory results were obtained, with a clear statistically significant increase in "growth" being observed in the second experiment at both rates whereas in the first run there was a significant reduction with the 1ppm rate and no effect with the 5ppm treatment.

Treatment	"Growth"
Rev. 25	11.96
Rev. 5	50.63
Rev. 1	43.95
Control	50.37
LSD5%	4.87
LSD1%	6.46
LSD0.1%	8.38

Treatment	"Growth"
Rev.25	6.26
Rev.5	58.07
Rev.1	53.45
Control	41.48
LSD5%	7.058
LSD1%	9.395
LSD 0.1%	12.237

Table 3.256. and 3.257. The mean "growth" of isolate 285 in treatments with 25, 5, and 1 ppm of Revysol® over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

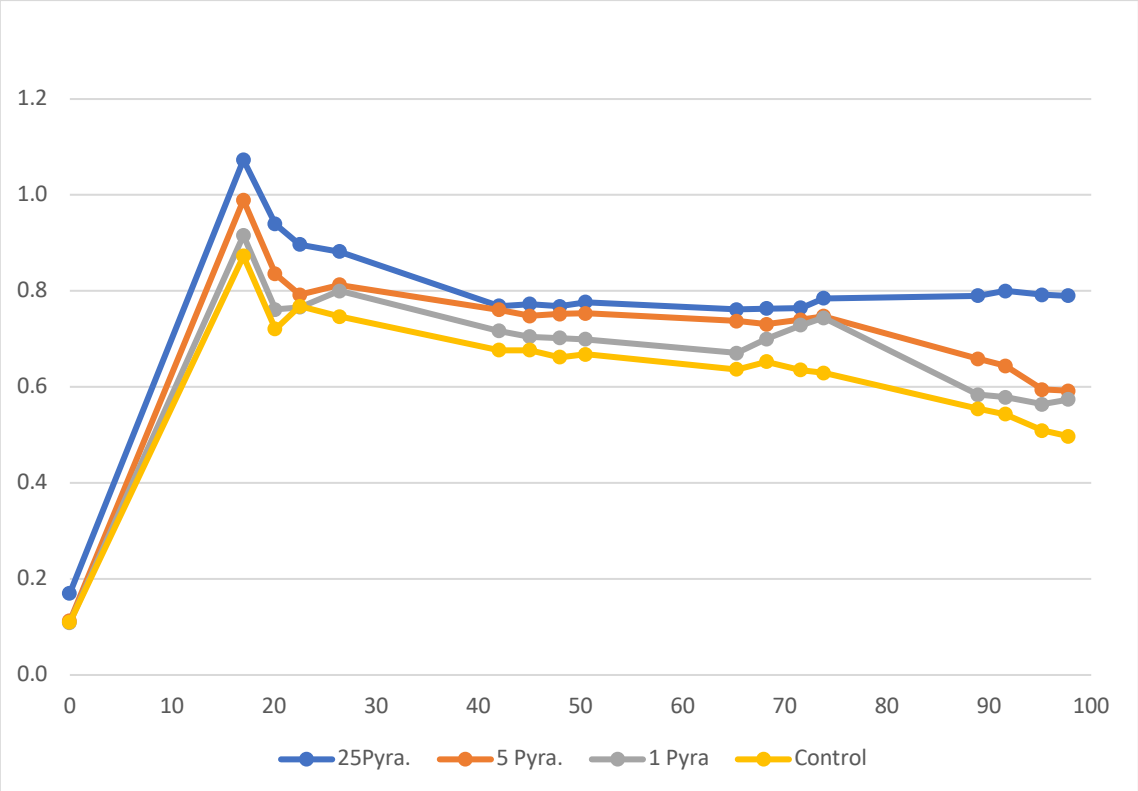


Figure 3.125. The OD curve of isolate 285 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

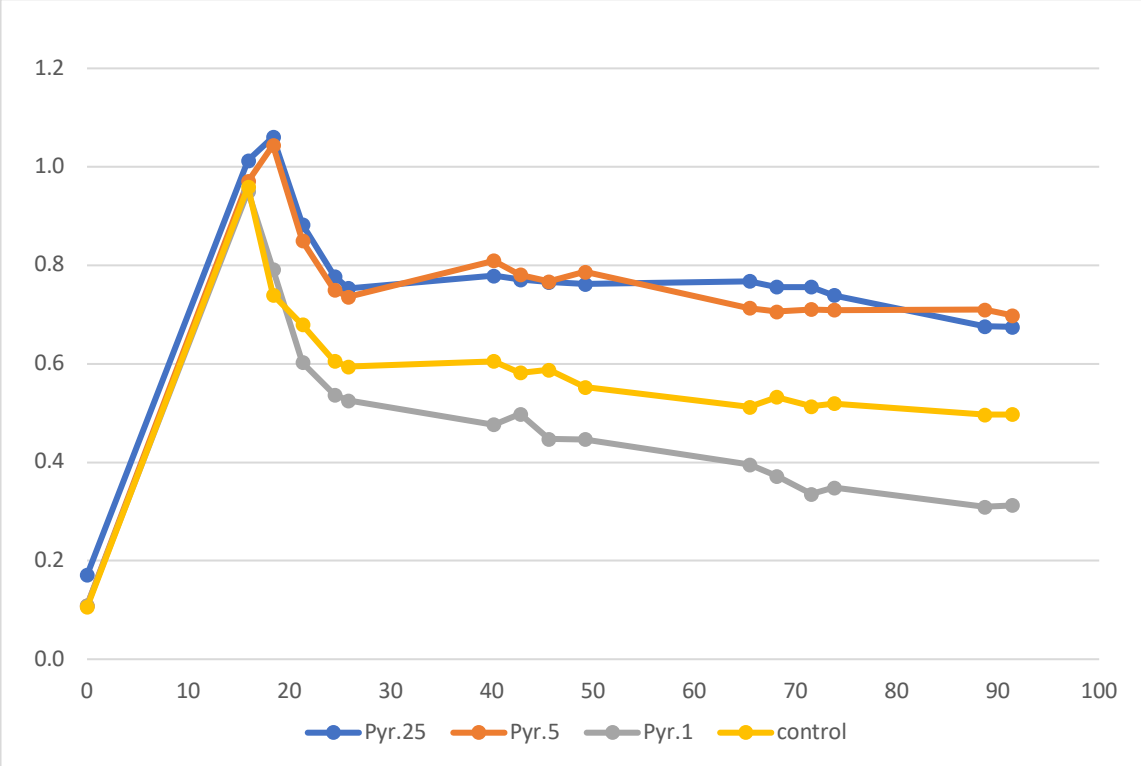


Figure 3.126. The OD curve of isolate 285 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

The isolate 285 was tested with the presence of the fungicide pyraclostrobin twice. In both trials the 25 ppm treatment achieved the highest peak value at 17.02 and 18.38 hours in the first and second trial respectively. In the first trial the OD values followed a decline after the peak which became more gradual until the test was finished (Fig. 3.125). Throughout the whole experiment, the OD values of the treatments were greater than those of the control resulting in statistically significant differences with the 5 and 25 ppm (Table 3.258) resulted in remarkedly greater "growth" of the 5 and 25 ppm treatments (Table 3.259).

In the second trial, after the decline in the OD value after reaching the peak, the 5 and 25 ppm treatments started to level off after 25.82 hours while the control and 1 ppm treatment continued to decline (Fig. 3.126). This resulted in very significant differences between the 5 and 25 ppm treatments and the control (Table 3.261). But with the 1 ppm the OD values started to decrease more steeply than those of the control with significant differences at some timings, particularly towards the end of the experiment (Table 3.259).
Nevertheless, the significantly higher "growth" of treatments with the 5 and 25 ppm suggests an enhanced "growth" of the strain 285 in the presence of the fungicide pyraclostrobin.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.170	0.112	0.109	0.110	1.2E-26	0.007	0.009	0.011
15.90	1.073	0.990	0.916	0.873	4.3E-21	0.096	0.128	0.166
18.90	0.940	0.835	0.762	0.721	4.0E-18	0.083	0.110	0.143
21.58	0.897	0.792	0.766	0.768	1.5E-21	0.069	0.092	0.119
24.52	0.882	0.813	0.800	0.747	1.3E-19	0.070	0.093	0.120
40.48	0.769	0.761	0.717	0.677	4.5E-17	0.059	0.078	0.102
43.03	0.773	0.748	0.705	0.677	5.7E-15	0.064	0.085	0.110
45.63	0.768	0.752	0.702	0.662	1.3E-16	0.065	0.086	0.112
47.80	0.768	0.752	0.702	0.662	2.5E-14	0.076	0.101	0.131
64.63	0.777	0.754	0.700	0.668	1.9E-19	0.068	0.090	0.116
66.75	0.762	0.737	0.671	0.637	8.1E-22	0.068	0.090	0.117
70.10	0.764	0.731	0.700	0.652	6.4E-24	0.064	0.084	0.109
72.37	0.765	0.740	0.729	0.635	1.0E-19	0.078	0.104	0.135
88.33	0.785	0.747	0.744	0.629	2.1E-15	0.090	0.120	0.155
90.53	0.790	0.659	0.584	0.555	3.0E-16	0.089	0.118	0.153
92.67	0.800	0.644	0.579	0.543	3.0E-16	0.092	0.122	0.158
96.08	0.790	0.592	0.574	0.497	8.5E-16	0.196	0.256	0.330

Table 3.258. The mean ODs at each assessment time for isolate 285 in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.171	0.109	0.109	0.106	2.1E-36	0.004	0.005	0.007
15.92	1.013	0.971	0.950	0.960	8.3E-33	0.058	0.077	0.101
18.38	1.061	1.044	0.792	0.740	1.0E-24	0.086	0.114	0.149
21.28	0.883	0.850	0.602	0.679	9.6E-22	0.069	0.092	0.120
24.45	0.778	0.750	0.537	0.605	1.8E-18	0.074	0.099	0.129
25.82	0.754	0.736	0.525	0.594	5.2E-19	0.073	0.097	0.126
40.17	0.779	0.810	0.477	0.605	4.6E-16	0.100	0.133	0.173
42.80	0.771	0.781	0.498	0.582	2.8E-14	0.107	0.142	0.185
45.62	0.766	0.767	0.447	0.588	1.2E-14	0.101	0.135	0.176
49.20	0.762	0.787	0.447	0.552	7.3E-13	0.108	0.144	0.188
65.43	0.768	0.713	0.396	0.512	5.2E-12	0.120	0.159	0.207
68.13	0.756	0.706	0.372	0.533	3.8E-14	0.108	0.143	0.186
71.57	0.756	0.711	0.335	0.513	1.6E-13	0.117	0.156	0.203
73.78	0.739	0.709	0.349	0.519	1.8E-14	0.106	0.141	0.184
88.67	0.676	0.710	0.309	0.497	1.2E-14	0.096	0.128	0.166
91.38	0.675	0.698	0.313	0.497	1.0E-12	0.106	0.141	0.184

Table 3.259. The mean ODs at each assessment time for isolate 285 in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In the one cross-screening test conducted with the isolate 285 and the fungicide fluxapyroxad, OD values reached a peak at 17.02 hours with the highest recorded peak with the 1 ppm treatment followed by a decline until the test was stopped (Fig. 3.127). Through the fall, the

steeper decline occurred with the 1 and 5 ppm treatments resulting in significant lower OD values with the 5 ppm treatment at some points (Table 3.262). Nevertheless, since the "growth" of the treatments did not significantly differ from the control it's assumed that the presence of the fungicide fluxapyroxad had little effect of the "growth" of the strain 285 (Table 3.263).

Treatment	"Growth"
Pyra. 25	58.96
Pyra. 5	58.40
Pyra. 1	54.62
Control	50.37
LSD5%	4.87
LSD1%	6.46
LSD0.1%	8.38

Treatment	"Growth"
Pyra. 25	51.88
Pyra. 5	56.12
Pyra. 1	32.13
Control	41.48
LSD5%	7.058
LSD1%	9.395
LSD 0.1%	12.237

Table 3.260. and 3.261. The mean "growth" of isolate 285 in treatments with 25, 5, and 1 ppm of pyraclostrobin over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

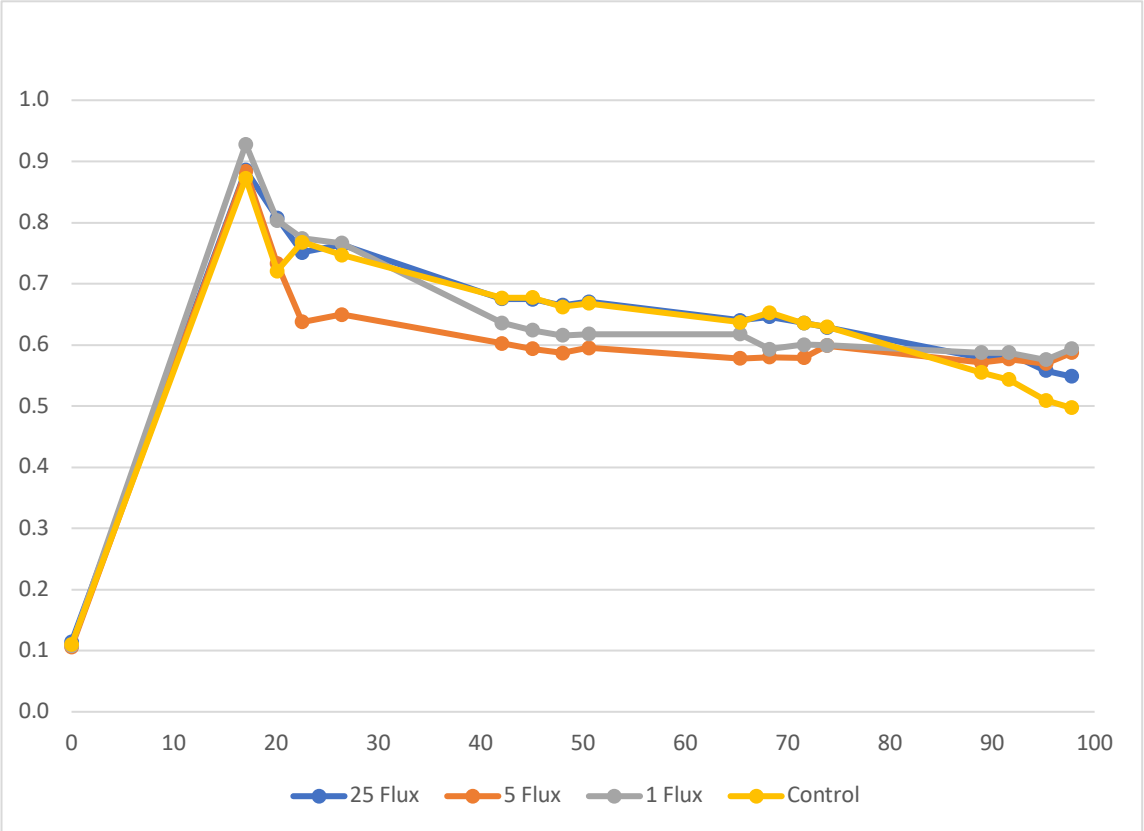


Figure 3.127. The OD curve of isolate 285 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.114	0.106	0.108	0.110	1.2E-26	0.007	0.009	0.011
15.90	0.886	0.884	0.928	0.873	4.3E-21	0.096	0.128	0.166
18.90	0.808	0.733	0.804	0.721	4.0E-18	0.083	0.110	0.143
21.58	0.751	0.638	0.774	0.768	1.5E-21	0.069	0.092	0.119
24.52	0.764	0.649	0.766	0.747	1.3E-19	0.070	0.093	0.120
40.48	0.675	0.602	0.636	0.677	4.5E-17	0.059	0.078	0.102
43.03	0.675	0.594	0.624	0.677	5.7E-15	0.064	0.085	0.110
45.63	0.665	0.587	0.616	0.662	1.3E-16	0.065	0.086	0.112
47.80	0.665	0.587	0.616	0.662	2.5E-14	0.076	0.101	0.131
64.63	0.671	0.595	0.618	0.668	1.9E-19	0.068	0.090	0.116
66.75	0.640	0.578	0.617	0.637	8.1E-22	0.068	0.090	0.117
70.10	0.646	0.580	0.593	0.652	6.4E-24	0.064	0.084	0.109
72.37	0.636	0.579	0.601	0.635	1.0E-19	0.078	0.104	0.135
88.33	0.628	0.599	0.599	0.629	2.1E-15	0.090	0.120	0.155
90.53	0.578	0.571	0.587	0.555	3.0E-16	0.089	0.118	0.153
92.67	0.586	0.577	0.587	0.543	3.0E-16	0.092	0.122	0.158
96.08	0.549	0.587	0.594	0.497	8.5E-16	0.196	0.256	0.330

Table 3.262. The mean ODs at each assessment time for isolate 285 in the presence of three concentrations of fluxapyroxad. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Flux. 25	51.01
Flux. 5	47.06
Flux. 1	50.41
Control	50.37
LSD5%	4.87
LSD1%	6.46
LSD0.1%	8.38

Table 3.263 The mean "growth" of isolate 285 in treatments with 25, 5, and 1 ppm of fluxapyroxad over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

3.2.4.15. Serenade®

The cross-screening with the Serenade® isolate (*Bacillus subtilis*) and the fungicide boscalid was carried out on two occasions. In the first trial the OD value increased gradually until around 70 hours or so where the peak values were reached after which the OD values started to level off (Fig. 3.128). The OD value with the 25 ppm boscalid treatment was significantly lower than the control form 68,60 hours onwards (Table 3.264). There was a tendency in the OD values of the 1 and 5 ppm treatments to be slightly higher than the control at most timings. Therefore, the

"growth" of treatments with the 1 and 5 ppm was numerically but not statistically significantly higher than the control (Table 3. 266).

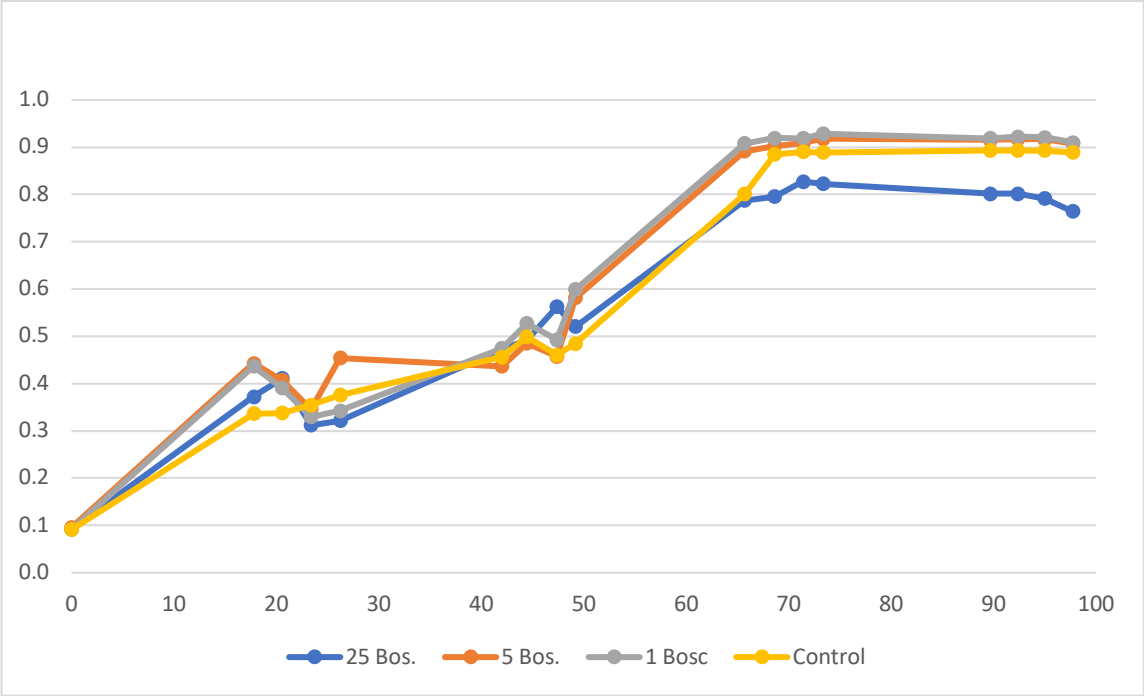


Figure 3.128. The OD curve of Serenade® over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

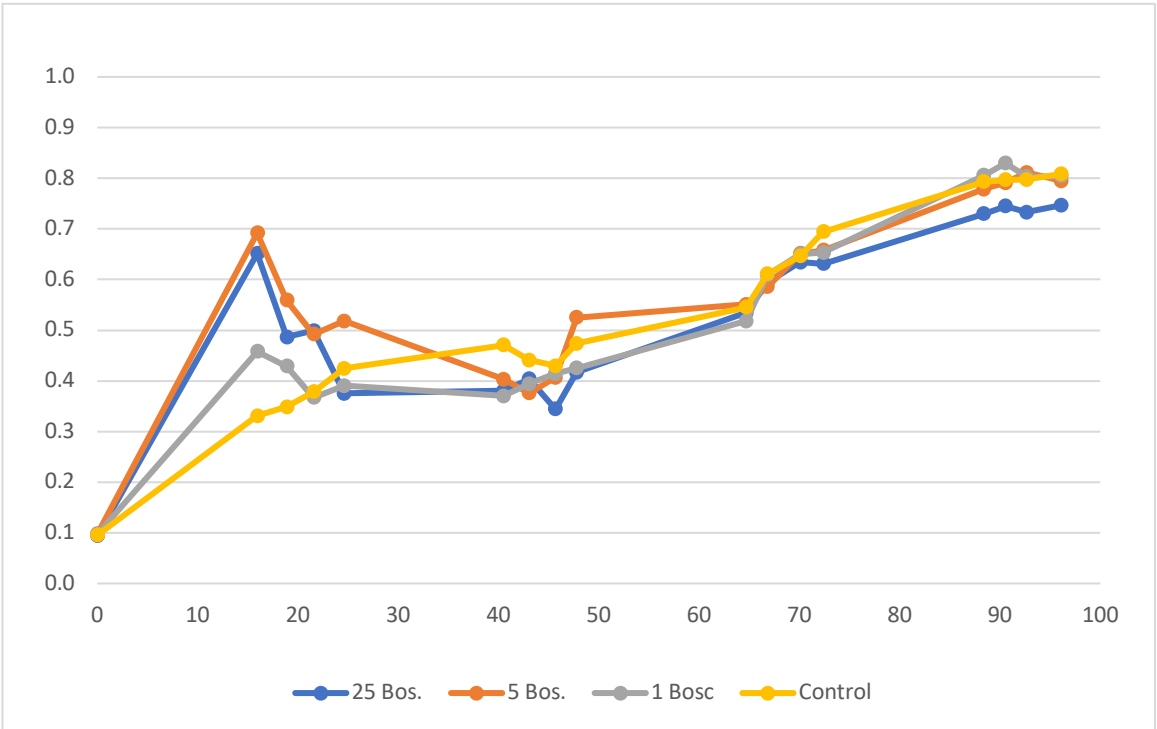


Figure 3.129. The OD curve of Serenade® over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.093	0.096	0.092	0.091	3.6E-23	0.006	0.008	0.010
17.78	0.373	0.443	0.437	0.337	2.4E-07	0.101	0.135	0.175
20.55	0.411	0.407	0.391	0.337	1.2E-05	0.105	0.140	0.181
23.35	0.312	0.346	0.330	0.355	3.3E-06	0.099	0.131	0.170
26.22	0.321	0.454	0.342	0.376	5.4E-06	0.121	0.160	0.208
42.00	0.473	0.437	0.475	0.456	3.3E-06	0.130	0.173	0.224
44.43	0.492	0.486	0.527	0.499	4.8E-08	0.125	0.166	0.216
47.35	0.563	0.458	0.493	0.460	8.0E-09	0.137	0.182	0.237
49.17	0.521	0.581	0.599	0.485	2.1E-09	0.130	0.172	0.224
65.65	0.787	0.892	0.908	0.801	8.2E-27	0.083	0.110	0.143
68.60	0.795	0.903	0.919	0.885	3.0E-24	0.089	0.118	0.153
71.38	0.828	0.909	0.919	0.890	6.6E-28	0.077	0.102	0.133
73.32	0.822	0.918	0.928	0.889	6.5E-29	0.076	0.102	0.132
89.63	0.802	0.915	0.919	0.893	1.1E-37	0.058	0.077	0.100
92.32	0.802	0.917	0.922	0.893	1.0E-39	0.054	0.072	0.094
94.98	0.792	0.916	0.921	0.893	7.6E-39	0.057	0.076	0.099
97.70	0.764	0.908	0.910	0.889	3.4E-38	0.058	0.077	0.100

Table 3.264. The mean ODs at each assessment time for Serenade® in the presence of three concentrations of boscalid. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.094	0.098	0.097	0.095	7.2E-09	0.012	0.015	0.020
15.95	0.651	0.692	0.458	0.331	1.6E-11	0.123	0.164	0.212
18.90	0.487	0.559	0.429	0.348	2.9E-10	0.086	0.114	0.148
21.57	0.499	0.492	0.367	0.379	4.5E-07	0.092	0.122	0.158
24.60	0.376	0.518	0.391	0.425	7.5E-10	0.072	0.096	0.124
40.48	0.380	0.403	0.370	0.470	1.1E-03	0.094	0.125	0.162
43.07	0.403	0.376	0.394	0.441	1.7E-02	0.104	0.137	0.178
45.68	0.345	0.407	0.414	0.430	4.1E-04	0.083	0.110	0.143
47.78	0.417	0.525	0.425	0.474	7.6E-08	0.085	0.113	0.147
64.70	0.535	0.551	0.518	0.545	4.0E-16	0.083	0.110	0.143
66.80	0.594	0.585	0.609	0.611	9.8E-20	0.080	0.107	0.138
70.08	0.635	0.652	0.650	0.646	9.0E-24	0.072	0.095	0.123
72.40	0.631	0.658	0.653	0.694	9.1E-22	0.082	0.108	0.140
88.37	0.730	0.779	0.806	0.793	4.3E-28	0.071	0.095	0.123
90.55	0.745	0.791	0.830	0.797	7.4E-27	0.076	0.101	0.131
92.67	0.733	0.811	0.803	0.798	1.0E-27	0.073	0.097	0.126
96.08	0.747	0.795	0.804	0.808	1.0E-30	0.065	0.087	0.113

Table 3.265. The mean ODs at each assessment time for Serenade® in the presence of three concentrations of boscalid. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

In the second trial, the boscalid treatments showed greater initial increases compared to the control leading to significant higher OD values of the 5 and 25 ppm treatments compared to the control within the first 24 hours (Table 3.265). The OD value with the treatments started to

decline after reaching an initial peak value at 15.95 hours. The treatments started again to gradually increase from 40.48 hours onwards. However, with the control the OD value was constantly increasing from the beginning of the experiment. (Fig. 3.129). Reflecting these results, the "growth" of treatments with the boscalid was not significantly different from the control although with the 5ppm treatment the value bordered on statistical significance mainly due to the differences occurring in the first 40 hours of the experiment (Table 3.267)

The numerically higher "growth" of the boscalid treatment 5 ppm in both trials could suggest a positive effect of the boscalid at this rate on the ''growth'' of the Serenade® isolate.

Treatment	"Growth"
Bosc. 25	44.67
Bosc. 5	50.68
Bosc. 1	50.96
Control	46.86
LSD5%	4.84
LSD1%	6.43
LSD0.1%	8.34

Treatment	"Growth"
Bosc. 25	39.31
Bosc. 5	43.47
Bosc. 1	38.21
Control	39.30
LSD5%	4.41
LSD1%	5.85
LSD0.1%	7.58

Table 3.266. and 3.267. The mean "growth" of Serenade® in treatments with 25, 5, and 1 ppm of boscalid over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

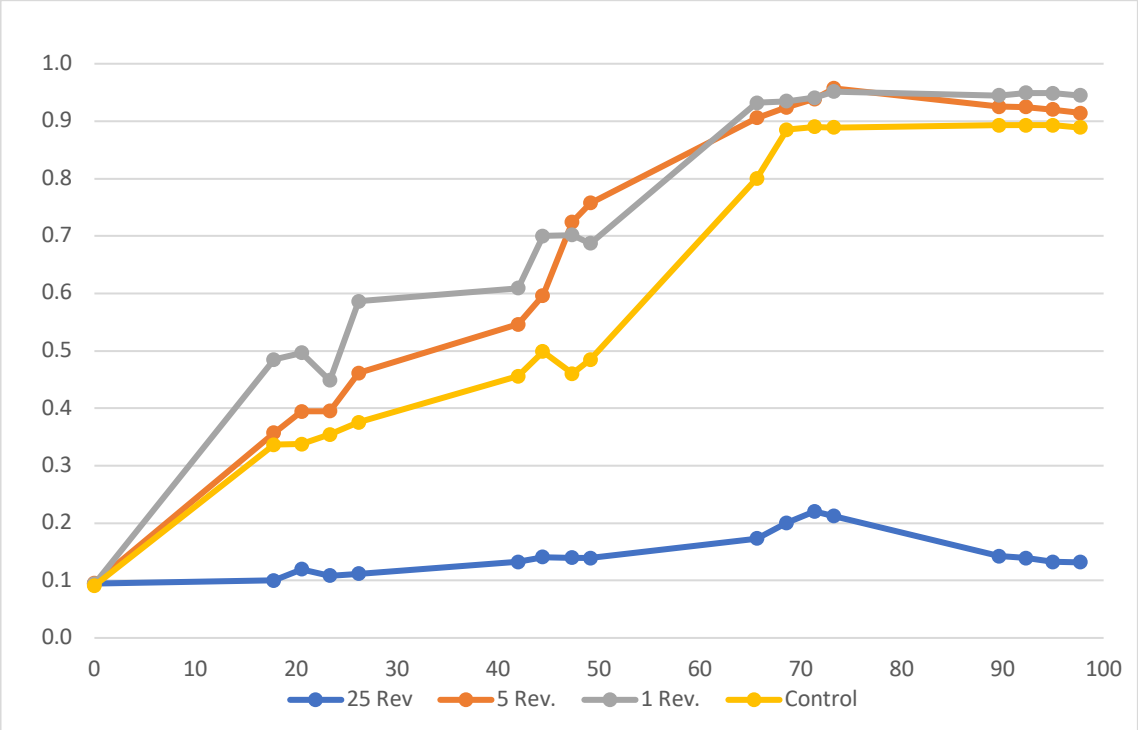


Figure 3.130. The OD curve of Serenade® over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

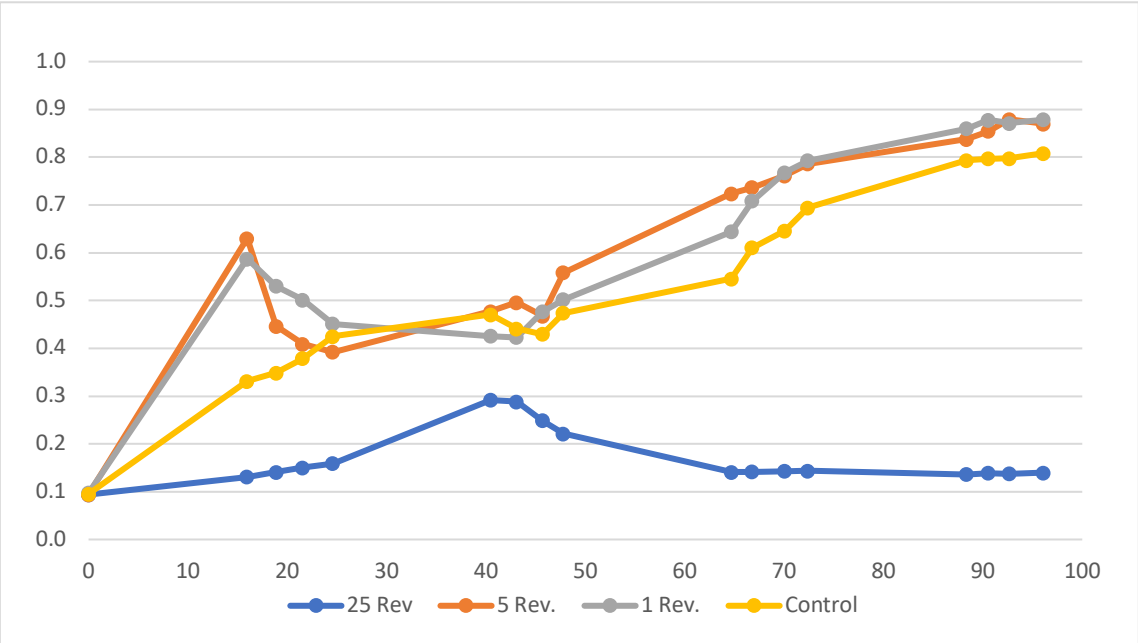


Figure 3.131. The OD curve of Serenade® over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

The cross-screening test with the Serenade® isolate (*Bacillus subtilis*) and the fungicide Revysol® was carried out twice. In both trials, the OD values showed similar tendencies. With the 25 ppm treatments the OD values showed minimal increase in the first run, and in the second it increased relatively little beyond the initial value at the start of experiment reflected in very significant lower ODs over the experimental time compared to the control and significantly lower "growth" than the control.

In the first run, the OD values increased gently until 68.60 hours where the ODs started to level off until the test was stopped (Fig. 3.130). The 1 and 5 ppm treatments had higher OD values throughout the whole experiment, and with the 1 ppm the OD value was significantly higher within the first 66 hours while the 5ppm treatment was significantly higher from 47 to 66 hours (Table 3.268). This resulted in the significantly higher "growth" of the 1 and 5 ppm treatments at the 0.1% and 1% level respectively (Table 3.270).

In the second trial, the 1 and 5 ppm treatments reached a first peak value at 15.95 hours followed by a decline. The OD value with the 5 ppm treatment at 24,60 hours and the 1 ppm treatment at 43.07 hours started again to increase gently for the remainder of the experiment whereas the control, similar to the first trial, was gently increasing throughout the whole experiment (Fig. 3. 131). However, the OD value of the control was lower than those with the 1 and 5 ppm treatments at most timings and with the 5 ppm treatment, the ODs were significantly different to the control at most of the timings and with the 5ppm treatment occasionally (Table 3.270).

This resulted in the significant higher "growth" of the 1 and 5 ppm at the 1% and 0.1 % level respectively compared to the control (Table 3.271).

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.095	0.096	0.094	0.091	3.6E-23	0.006	0.008	0.010
17.78	0.100	0.357	0.485	0.337	2.4E-07	0.101	0.135	0.175
20.55	0.120	0.395	0.497	0.337	1.2E-05	0.105	0.140	0.181
23.35	0.109	0.395	0.449	0.355	3.3E-06	0.099	0.131	0.170
26.22	0.112	0.461	0.587	0.376	5.4E-06	0.121	0.160	0.208
42.00	0.132	0.546	0.609	0.456	3.3E-06	0.130	0.173	0.224
44.43	0.141	0.597	0.700	0.499	4.8E-08	0.125	0.166	0.216
47.35	0.140	0.725	0.702	0.460	8.0E-09	0.137	0.182	0.237
49.17	0.139	0.757	0.687	0.485	2.1E-09	0.130	0.172	0.224
65.65	0.173	0.906	0.932	0.801	8.2E-27	0.083	0.110	0.143
68.60	0.201	0.924	0.935	0.885	3.0E-24	0.089	0.118	0.153
71.38	0.220	0.939	0.941	0.890	6.6E-28	0.077	0.102	0.133
73.32	0.212	0.957	0.951	0.889	6.5E-29	0.076	0.102	0.132
89.63	0.142	0.925	0.945	0.893	1.1E-37	0.058	0.077	0.100
92.32	0.139	0.925	0.949	0.893	1.0E-39	0.054	0.072	0.094
94.98	0.132	0.920	0.949	0.893	7.6E-39	0.057	0.076	0.099
97.70	0.132	0.914	0.945	0.889	3.4E-38	0.058	0.077	0.100

Table 3.268. The mean ODs at each assessment time for Serenade® in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.094	0.094	0.097	0.095	7.2E-09	0.012	0.015	0.020
15.95	0.131	0.630	0.587	0.331	1.6E-11	0.123	0.164	0.212
18.90	0.141	0.447	0.530	0.348	2.9E-10	0.086	0.114	0.148
21.57	0.150	0.409	0.501	0.379	4.5E-07	0.092	0.122	0.158
24.60	0.159	0.392	0.451	0.425	7.5E-10	0.072	0.096	0.124
40.48	0.292	0.477	0.426	0.470	1.1E-03	0.094	0.125	0.162
43.07	0.289	0.496	0.423	0.441	1.7E-02	0.104	0.137	0.178
45.68	0.249	0.468	0.477	0.430	4.1E-04	0.083	0.110	0.143
47.78	0.222	0.558	0.502	0.474	7.6E-08	0.085	0.113	0.147
64.70	0.141	0.724	0.644	0.545	4.0E-16	0.083	0.110	0.143
66.80	0.141	0.737	0.709	0.611	9.8E-20	0.080	0.107	0.138
70.08	0.143	0.761	0.768	0.646	9.0E-24	0.072	0.095	0.123
72.40	0.144	0.786	0.793	0.694	9.1E-22	0.082	0.108	0.140
88.37	0.137	0.838	0.860	0.793	4.3E-28	0.071	0.095	0.123
90.55	0.139	0.854	0.878	0.797	7.4E-27	0.076	0.101	0.131
92.67	0.138	0.879	0.871	0.798	1.0E-27	0.073	0.097	0.126
96.08	0.140	0.870	0.878	0.808	1.0E-30	0.065	0.087	0.113

Table 3.269. The mean ODs at each assessment time for Serenade® in the presence of three concentrations of Revysol®. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Rev. 25	4.46
Rev. 5	54.28
Rev. 1	58.12
Control	46.86
LSD5%	4.84
LSD1%	6.43
LSD0.1%	8.34

Treatment	"Growth"
25 Rev	7.01
5 Rev.	47.42
1 Rev.	46.13
Control	39.30
LSD5%	4.41
LSD1%	5.85
LSD0.1%	7.58

Table 3.270. and 3.271. The mean "growth" of Serenade® in treatments with 25, 5, and 1 ppm of Revysol® over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

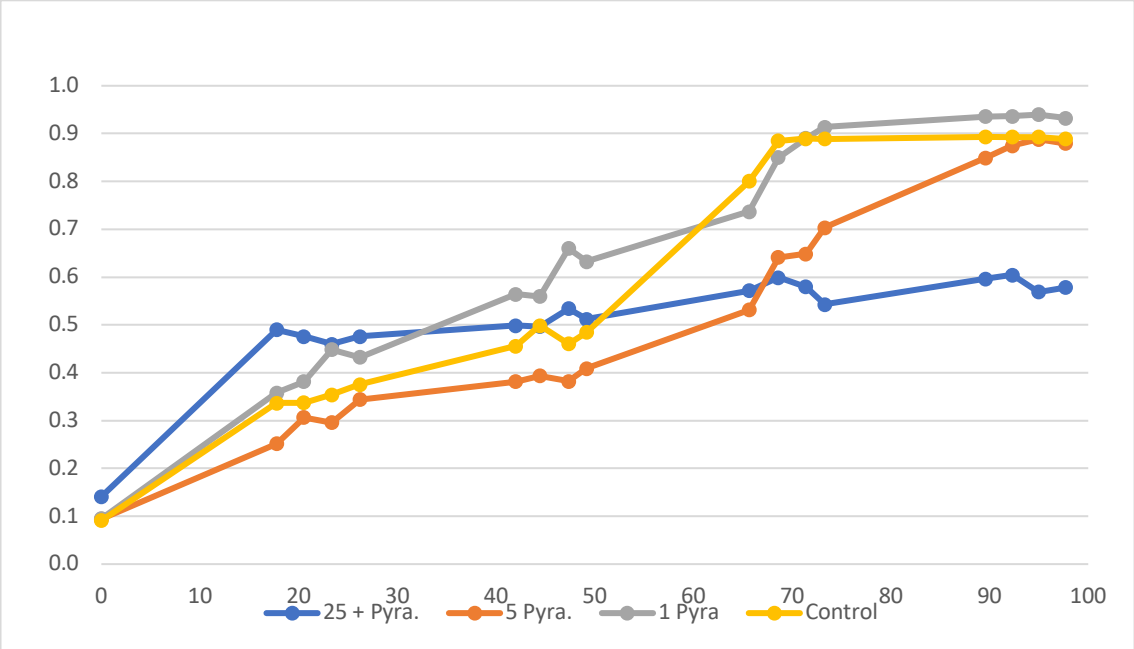


Figure 3.132. The OD curve of Serenade® over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

The observation that the "growth" of the treatments with the 1 and 5 ppm was significantly greater than the control in both trial points in the direction of the enhanced "growth" of the strain *B. subtilis* in the presence of the fungicide Revysol® at the rate of 1 and 5 ppm, while the rate of 25 ppm represents an inhibitory effect on the "growth".

The cross-screening test with the Serenade® isolate and the fungicide pyraclostrobin was conducted twice. In the first trial, the OD value with the 25 ppm treatment after initially increasing more rapidly showed only a slight increase from 17.78 hours onwards (Fig. 3.132). Meanwhile, the OD values with the 1 and 5 ppm treatments and the control increased to a greater extent. As a result, very significantly lower OD values of the 25 ppm treatment occurred from 65.70 hours onwards (Table 3.271). The OD values of the 5 ppm treatment were lower than those of the control throughout the experiment until after 68.6 hours when the ODs of the 1ppm treatment and the control started to plateau out while those of the 5ppm treatment

continued to increase. Nevertheless, the ODs of the 5 ppm treatment were significantly lower between 65.70 and 89.60 hours. Reflecting this, the "growth" of the 5 and 25 ppm treatments were significantly lower than the control at the 1% and 0.1% level. The "growth" of the 1 ppm treatment was higher than the control and bordered on statistical significance (Table 3.273).

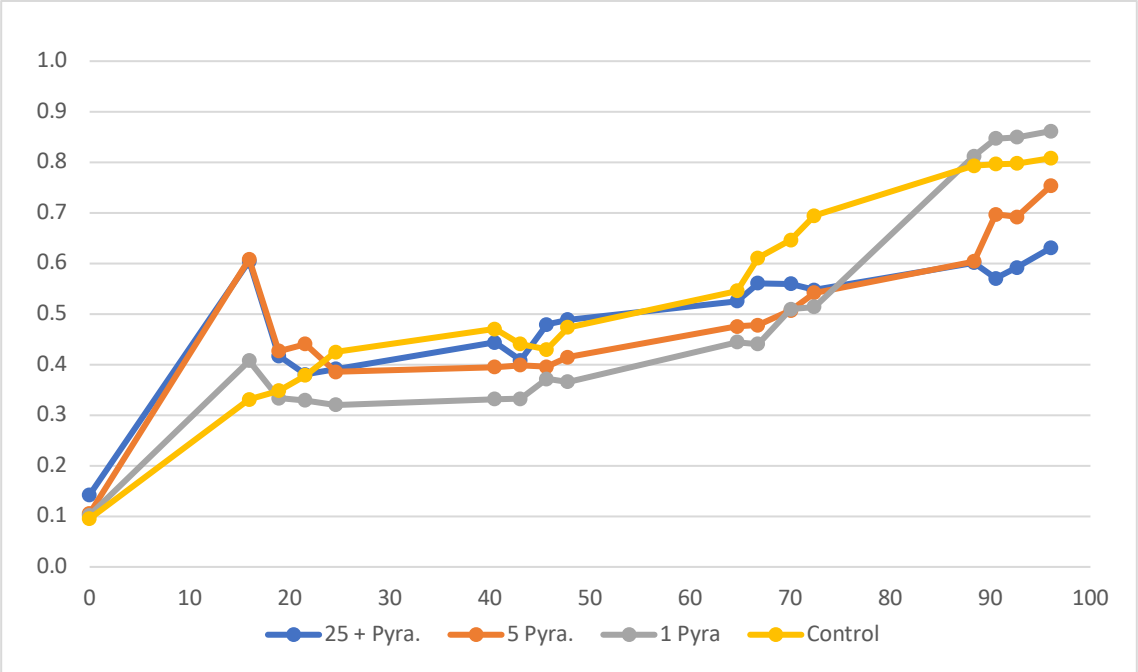


Figure 3.133. The OD curve of Serenade® over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.141	0.094	0.095	0.091	3.6E-23	0.006	0.008	0.010
17.78	0.490	0.252	0.358	0.337	2.4E-07	0.101	0.135	0.175
20.55	0.476	0.307	0.382	0.337	1.2E-05	0.105	0.140	0.181
23.35	0.460	0.296	0.448	0.355	3.3E-06	0.099	0.131	0.170
26.22	0.476	0.344	0.432	0.376	5.4E-06	0.121	0.160	0.208
42.00	0.499	0.382	0.564	0.456	3.3E-06	0.130	0.173	0.224
44.43	0.497	0.394	0.560	0.499	4.8E-08	0.125	0.166	0.216
47.35	0.534	0.383	0.660	0.460	8.0E-09	0.137	0.182	0.237
49.17	0.512	0.409	0.633	0.485	2.1E-09	0.130	0.172	0.224
65.65	0.572	0.532	0.737	0.801	8.2E-27	0.083	0.110	0.143
68.60	0.599	0.641	0.850	0.885	3.0E-24	0.089	0.118	0.153
71.38	0.580	0.649	0.890	0.890	6.6E-28	0.077	0.102	0.133
73.32	0.543	0.704	0.914	0.889	6.5E-29	0.076	0.102	0.132
89.63	0.597	0.850	0.936	0.893	1.1E-37	0.058	0.077	0.100
92.32	0.605	0.875	0.936	0.893	1.0E-39	0.054	0.072	0.094
94.98	0.569	0.888	0.940	0.893	7.6E-39	0.057	0.076	0.099
97.70	0.579	0.880	0.932	0.889	3.4E-38	0.058	0.077	0.100

Table 3.271. The mean ODs at each assessment time for Serenade® in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.143	0.106	0.102	0.095	7.2E-09	0.012	0.015	0.020
15.95	0.604	0.608	0.408	0.331	1.6E-11	0.123	0.164	0.212
18.90	0.417	0.427	0.334	0.348	2.9E-10	0.086	0.114	0.148
21.57	0.381	0.441	0.329	0.379	4.5E-07	0.092	0.122	0.158
24.60	0.391	0.386	0.321	0.425	7.5E-10	0.072	0.096	0.124
40.48	0.444	0.395	0.332	0.470	1.1E-03	0.094	0.125	0.162
43.07	0.408	0.399	0.333	0.441	1.7E-02	0.104	0.137	0.178
45.68	0.479	0.396	0.371	0.430	4.1E-04	0.083	0.110	0.143
47.78	0.489	0.415	0.366	0.474	7.6E-08	0.085	0.113	0.147
64.70	0.525	0.476	0.444	0.545	4.0E-16	0.083	0.110	0.143
66.80	0.561	0.478	0.441	0.611	9.8E-20	0.080	0.107	0.138
70.08	0.560	0.507	0.510	0.646	9.0E-24	0.072	0.095	0.123
72.40	0.548	0.542	0.514	0.694	9.1E-22	0.082	0.108	0.140
88.37	0.602	0.604	0.812	0.793	4.3E-28	0.071	0.095	0.123
90.55	0.570	0.697	0.847	0.797	7.4E-27	0.076	0.101	0.131
92.67	0.592	0.692	0.850	0.798	1.0E-27	0.073	0.097	0.126
96.08	0.631	0.754	0.862	0.808	1.0E-30	0.065	0.087	0.113

Table 3.272. The mean ODs at each assessment time for Serenade® in the presence of three concentrations of pyraclostrobin. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Pyra. 25	34.59
Pyra. 5	37.23
Pyra. 1	50.83
Control	46.86
LSD5%	4.84
LSD1%	6.43
LSD0.1%	8.34

Treatment	"Growth"
Pyra. 25	32.51
Pyra. 5	34.47
Pyra. 1	32.60
Control	39.30
LSD5%	4.41
LSD1%	5.85
LSD0.1%	7.58

Table 3.273. and 3.274. The mean "growth" of Serenade® in treatments with 25, 5, and 1 ppm of pyraclostrobin over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

In the second run of the experiment apart from initial small "peaks" in the first 24 hours when some significantly greater ODs for the 25 and 5ppm pyraclostrobin treatments were observed, the ODs of the treatments and the control increased steadily throughout the experiment (Fig. 3.133). The ODs of all three doses of the pyraclostrobin treatments were lower than those of the control and in many cases significantly so, towards the middle of the experiment in the case of the 1ppm treatment and in the later stages of the experiment for the other two doses (Table 3.272). This resulted in significantly lower "growth" of all three pyraclostrobin treatments compared to the control (Table 3.273).

The results of the two runs of this experiment suggest that pyraclostrobin tends to inhibit the growth of the Serenade® isolate.

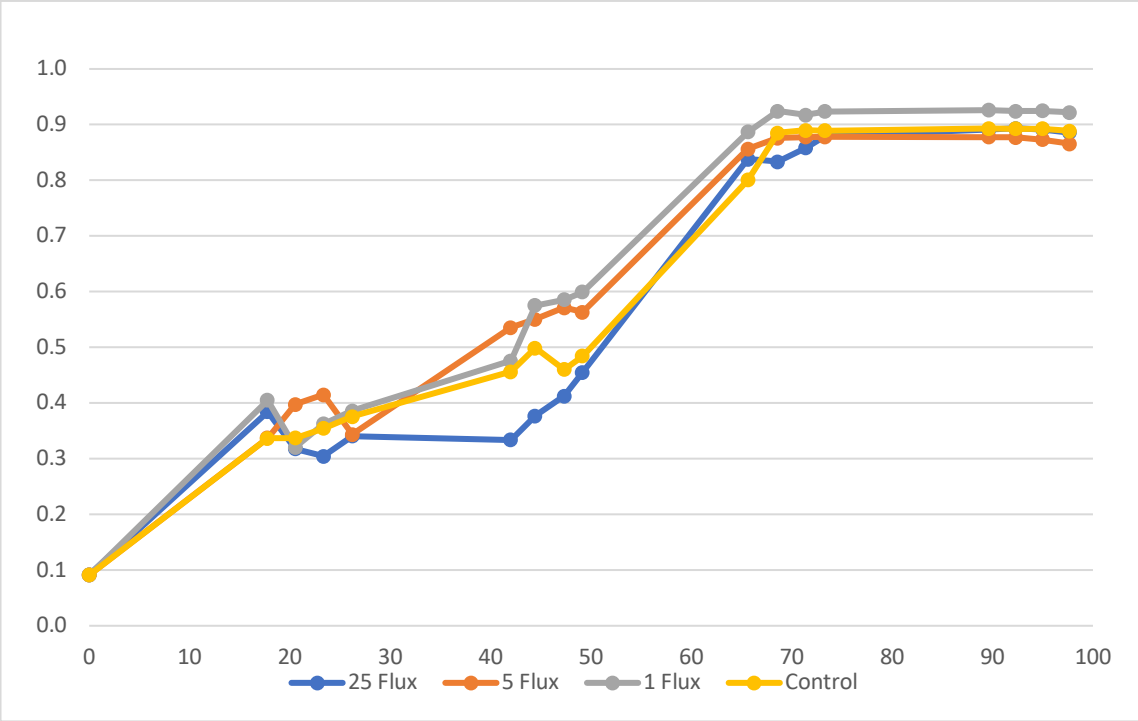


Figure 3.135. The OD curve of Serenade® over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

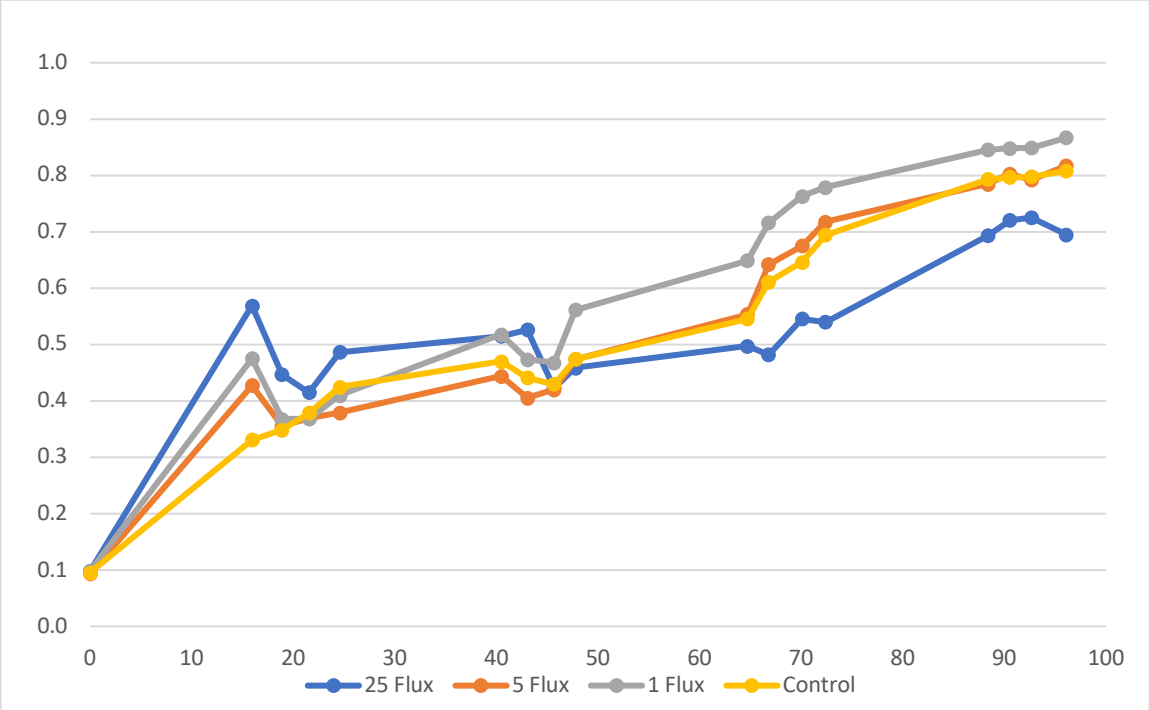


Figure 3.135. The OD curve of Serenade® over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.092	0.091	0.091	0.091	3.6E-23	0.006	0.008	0.010
17.78	0.384	0.337	0.405	0.337	2.4E-07	0.101	0.135	0.175
20.55	0.318	0.397	0.320	0.337	1.2E-05	0.105	0.140	0.181
23.35	0.305	0.415	0.363	0.355	3.3E-06	0.099	0.131	0.170
26.22	0.341	0.343	0.386	0.376	5.4E-06	0.121	0.160	0.208
42.00	0.334	0.536	0.476	0.456	3.3E-06	0.130	0.173	0.224
44.43	0.377	0.551	0.576	0.499	4.8E-08	0.125	0.166	0.216
47.35	0.413	0.571	0.586	0.460	8.0E-09	0.137	0.182	0.237
49.17	0.455	0.563	0.599	0.485	2.1E-09	0.130	0.172	0.224
65.65	0.838	0.856	0.887	0.801	8.2E-27	0.083	0.110	0.143
68.60	0.834	0.876	0.924	0.885	3.0E-24	0.089	0.118	0.153
71.38	0.858	0.878	0.918	0.890	6.6E-28	0.077	0.102	0.133
73.32	0.880	0.878	0.924	0.889	6.5E-29	0.076	0.102	0.132
89.63	0.891	0.878	0.926	0.893	1.1E-37	0.058	0.077	0.100
92.32	0.894	0.877	0.925	0.893	1.0E-39	0.054	0.072	0.094
94.98	0.892	0.873	0.925	0.893	7.6E-39	0.057	0.076	0.099
97.70	0.886	0.866	0.922	0.889	3.4E-38	0.058	0.077	0.100

Table 3.275. The mean ODs at each assessment time for Serenade® in the presence of three concentrations of fluxapyroxad. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Ass. (h)	Flux. 25	Flux 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.098	0.094	0.098	0.095	7.2E-09	0.012	0.015	0.020
15.95	0.569	0.427	0.475	0.331	1.6E-11	0.123	0.164	0.212
18.90	0.447	0.355	0.368	0.348	2.9E-10	0.086	0.114	0.148
21.57	0.415	0.369	0.368	0.379	4.5E-07	0.092	0.122	0.158
24.60	0.486	0.379	0.410	0.425	7.5E-10	0.072	0.096	0.124
40.48	0.515	0.444	0.518	0.470	1.1E-03	0.094	0.125	0.162
43.07	0.527	0.405	0.474	0.441	1.7E-02	0.104	0.137	0.178
45.68	0.423	0.420	0.468	0.430	4.1E-04	0.083	0.110	0.143
47.78	0.459	0.474	0.562	0.474	7.6E-08	0.085	0.113	0.147
64.70	0.498	0.554	0.649	0.545	4.0E-16	0.083	0.110	0.143
66.80	0.482	0.642	0.716	0.611	9.8E-20	0.080	0.107	0.138
70.08	0.545	0.675	0.763	0.646	9.0E-24	0.072	0.095	0.123
72.40	0.540	0.718	0.779	0.694	9.1E-22	0.082	0.108	0.140
88.37	0.693	0.785	0.846	0.793	4.3E-28	0.071	0.095	0.123
90.55	0.721	0.803	0.848	0.797	7.4E-27	0.076	0.101	0.131
92.67	0.725	0.792	0.849	0.798	1.0E-27	0.073	0.097	0.126
96.08	0.695	0.817	0.867	0.808	1.0E-30	0.065	0.087	0.113

Table 3.276. The mean ODs at each assessment time for Serenade® in the presence of three concentrations of fluxapyroxad. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The cross-screening test with the Serenade® isolate *(B. subtilis)* and the fungicide fluxapyroxad was conducted twice. In the first trial, the 1 and 25 ppm fluxapyroxad treatments after an initial

increase and reaching a first peak at 17.78 hours started to decline. Thereafter, the 1 ppm treatment at 20.55 hours and later the 25 ppm treatment at 42 hours after levelling off started to increase again. In contrast, the control and the 5 ppm consistently increased until around 70 hours or so where the OD value with the control and all three treatments levelled off for the remainder of the test (Fig. 3.134). The OD value with the 1 ppm treatment was numerically higher than the control at most timings and with the 5 ppm treatment at some points resulting in numerically higher "growth" of the 1 and 5 ppm treatments with the 1 ppm treatment bordering on statistical significance (Table 3.275, 3.277).

In the second run (Fig. 3.135), the fluxapyroxad treatments after an initial increase declined at 15.95 hours. Although during this initial 15 hours, the highest OD values were recorded with the 25 ppm treatment after the decline its OD value started to increase more slowly than the other two treatments and the control reflected in statistically significantly lower OD values from 70.08 hours onwards (Table 3.276). With the 1 ppm treatment, the OD value was at almost all timings higher than that of the control resulting in significantly higher "growth" than the control at the 5% level (Table 3. 278).

The consistently higher "growth" values with the 1 ppm treatment in both runs of the experiment suggest a slight promotion of growth by fluxapyroxad.

Treatment	"Growth"
Flux. 25	44.94
Flux. 5	48.81
Flux. 1	51.22
Control	46.86
LSD5%	4.84
LSD1%	6.43
LSD0.1%	8.34

Treatment	"Growth"
Flux. 25	38.80
Flux. 5	39.96
Flux. 1	44.94
Control	39.30
LSD5%	4.41
LSD1%	5.85
LSD0.1%	7.58

Table 3.277 and 3.278. The mean "growth" of Serenade® in treatments with 25, 5, and 1 ppm of fluxapyroxad over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

3.2.4.16. MBI600

In the one cross-screening test conducted with MBI600 and the fungicide boscalid, the OD values reached a peak after an initial increase between 21 and 24 hours followed by a gradual leveling off for the remainder of the experiment (Fig. 3.136). It should be noted that the OD values of the 1 and 5ppm treatments did seem to increase during this period more than the control or the 25ppm treatment resulting in significant ODs at many timings (Table 3.279). This

resulted in significantly enhanced "growth" of the treatments with 1 and 5 ppm boscalid at the 5% level (Table 3.281) suggesting a positive effect of the fungicide boscalid at 1 and 5 ppm on the "growth" of the strain.

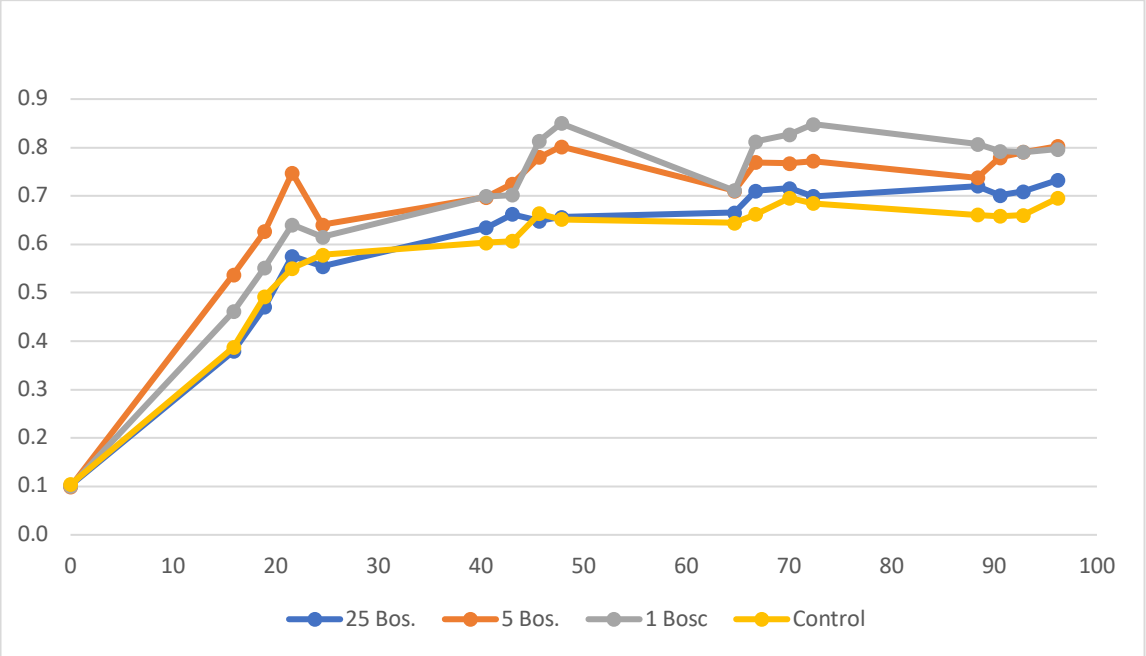


Figure 3.136. The OD curve of MBI 600 over 4 days. 3 doses of boscalid 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Bosc. 25	Bosc. 5	Bosc. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.102	0.099	0.101	0.104	1.2E-03	0.010	0.013	0.017
15.87	0.379	0.538	0.462	0.387	9.8E-06	0.089	0.119	0.154
18.90	0.470	0.626	0.552	0.492	1.5E-04	0.098	0.130	0.168
21.58	0.576	0.747	0.641	0.551	4.2E-08	0.096	0.127	0.165
24.53	0.555	0.641	0.615	0.578	2.2E-07	0.075	0.099	0.129
40.47	0.634	0.697	0.700	0.603	1.6E-04	0.082	0.109	0.141
43.05	0.662	0.725	0.702	0.607	6.6E-06	0.087	0.116	0.150
45.67	0.648	0.780	0.814	0.663	1.8E-04	0.097	0.129	0.167
47.80	0.656	0.802	0.850	0.652	3.4E-05	0.102	0.135	0.175
64.67	0.666	0.711	0.711	0.645	2.5E-03	0.093	0.124	0.161
66.75	0.711	0.769	0.812	0.663	4.6E-03	0.114	0.151	0.195
70.05	0.716	0.767	0.827	0.696	1.7E-02	0.110	0.145	0.189
72.37	0.699	0.772	0.848	0.685	4.0E-03	0.109	0.144	0.187
88.35	0.720	0.738	0.807	0.661	6.8E-03	0.107	0.142	0.184
90.55	0.701	0.779	0.792	0.658	2.5E-03	0.108	0.144	0.186
92.80	0.709	0.790	0.791	0.660	3.3E-03	0.111	0.147	0.191
96.13	0.732	0.803	0.796	0.695	4.6E-02	0.120	0.159	0.207

Table 3.279. The mean ODs at each assessment time for MBI600 in the presence of three concentrations of boscalid. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Bosc. 25	46.06
Bosc. 5	54.09
Bosc. 1	54.63
Control	44.42
LSD5%	6.16
LSD1%	8.17
LSD0.1%	10.60

Table 3.280. The mean "growth" of MBI600 in treatments with 25, 5, and 1 ppm of boscalid over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

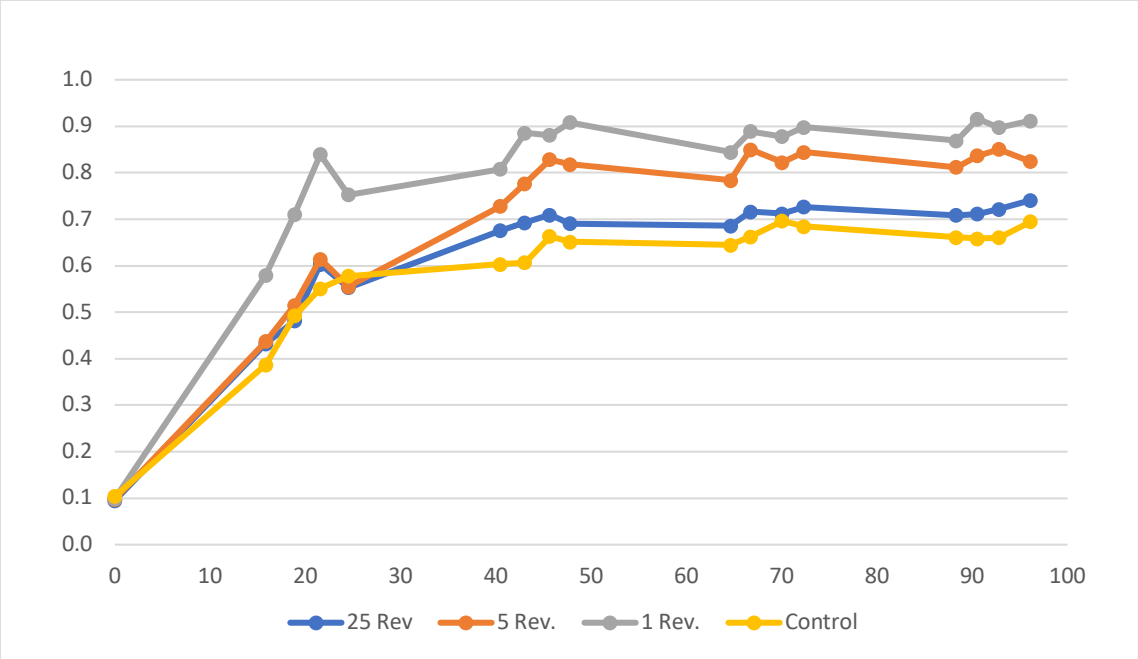


Figure 3.137. The OD curve of MBI 600 over 4 days. 3 doses of Revysol® 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

The cross-screening test with MBI600 and the fungicide Revysol® showed the similar tendency in OD values to the test with boscalid; after an initial increase the OD values achieved a peak point at 21.58 hours and then continued to gradually level off until the test was stopped (Fig. 3.137). The OD values with the 1 and 5 ppm treatments were significantly greater than those of the control over the whole experiment and from the 40.47 hours respectively. The level of significance with the 1ppm treatment was invariably higher than that for the 5ppm treatment. With the 25 ppm treatment a numerically higher OD value was recorded from 40.47 hours onwards (Table 3.281). The "growth" of the treatments with the 1 and 5 ppm Revysol® were significantly greater than the control. Furthermore, the 25ppm treatment also showed a

numerically higher "growth" (Table 3.282). This experiment suggests the growth of the strain MBI600 is enhanced in the presence of the fungicide Revysol®.

Ass. (h)	Rev. 25	Rev. 5	Rev. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.095	0.098	0.099	0.104	1.2E-03	0.010	0.013	0.017
15.87	0.432	0.437	0.580	0.387	9.8E-06	0.089	0.119	0.154
18.90	0.481	0.515	0.711	0.492	1.5E-04	0.098	0.130	0.168
21.58	0.603	0.614	0.840	0.551	4.2E-08	0.096	0.127	0.165
24.53	0.553	0.555	0.752	0.578	2.2E-07	0.075	0.099	0.129
40.47	0.676	0.728	0.808	0.603	1.6E-04	0.082	0.109	0.141
43.05	0.693	0.777	0.886	0.607	6.6E-06	0.087	0.116	0.150
45.67	0.709	0.828	0.881	0.663	1.8E-04	0.097	0.129	0.167
47.80	0.691	0.818	0.908	0.652	3.4E-05	0.102	0.135	0.175
64.67	0.686	0.784	0.844	0.645	2.5E-03	0.093	0.124	0.161
66.75	0.716	0.850	0.889	0.663	4.6E-03	0.114	0.151	0.195
70.05	0.712	0.823	0.878	0.696	1.7E-02	0.110	0.145	0.189
72.37	0.727	0.844	0.898	0.685	4.0E-03	0.109	0.144	0.187
88.35	0.708	0.812	0.869	0.661	6.8E-03	0.107	0.142	0.184
90.55	0.711	0.837	0.916	0.658	2.5E-03	0.108	0.144	0.186
92.80	0.722	0.850	0.898	0.660	3.3E-03	0.111	0.147	0.191
96.13	0.741	0.824	0.912	0.695	4.6E-02	0.120	0.159	0.207

Table 3.281. The mean ODs at each assessment time for MBI600 in the presence of three concentrations of Revysol® LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Rev. 25	48.68
Rev. 5	55.18
Rev. 1	63.77
Control	44.42
LSD5%	6.16
LSD1%	8.17
LSD0.1%	10.60

Table 3.282. The mean "growth" of MBI600 in treatments with 25, 5, and 1 ppm of Revysol® over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

The cross-screening test with the strain MBI600 and the fungicide pyraclostrobin showed a gradual increase in the OD value until 47.80 hours when the OD values started to level off (Fig. 3.138). The biggest difference in the OD values was observed between the control and the 1 ppm treatment which reached statistical significance on a number of occasions (Table 3.283). Accordingly, the "growth" value for this treatment was significantly higher than the control at the 5% level. With the 5 ppm treatment, higher OD values were recorded at some timings

leading to a numerically higher "growth" value (Table 3.284). These observations suggest that the presence of 1 and 5ppm of the fungicide pyraclostrobin can enhance the growth of the strain.

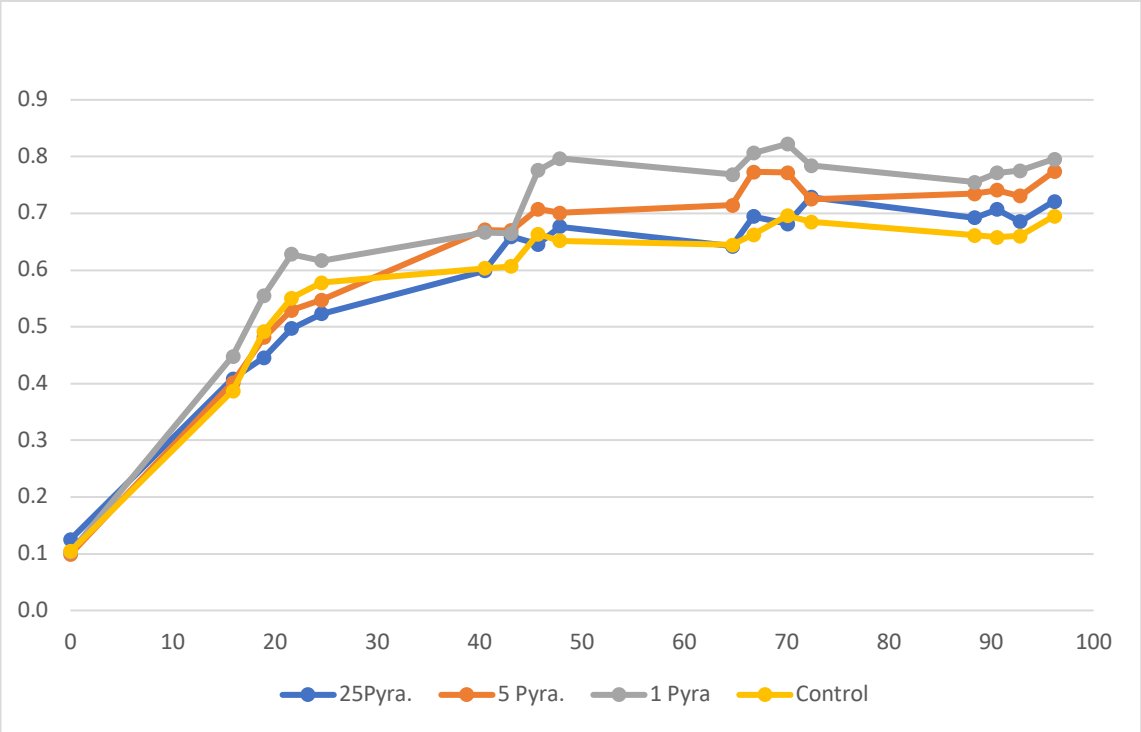


Figure 3. 138. The OD curve of MBI 600 over 4 days. 3 doses of pyraclostrobin 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Pyra. 25	Pyra. 5	Pyra. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.125	0.099	0.103	0.104	1.2E-03	0.010	0.013	0.017
15.87	0.409	0.402	0.448	0.387	9.8E-06	0.089	0.119	0.154
18.90	0.446	0.481	0.555	0.492	1.5E-04	0.098	0.130	0.168
21.58	0.497	0.529	0.628	0.551	4.2E-08	0.096	0.127	0.165
24.53	0.523	0.547	0.617	0.578	2.2E-07	0.075	0.099	0.129
40.47	0.599	0.671	0.667	0.603	1.6E-04	0.082	0.109	0.141
43.05	0.659	0.670	0.665	0.607	6.6E-06	0.087	0.116	0.150
45.67	0.646	0.708	0.776	0.663	1.8E-04	0.097	0.129	0.167
47.80	0.677	0.701	0.797	0.652	3.4E-05	0.102	0.135	0.175
64.67	0.642	0.714	0.768	0.645	2.5E-03	0.093	0.124	0.161
66.75	0.695	0.773	0.806	0.663	4.6E-03	0.114	0.151	0.195
70.05	0.681	0.772	0.822	0.696	1.7E-02	0.110	0.145	0.189
72.37	0.728	0.725	0.784	0.685	4.0E-03	0.109	0.144	0.187
88.35	0.692	0.734	0.755	0.661	6.8E-03	0.107	0.142	0.184
90.55	0.707	0.741	0.771	0.658	2.5E-03	0.108	0.144	0.186
92.80	0.686	0.731	0.775	0.660	3.3E-03	0.111	0.147	0.191
96.13	0.721	0.774	0.796	0.695	4.6E-02	0.120	0.159	0.207

Table 3.283. The mean ODs at each assessment time for MBI600 in the presence of three concentrations of pyraclostrobin LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

The cross-screening test conducted with MBI600 and the fungicide fluxapyroxad showed an initial steep increase in the OD values and a more gentile increase until the test was stopped (Fig. 3.139). The control showed the lowest OD values throughout the whole experiment leading to significant greater OD values for the 25 ppm treatment and numerically greater OD values with the 1 and 5 ppm treatments compared to the control (Table 3.285). This resulted in a markedly greater "growth" value for the 25 ppm treatment and also numerically (bordering on statistical significance) higher "growth" of the 1 and 5 ppm treatments, suggesting that the presence of the fungicide fluxapyroxad is enhancing the growth of MBI600 (Table 3.286).

Treatment	"Growth"
Pyra. 25	43.15
Pyra. 5	48.66
Pyra. 1	52.64
Control	44.42
LSD5%	6.16
LSD1%	8.17
LSD0.1%	10.60

Table 3.284. The mean "growth" of MBI600 in treatments with 25, 5, and 1 ppm of pyraclostrobin over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

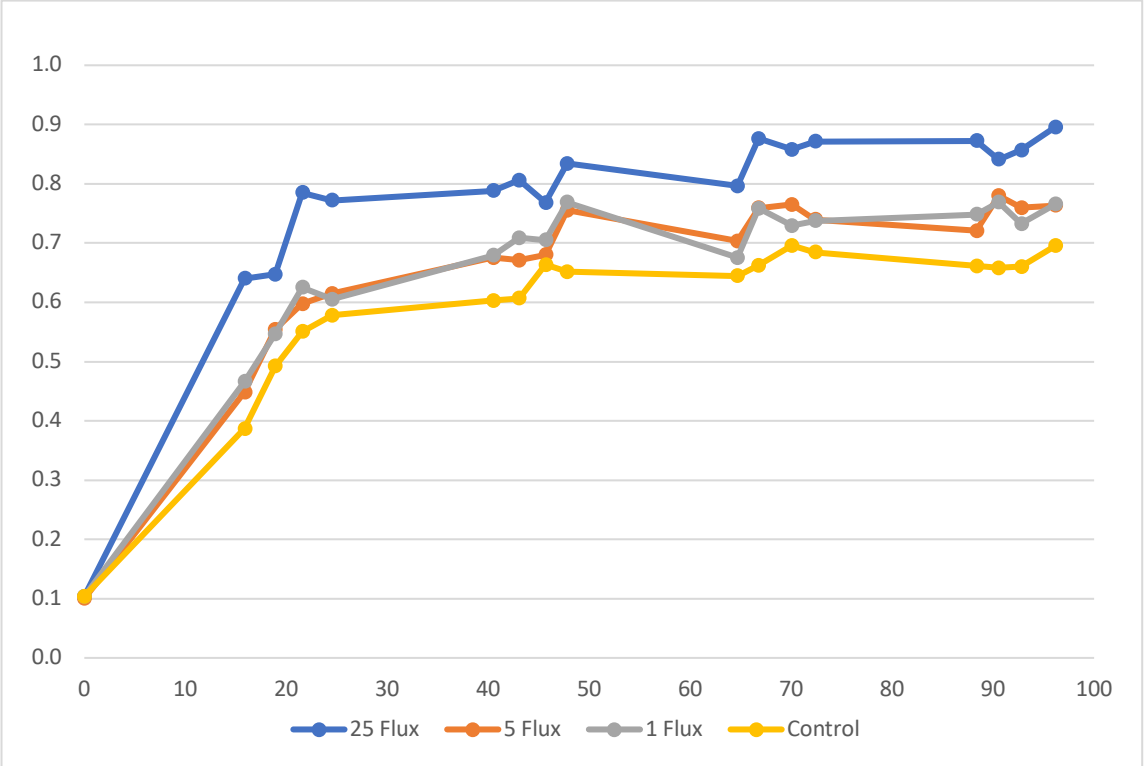


Figure 3.139. The OD curve of MBI 600 over 4 days. 3 doses of fluxapyroxad 1,5, and 25 ppm shown with different colored curves. OD values as Y-axis and assessment time intervals in hours as X-axis.

Ass. (h)	Flux. 25	Flux. 5	Flux. 1	Control	P-value	5% LSD	1%LSD	0.1%LSD
0.00	0.104	0.100	0.104	0.104	1.2E-03	0.010	0.013	0.017
15.87	0.640	0.449	0.466	0.387	9.8E-06	0.089	0.119	0.154
18.90	0.647	0.555	0.547	0.492	1.5E-04	0.098	0.130	0.168
21.58	0.785	0.597	0.625	0.551	4.2E-08	0.096	0.127	0.165
24.53	0.772	0.615	0.605	0.578	2.2E-07	0.075	0.099	0.129
40.47	0.788	0.675	0.679	0.603	1.6E-04	0.082	0.109	0.141
43.05	0.806	0.671	0.709	0.607	6.6E-06	0.087	0.116	0.150
45.67	0.768	0.680	0.705	0.663	1.8E-04	0.097	0.129	0.167
47.80	0.834	0.755	0.769	0.652	3.4E-05	0.102	0.135	0.175
64.67	0.797	0.703	0.676	0.645	2.5E-03	0.093	0.124	0.161
66.75	0.876	0.759	0.758	0.663	4.6E-03	0.114	0.151	0.195
70.05	0.858	0.765	0.730	0.696	1.7E-02	0.110	0.145	0.189
72.37	0.871	0.739	0.737	0.685	4.0E-03	0.109	0.144	0.187
88.35	0.873	0.721	0.749	0.661	6.8E-03	0.107	0.142	0.184
90.55	0.841	0.780	0.769	0.658	2.5E-03	0.108	0.144	0.186
92.80	0.857	0.760	0.732	0.660	3.3E-03	0.111	0.147	0.191
96.13	0.896	0.764	0.766	0.695	4.6E-02	0.120	0.159	0.207

Table 3.285. The mean ODs at each assessment time for MBI600 in the presence of three concentrations of fluxapyroxad. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

Treatment	"Growth"
Flux. 25	61.36
Flux. 5	50.58
Flux. 1	50.45
Control	44.42
LSD5%	6.16
LSD1%	8.17
LSD0.1%	10.60

Table 3.286. The mean "growth" of MBI600 in treatments with 25, 5, and 1 ppm of fluxapyroxad over the four-day experimental period. Significant increases in "growth" compared to the control at the 5, 1, and 0.1 % level are highlighted in shades of green and significantly decreased growth in shades of oranges according to the degree of significance.

3.3 Confirmation of Fungicide Degradation accompanying the Enhanced Growth in the Presence of the Fungicide.

To test the hypothesis that the increased growth reflected in the increased OD measurements in the presence of the fungicide is a result of the bacteria utilising the fungicide as an additional nutrient source, it was initially attempted to develop a method using UV-VIS spectrometry to measure the quantity of the fungicide within the culture medium. In doing so, the first step was to establish a reliable correlation between the amount of fungicide present and the UV-Vis

absorbance. Since the fungicide was always added to the medium in acetoneic solution in the other experiments conducted, an acetoneic solution was initially used here.

Initial experiments were conducted with fluxapyroxad. The sample consisted of 5 mls of salt medium with glucose and 0.1 ml of an acetoneic solution of fluxapyroxad to give a final rate of 2.5 ppm in the 5 mls medium. The machine was set to the wavelength 203 nm, the region of maximum absorbance for fluxapyroxad (fluxapyroxad). For the blank 0.1 ml acetone without any fungicide was added to the medium (Table 3.287).

Salt Medium + Fluxapyroxad (ppm)	OD: 203 nm
3.90625	0.009
7.8125	0.053
15.625	0.061
31.25	0.024
62.5	0.114
125	0.164
250	0.280
500	0.347
750	0.290
Correlation Coefficient	0.323

Salt Medium + Fluxapyroxad (ppm)	OD: 203 nm
3.90625	0.009
7.8125	0.053
15.625	0.061
31.25	0.024
62.5	0.114
125	0.164
250	0.280
500	0.347
750	0.290
Correlation Coefficient	0.849

Table 3.287. The ODs of different fluxapyroxad concentrations in salt medium at wavelength 203nm and the resulting correlation coefficient.

After excluding the highest and lowest doses, the obtained correlation coefficient of 0.849 appears to be quite reasonable. The result obtained with the 500 ppm concentration seems to be somewhat anomalous and in any event this concentration is way in excess of the concentrations found to be optimal for the growth of the isolates. Accordingly, if the highest concentration, 500 ppm, is omitted the correlation coefficient is further improved to 0.948.

The next experiments were set up with the purpose of determining whether other solvents might be preferable to acetone as a solvent for the fungicide fluxapyroxad for the purpose of determining UV absorbances. It is known that acetone absorbs over a broad range of UV wavelengths (cut off 330 nm) while the absorbance of methanol and ethanol is restricted to a smaller range (cut off 210 and 205 nm respectively).

Three rates of fluxapyroxad 62.5, 125, and 250 ppm were used. Following the protocol of previous test, 0.1 ml of the appropriate methanolic solution of fluxapyroxad was added into the medium. The OD values were measured using the salt medium containing 0.1ml methanol as a blank at 203 nm (Table 3.288).

Salt Medium + Fluxapyroxad (ppm)	OD: 203 nm
62.5	0.04
125	0.08
250	0.25
Correlation Coefficient	0.99

Table 3.288. The ODs at wavelength 203nm of different fluxapyroxad concentrations in salt medium using methanol as a solvent and the resulting correlation coefficient.

These encouraging results were then followed up with a more extensive design. The experimental design with methanol as a solvent for the fungicide was employed. The experiment was performed with three different "diluents"; water, salt medium, and salt medium supplemented with the glucose. For example, the 32 ppm fluxapyroxad was made up in 10 mls methanol (the highest dose) and the lower doses were composed by diluting the previous dose 50:50 with three solutions (Table 3.289). The main reason to set up this experiment was to examine the efficacy of different diluents used to make up a broad range of fungicide doses on the UV absorbance.

The correlation coefficients obtained in all three cases were acceptable and only differed marginally. The best correlation coefficient numerically was water, followed by salt medium, and then salt medium plus glucose (Table 3.290).

Fluxapyroxad (203 nm)	
Blank	Sample
100% Methanol	31.25 ppm (In 100 % Methanol)
50% Methanol + 50% (Water/Salt Medium/Salt Medium+Glucose)	15 625ppm (In 50% 31 ppm+ 50% Water)
250 μl Methanol + 750 μl (Water/Salt Medium/Salt Medium+Glucose)	7.8125 ppm (In 50% 15 ppm+ 50% Water)
125 μl Methanol + 825 μl (Water/Salt Medium/Salt Medium+Glucose)	3.90625 ppm. (In 50% 7.5 ppm+ 50% Water)
62.5 μl Methanol + 937.5 μl (Water/Salt Medium/Salt Medium+Glucose)	1.953125 ppm. (In 50% 3.8 ppm+ 50% Water)

Table 3.289. The treatments used to examine the effects of different diluents on the OD of fluxapyroxad at 203 nm using methanol as a solvent for the fluxapyroxad.

If another solvent was substituted for acetone, it would be necessary to demonstrate that there was no influence of the presence of the replacement solvent in the medium on bacterial growth. Therefore, a microtiter plate test was set up. Each well consisted of 150 microliters salt medium supplemented with glucose and 3 microliters of acetone, methanol, or ethanol with and without fluxapyroxad to give a final fluxapyroxad concentration in the wells of either 5 or 0 ppm. The treatments were all set up in duplicate, each with 6 replicates and one of the duplicates

inoculated with 1 microliter of a bacterial "solution" which had been adjusted to an optical density of 0.3. In addition, treatments with no solvents added with and without inoculation were set up each with 12 replicates (Table 3.291).

Water	
PPM	OD
31.25	1.93
15.625	0.85
7.8125	0.45
3.90625	0.23
1.9531250	0.12
Corr.	0.998

Salt Medium	
PPM	OD
31.00	1.63
15.00	1.02
7.50	0.58
3.80	0.27
1.90	0.13
Corr.	0.990

Salt Medium + Glucose	
PPM	OD
31.00	1.63
15.00	1.09
7.50	0.47
3.80	0.24
1.90	0.04
Corr.	0.981

Table 3.290. The effects of different diluents on the OD of fluxapyroxad at 203 nm using methanol as a solvent for the fluxapyroxad. Means of xx replicates are shown.

Water	water
0 ppm (Acetone) + Bacteria	5 ppm fluxapyroxad (Acetone) + Bacteria
0 ppm (Ethanol) + Bacteria	5 ppm fluxapyroxad (Ethanol) + Bacteria
0 ppm (Methanol)+Bacteria	5 ppm (Methanol+ Bacteria
(Salt Medium + Bacteria)	(Salt Medium + Bacteria)
Salt Medium	Salt Medium
Water	water

Table 3.291. The treatments used to examine the effects of acetone, ethanol, and methanol as solvents to dissolve fluxapyroxad on the integrated average OD of *Bacillus subtilis* DMS10.

The results demonstrated that the bacteria grew similarly in the presence of all three solvents. The only treatment showing a statistically significant difference from the others was the acetonic treatment containing 0 ppm of fluxapyroxad where a significant decrease in the integrated average OD was seen. However, the 5 ppm treatment did not significantly deviate from the others (Table 3.293). It should be mentioned that the variance in the 0ppm fluxapyroxad treatment containing acetone was significantly higher than the treatment with the 5ppm fluxapyroxad treatment containing acetone (Table 3.293). Previous studies (Gabriel Dyrda et al. 2019 The effect of organic solvents on selected microorganisms and model liposome membrane) and (Soltani 2019) indicated an apparent bacterial growth inhibition in the presence of pure acetonic solution, which also happened in this experiment. As it's shown in the (Table 3.292), the OD value of three replicates of treatment with pure acetone didn't increase at all.

Nevertheless, since the intention was to replace the acetone with another solvent for the UV-VIS spectrometry, this result was not further investigated.

Treatments	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6
Acetone + 0 + F	31.9	42.7	31.1	30.1	27.5	29.9
Acetone + B	0.4	0.0	5.3	25.5	29.9	33.6
Ethanol + B + F	25.2	29.9	28.3	29.1	40.9	33.3
Ethanol + B	24.8	28.2	30.9	19.8	30.7	31.6
Methanol + B + F	26.4	28.4	30.1	31.1	27.4	27.2
Methanol + B	16.2	30.7	30.5	25.1	32.1	24.6
Medium + B	28.8	28.1	27.8	30.9	26.9	25.7
	15.69	17.83	16.49	30.04	34.76	27.30
	24.42	31.45	35.90	24.96	44.34	29.47
	32.73	40.40	38.36	32.54	41.12	50.66

Table 3.292. The effects of acetone. ethanol and methanol as solvents to dissolve fluxapyroxad on the "growth'' of *Bacillus subtilis* DMS10 *(B)* shown.

Groups	Mean	Variance
Medium + B+ Fluxapyroxad (Acetone)	32.200	28.67116
Medium + Acetone + B	15.795	240.7487
Medium + B+ Fluxapyroxad (Ethanol)	31.107	29.88303
Medium + Ethanol + B	27.663	21.01534
Medium +B+ Fluxapyroxad (Methanol)	28.418	3.33468
Medium + Methanol + B	26.511	35.47692
Medium + *B*	30.689	71.07701

LSD	5%	1%	0.1%
6v6	9.290	12.355	15.653
6v24	7.344	9.768	12.705

Table 3.293. The effects of acetone, ethanol, and methanol as solvents to dissolve fluxapyroxad on the "growth'' of *Bacillus subtilis* DMS10. Mean "growth'' values are shown.

The next step was to establish whether a calibration curve for the three fungicides fluxapyroxad, boscalid and Revysol® could be established using methanol as a solvent at wavelengths of 203, 207, and 202 nm respectively, the respective wavelengths for maximum absorbance of the three fungicides (quote source also above for fluxapyroxad). Seven doses of the fungicides (0.5, 1.25, 2.5, 5, 10, 25, and 50 ppm) were used.

The first experiment was conducted with fluxapyroxad. 50 mg of the fungicide was added into 10 mls methanol. then, 40 μl of the methanol solution was added into the 2-ml salt medium (Table 3.294). However, the set-up of the experiments with the other two fungicides was amended to check whether the dilution method influenced the results. In the case of boscalid, 12.5 mg boscalid was dissolved in 2-ml methanol and then serial dilutions made in each of the three diluents water, salt medium and salt medium supplemented with glucose (Table 3.296).

With Revysol® however the fungicide was firstly dissolved, and the serial dilutions made in methanol and then 0.1 of each stock solution was added into 5-ml of the three solutions (Table 3.295).

Fluxapyroxad (ppm)	OD
50	1.168
25	1.035
10	0.591
5	0.267
2.5	0.125
1.25	0.071
0.5	0.025
Corr. Coef.	0.894

Table 3.294. ODs at 203nm of 0.1, 0.5, 1.25, 2.5, 5, 10, 25, and 50 ppm fluxapyroxad solutions in salt medium at 203 nm.

Revysol® (ppm)	Water	SM	SM+G
100	0.881	0.870	0.562
50	0.942	0.695	0.505
25	1.072	0.672	0.416
10	0.334	0.459	0.263
5	0.271	0.339	0.221
2.5	NM	0.199	0.095
1.25	NM	0.192	0.069
0.5	NM	0.180	0.034
0.1	NM	0.123	0.056
STDE	0.37	0.27	0.20
Corr. Coef.	0. 87	0.886	0.872

Table 3.295. OD at 202 nm of 0.1, 0.5, 1.25, 2.5, 5, 10, 25, and 50 ppm Revysol® in three different media; water, Salt Medium, and Salt Medium supplemented with the glucose at 202 nm. Means of 3 replicates are shown.
*NM.: Not Mensurable

The results were promising although the correlation coefficients were less than 0.9. Nevertheless, the experiment was repeated with the identical order to check the stability of results. From each fungicide 25 mg was dissolved in 10mls methanol to make up the rate of 50 ppm and then the lower doses were made up through serial dilutions in methanol solution, 40 µl of which was added into 2 mls of each solution (salt medium, salt medium with glucose, and water, Revysol® was tested only in the Salt Medium).

Boscalid (ppm)	Water	SM	SM+G
100	2.846	2.331	1.606
50	2.719	1.86	1.334
25	2.259	1.706	1.483
10	0.649	0.95	0.274
5	0.265	0.292	0.43
2.5	0.162	0.22	0.214
1.25	0.029	0.189	0.061
0.5	0.025	0.121	0.046
0.1	-0.002	0.079	0.021
Corr. Coef.	0.878	0.898	0.847

Table 3.296. ODs at 207 nm of 0.1, 0.5, 1.25, 2.5, 5, 10, 25, and 50 ppm boscalid in three different media; water, Salt Medium, and Salt Medium supplemented with glucose are shown.

Flux.	Water			SM			SM+G		
ppm	a	b	c	a	b	c	a	b	c
50	0.416	0.423	0.445	0.015	0.178	0.163	0.053	0.046	0.117
25	0.510	0.436	0.410	0.043	0.121	0.079	0.116	0.058	0.065
10	0.731	0.752	0.637	0.078	0.084	0.085	0.032	0.042	0.105
5	0.349	0.369	0.327	0.128	0.082	0.112	0.167	0.011	0.042
2.5	0.189	0.168	0.144	0.100	0.180	0.143	0.191	-0.017	0.147
1.2	0.087	0.069	0.057	0.113	0.105	0.089	0.072	0.024	0.051
0.6	0.044	0.023	0.013	0.163	0.170	0.069	0.057	-0.006	0.081
std	0.241	0.252	0.210	0.025	0.045	0.032	0.064	0.022	0.040
Corr.	0.443	0.423	0.543	0.889-0.885	0.305	0.537	-0.295	0.681	0.246

Table 3.297. ODs at 203nm of 0.6, 1.25, 2.5, 5, 10, 25, and 50 ppm fluxapyroxad in three different media; water, Salt Medium, and Salt Medium supplemented with glucose. Means of 3 replicates are shown.

Boscalid	Water			SM			SM+G		
ppm	a	b	c	a	b	c	a	b	c
50	0.400	0.402	0.387	0.239	0.010	0.111	0.195	-0.078	NM
25	0.385	0.444	0.404	0.133	0.060	0.100	0.119	0.219	0.038
10	0.447	0.676	0.494	0.134	0.103	0.119	0.167	-0.053	0.082
5	0.383	0.404	0.399	0.133	0.127	0.155	0.125	0.050	-0.024
2.5	0.223	0.234	0.241	0.087	0.081	0.055	0.115	0.069	0.202
1.2	0.119	0.204	0.122	0.091	-0.072	0.006	0.024	0.068	-0.077
0.6	0.081	0.069	0.063	0.108	0.043	-0.066	0.050	-0.001	0.047
std	0.133	0.201	0.154	0.020	0.072	0.0752	0.046	0.106	--------
Corr.	0.575	0.374	0.494	0.932	-0.130	0.437	0.708	-0.200	-0.024

Table 3.298. ODs at 207nm of 0.6, 1.25, 2.5, 5, 10, 25, and 50 ppm boscalid in three different media; water, Salt Medium, and Salt Medium supplemented with glucose Means of 3 replicates are shown. *NM.: Not Mensurable.

Revysol® (194 nm)	SM		
ppm	a	b	c
50	0.121	-0.058	0.110
25	0.102	-0.062	0.176
10	0.166	-0.069	0.209
5	0.081	0.122	0.150
2.5	0.225	-0.053	0.235
1.25	0.023	-0.022	0.081
0.6	0.066	-0.080	0.049
std	0.072	0.074	0.074
Corr.	0.100	-0.228	-0.021

Table 3.299. ODs at 194nm of 0.6, 1.25, 2.5, 5, 10, 25, and 50 ppm Revysol® in salt medium. Means of 3 replicates are shown.

Despite of the first trial, in the second trial the divergence between values observed not only within doses but also within replicates were markedly significant (Table 3.298, 3.299).

Thereby, due to the inconsistencies observed, the OD of the salt medium supplemented with glucose was measured at wavelength of 207 nm. Although it was expected to get zero absorbance since there was no boscalid present in the solution, the OD of the medium containing sample was 2.3 AU., suggesting that the medium was absorbing at this wavelength. In an attempt to reduce the absorbance by the medium, the medium was diluted 1:3 with water (0.5-ml salt medium diluted in 1.5-ml water). Then 40 microliters methanolic solution of boscalid 2.5, 5, 10, and 25 ppm was added into the diluted medium. 40 microliters methanol added into the diluted medium was used as a blank and absorbances were measured at 207 nm. The high consistency between values (correlation ecoefficiency level at 0.97) confirmed the result in the experiment above showing the absorbance of medium at wavelength 207 (Table 3.300).

Boscalid (ppm)	OD
2.5	0.136
5	0.261
10	0.448
25	0.731
Corr.	0.979

Table 3.300. The ODs at 207n of 25,5, 10, and 25 ppm boscalid added into the diluted medium. Means of y replicates are shown.

An experiment was carried out in glass tubes to examine the robustness of the correlation between the concentration of fungicide and absorbance in the presence of bacteria. 0, 2.5, 5, 10 and 25 ppm treatments were prepared by making up a stock solution of boscalid for the higher

concentration and then carrying out serial dilutions with methanol to provide the right quantity for each well in the 0.3 mls of the methanolic solution added to the well. The medium used was salt medium supplemented. Each treatment was replicated five times and was set up in duplicate with one set of the treatments being inoculated with 0.1 ml inoculum.

After approximately 24 hours, the ODs of treatments 1 and 3 (Table 3.301) were measured and the medium then centrifuged at 18000 rcf for 2 minutes and then the ODs of the supernatants were measured at 207 nm. The centrifugation was seen to reduce the ODs below their initial values (Table 3.302). This could indicate that the bacteria are influencing the absorbance or that fungicide particles might be partially taken out from the sample through the centrifugation procedure. It is also noticeable that there is a high degree of variability between the different replicates of each treatment.

Treat. 1	5-ml Medium+0.3-ml Bosc. 25 ppm+ 0.1-ml Inoculum	5-ml Medium+0.3-ml Bosc. 25 ppm	Treat. 6
Treat. 2	5-ml Medium+0.3-ml Bosc. 10 ppm+ 0.1-ml Inoculum	5-ml Medium+0.3-ml Bosc. 10 ppm	Treat. 7
Treat. 3	5-ml Medium+0.3-ml Bosc. 5 ppm+ 0.1-ml Inoculum	5-ml Medium+0.3-ml Bosc. 5 ppm	Treat. 8
Treat. 4	5-ml Medium+0.3-ml Bosc. 2.5 ppm+ 0.1-ml Inoculum	5-ml Medium+0.3-ml Bosc. 2.5 ppm	Treat. 9
Treat. 5	5-ml Medium+0.3-ml diluted methanolic solution + 0.1-ml Inoculum	5-ml Medium+0.3-ml diluted methanolic solution	Treat. 10

Table 3.301. The treatments used to examine the robustness of the correlation between boscalid concentration and OD at 207 nm.

Treatment	R1	R2	R3	R4	R5	Average
Treat. 1 before centrifuge	0.052	0.564	0.226	0.202	0.221	0.253
Treat.1 after centrifuge	0.219	0.116	0.162	0.234	0.266	0.199
Treat.3 before centrifuge	0.613	0.373	0.6	0.266	0.614	0.493
Treat.3 after centrifuge	0.428	0.105	0.378	0.003	0.338	0.250

Table 3.302. ODs at 207nm of selected treatments before and after centrifugation in the boscalid concentration and OD correlation test.

Following these series of experiments, the method was considered not insufficiently robust to enable the reliable quantification of the fungicide content by UV-VIS directly within the media in situ. Therefore, it was decided to switch from UV-VIS spectrometry to a more detailed HPLC analytical method with UV-VIS detection.

Accordingly, based on a BASF method an experiment was set up with isolate 53 which had led to high and consistent growth enhancement in the presence of boscalid in previous experiments. Four treatments each with 15 replicates was set up within a microtiter plate. The four treatments

consisted of the salt medium supplemented with glucose with and without 5 ppm boscalid and each of these two treatments with and without the inoculation of the isolate. In addition to the in-plate replicates, each plate was replicated four times (Table 3.303).

water	water	water	water
water	Boscalid 5ppm inoculated	Boscalid 5 ppm uninoculated	water
water	Boscalid 5ppm inoculated	Boscalid 5 ppm uninoculated	water
water	Boscalid 5ppm inoculated	Boscalid 5 ppm uninoculated	water
water	Boscalid 0ppm inoculated	Boscalid 0 ppm uninoculated	water
water	Boscalid 0ppm inoculated	Boscalid 0 ppm uninoculated	water
water	Boscalid 0ppm inoculated	Boscalid 0 ppm uninoculated	water
water	water	water	water

Table 3.303. The layout of treatments in the 96 well microtiter plate for the boscalid degradation experiment for HPLC analysis.

In this experiment the 5ppm rate was chosen as the rate that had shown, the most significant growth increase over multiple tests. The test was set up within a 96 well microtiter plate The OD of the treatments was followed over 50 hours at the end of which 100 µL of medium was pipetted out from each well and then pipetted into a 2-ml Eppendorf tube. The 100 µL from each of the 15 replicates were bulked in one Eppendorf tube. and then transferred into the freezer maintained in -80 °C.

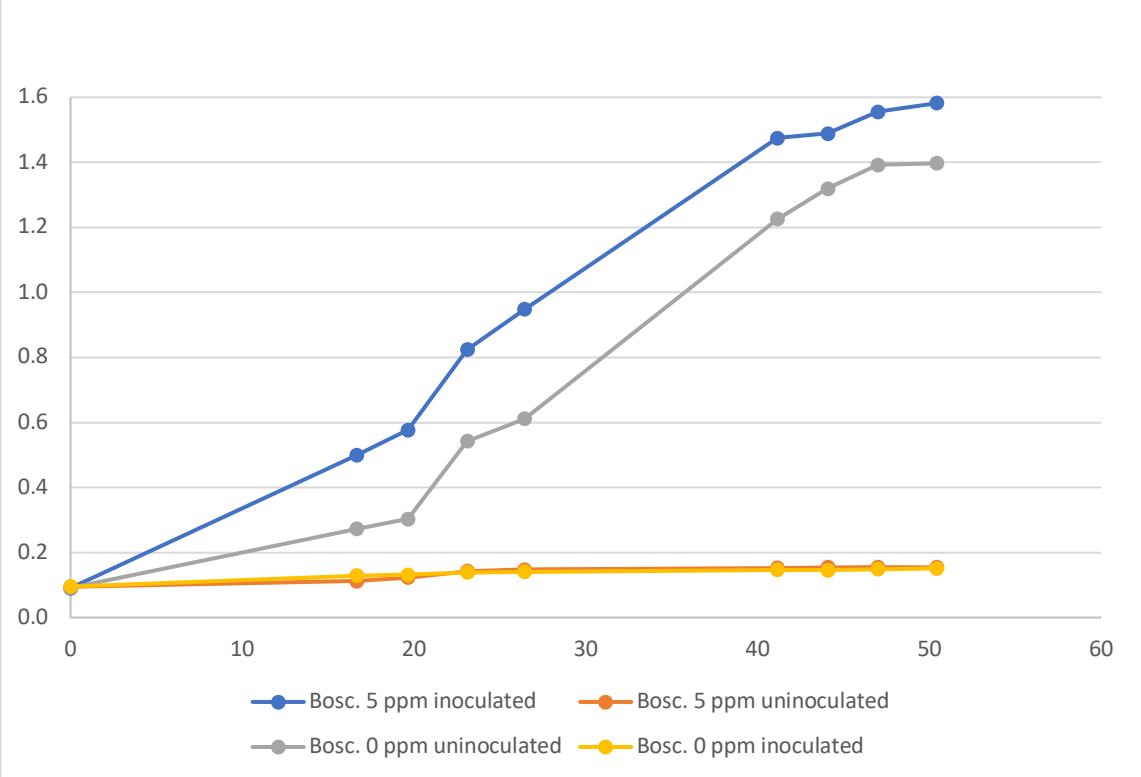


Figure 3.140. The OD curve of isolate 53 over 50 hours for plate 1 in the boscalid degradation experiment for HPLC analysis. OD values as Y-axis and assessment time intervals in hours as X-axis are illustrated.

The frozen samples were then removed from the freezer and maintained at room temperature for 5 minutes until they were completely thawed. They were then centrifuged for 2 minutes at 18000 rcf. Thereafter, 400 µL of each sample was transferred into an Eppendorf tube. 100 µL acetonitrile was then added into the sample giving a final concentration of 20 % acetonitrile. After mixing the samples with a vortex mixer, they were allowed to settle for 2 minutes to let any salts precipitate. Soon after, the samples were centrifuged again for 2 minutes at 18000 rcf. Finally, 450 µL of the sample was transferred into a labelled HPLC-vial.

In all four plates after an initial increase, the OD values increased for the first 44 to 47 hours and thereafter declined. The 5ppm boscalid treatment showed higher OD values than the control over the whole experimental period except with the second and fourth plates where the OD value with the control exceeded the one with the treatment from 46.98 and 41.10 hours onwards respectively (Fig. 3.140-143, Table 3.304, 3.305, 3.306, 3.307). Nevertheless, in the first test the OD value of the 5ppm treatment was very significantly increased (0.1% level) throughout the whole experiment and in the other three runs the OD value was significantly greater than with the control in many of the timings. This resulted in significantly greater "growth" of the treatment with boscalid 5 ppm at the 0.1 % level in first three runs and at the 1% level in the last run (Table 3.308, 3.309, 3.310, 3.311).

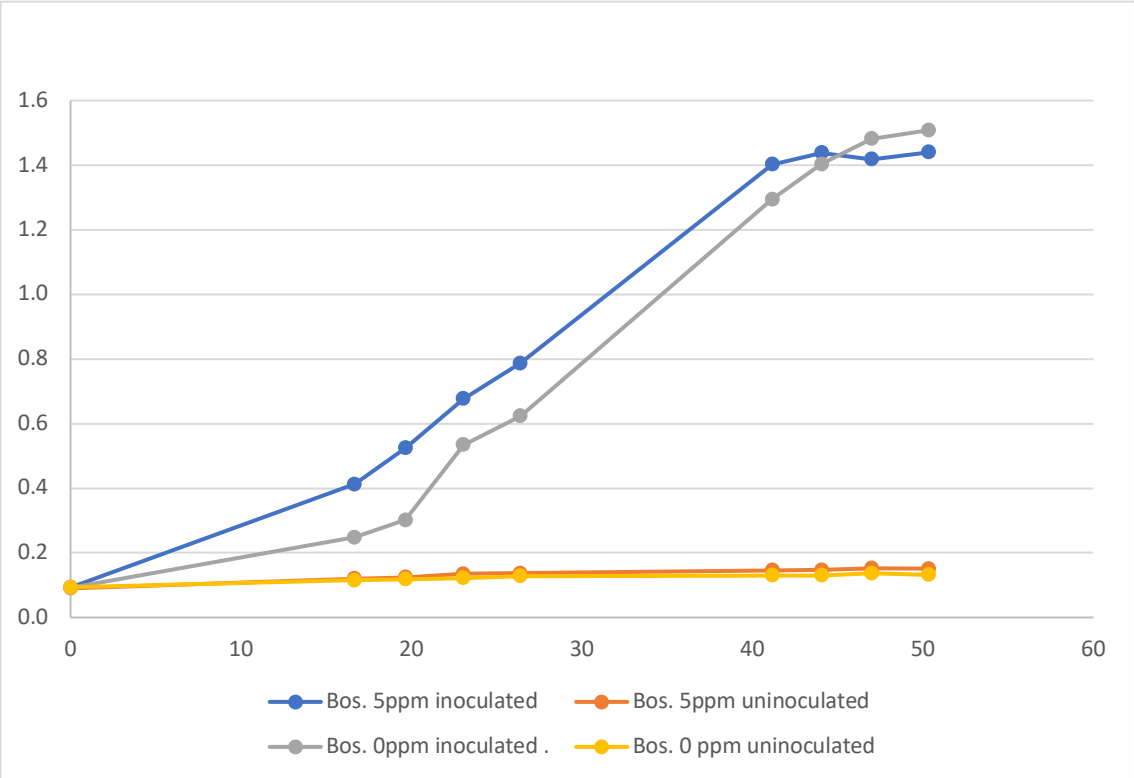


Figure 3.141. The OD curve of isolate 53 over 50 hours for plate 2 in the boscalid degradation experiment for HPLC analysis. OD values as Y-axis and assessment time intervals in hours as X-axis are illustrated.

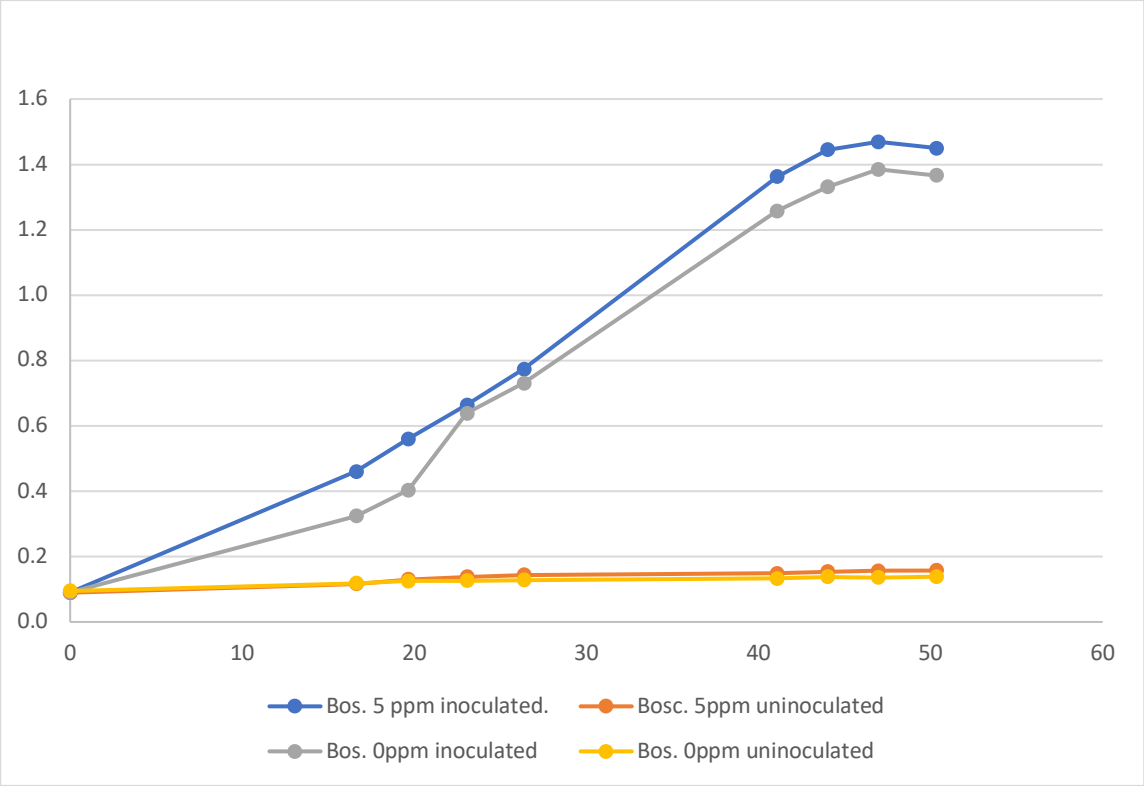


Figure 3.142. The OD curve of isolate 53 over 50 hours for plate 3 in the boscalid degradation experiment for HPLC analysis. OD values as Y-axis and assessment time intervals in hours as X-axis are illustrated.

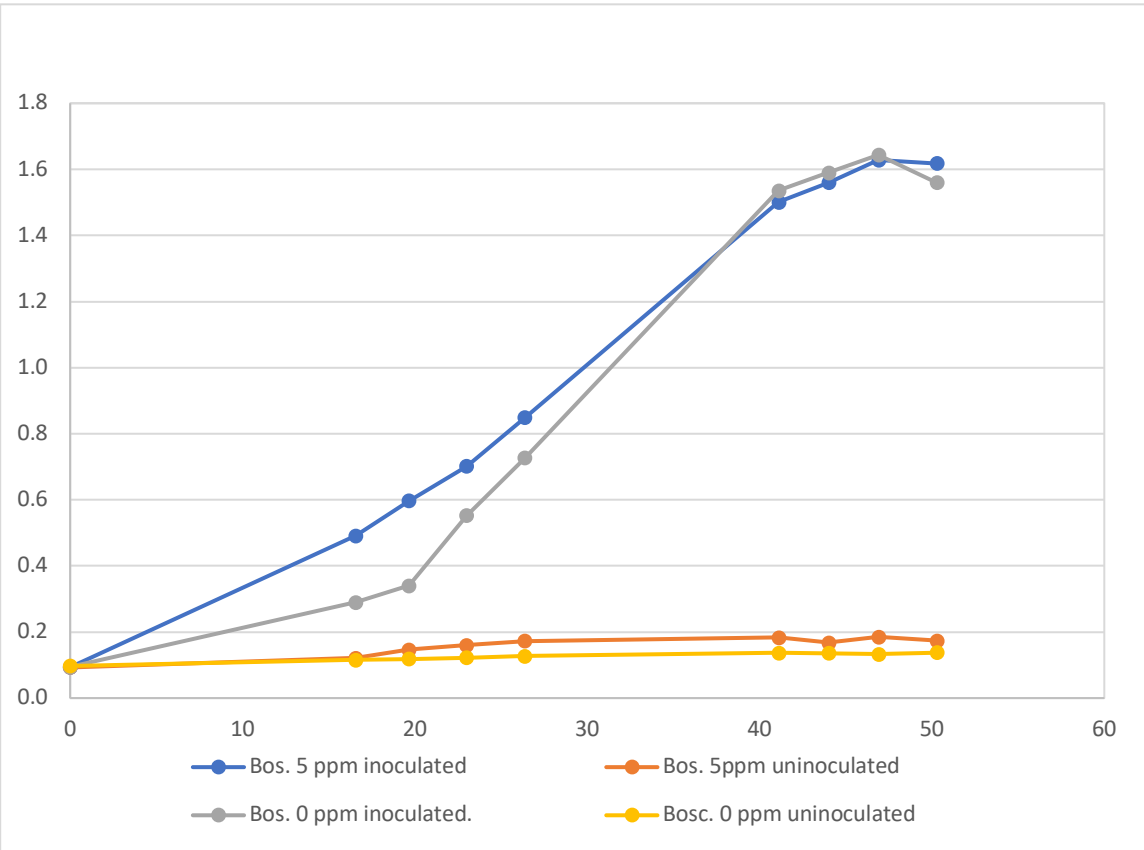


Figure 3.143. The OD curve of isolate 53 over 4 days for plate 3 in the boscalid degradation experiment for HPLC analysis. OD values as Y-axis and assessment time intervals in hours as X-axis are illustrated.

Ass. (h)	Bosc. 5ppm inoculated	Bosc. 5ppm uninoculated	Bosc. 0ppm inoculated	Bosc. 0ppm uninoculated	5% LSD	1% LSD	0.1%LSD
0.00	0.091	0.095	0.091	0.095	0.005	0.006	0.008
16.63	0.499	0.112	0.273	0.129	0.054	0.073	0.096
19.63	0.577	0.123	0.303	0.132	0.062	0.082	0.108
23.08	0.824	0.142	0.542	0.139	0.054	0.072	0.095
26.42	0.948	0.147	0.612	0.140	0.063	0.084	0.111
41.15	1.474	0.153	1.225	0.146	0.071	0.095	0.124
44.07	1.488	0.154	1.320	0.145	0.067	0.089	0.117
46.98	1.555	0.155	1.392	0.149	0.082	0.109	0.143
50.40	1.582	0.154	1.397	0.151	0.065	0.086	0.114

Table 3.304. The mean ODs at each assessment time for isolate 53 with and without 5ppm boscalid over 50 hours for plate 1 in the boscalid degradation experiment. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance.

Ass. (h)	Bosc. 5ppm inoculated	Bosc. 5ppm uninoculated	Bosc. 0ppm inoculated	Bosc. 0ppm uninoculated	5%LSD	1%LSD	0.1%LSD
0.00	0.093	0.090	0.092	0.093	0.003	0.004	0.005
16.67	0.412	0.120	0.248	0.115	0.042	0.056	0.073
19.65	0.525	0.124	0.302	0.118	0.059	0.079	0.104
23.05	0.677	0.135	0.534	0.123	0.045	0.060	0.079
26.38	0.787	0.137	0.624	0.128	0.055	0.073	0.096
41.17	1.403	0.146	1.295	0.130	0.060	0.080	0.105
44.07	1.438	0.147	1.403	0.131	0.074	0.099	0.130
46.98	1.419	0.152	1.481	0.137	0.104	0.139	0.182
50.35	1.440	0.151	1.508	0.132	0.109	0.146	0.191

Table 3.305. The mean ODs at each assessment time for isolate 53 with and without 5ppm boscalid over 50 hours for plate 2 in the boscalid degradation experiment. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance.

Ass. (h)	Bosc. 5ppm inoculated	Bosc. 5ppm uninoculated	Bosc. 0ppm inoculated	Bosc. 0ppm uninoculated	5% LSD	1% LSD	0.1% LSD
0.00	0.090	0.090	0.091	0.095	0.002	0.003	0.004
16.57	0.460	0.117	0.324	0.118	0.060	0.080	0.105
19.65	0.560	0.130	0.403	0.125	0.072	0.096	0.126
23.00	0.664	0.138	0.638	0.127	0.038	0.051	0.067
26.37	0.773	0.144	0.731	0.129	0.047	0.063	0.083
41.10	1.363	0.149	1.258	0.134	0.077	0.103	0.136
44.00	1.445	0.154	1.331	0.138	0.070	0.094	0.124
46.90	1.469	0.156	1.385	0.136	0.076	0.102	0.134
50.28	1.450	0.157	1.366	0.139	0.077	0.103	0.135

Table 3.306. The mean ODs at each assessment time for isolate 53 with and without 5ppm boscalid over 50 hours for plate 3 in the boscalid degradation experiment. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance.

Ass. (h)	Bosc. 5ppm inoculated	Bosc. 5ppm uninoculated	Bosc. 0ppm inoculated	Bosc. 0ppm uninoculated	5% LSD	1% LSD	0.1% LSD
0.00	0.093	0.093	0.095	0.097	0.003	0.004	0.005
16.57	0.491	0.121	0.290	0.116	0.088	0.118	0.155
19.65	0.597	0.146	0.340	0.118	0.103	0.138	0.181
23.00	0.701	0.160	0.553	0.122	0.096	0.129	0.169
26.37	0.849	0.172	0.727	0.127	0.104	0.139	0.182
41.10	1.501	0.183	1.536	0.137	0.075	0.101	0.132
44.00	1.560	0.168	1.590	0.136	0.069	0.092	0.121
46.90	1.628	0.185	1.644	0.133	0.069	0.092	0.121
50.28	1.618	0.173	1.560	0.137	0.079	0.105	0.139

Table 3.307. The mean ODs at each assessment time for isolate 53 with and without 5ppm boscalid over 50 hours for plate 4 in the boscalid degradation experiment. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance.

Treatment (Plate 1)	"Growth"
Bosc. 5ppm inoculated	39.274
Bosc. 5 ppm uninoculated	1.880
Bosc. 0ppm inoculated	28.631
Bosc. 0ppm uninoculated	1.713
LSD 5%	2.078
LSD 1%	2.778
LSD 0.1%	3.652

Treatment (Plate 2)	"Growth"
Bosc. 5ppm inoculated	34.697
Bosc. 5 ppm uninoculated	1.930
Bosc. 0ppm inoculated	29.702
Bosc. 0ppm uninoculated	1.241
LSD 5%	1.668
LSD 1%	2.230
LSD 0.1%	2.932

Treatment (Plate 3)	"Growth"
Bosc. 5ppm inoculated	35.088
Bosc. 5 ppm uninoculated	2.087
Bosc. 0ppm inoculated	31.093
Bosc. 0ppm uninoculated	1.313
LSD 5%	1.830
LSD 1%	2.446
LSD 0.1%	3.216

Treatment (Plate 4)	"Growth"
Bosc. 5ppm inoculated	38.474
Bosc. 5 ppm uninoculated	2.842
Bosc. 0ppm inoculated	34.358
Bosc. 0ppm inoculated	1.161
LSD 5%	2.711
LSD 1%	3.624
LSD 0.1%	4.764

Table 3.308, 3.309, 3.310, and 3.311. The mean "growth" of isolate 53 with and without 5ppm boscalid over 50 hours in the boscalid degradation experiment. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance.

The sample

	cal_c	Area	Area	Area
Lab_ID	[µg/L]	Bosc.	Bosc.	Bosc.
Bosc. 10	10	6.7	5.3	6.4
Bosc. 50	50	23.0	43.7	20.8
Bosc. 100	100	46.8		41.9
Bosc. 500	500	207.1	226.7	234.8
Bosc. 1000	1000	437.6	449.4	452.3
Bosc. 5000	5000	2272.5	2276.8	2266.0

Table 3.312. Calibration of HPLC system for boscalid with rates of 10, 50, 100, 500, 1000, 5000 ppm.

	28.04.22	29.04.22	02.05.22
Intercept	0	0	0
Real intercept	-6.1	4.8	0.7
Slope	0.453	0.455	0.453
R^2 (OLS)	0.9999	0.9999	1.0000

Table 3.313. The HPLC method validation Linearity Curve with a Slope Intercept.

Vial_ID	Plate	Sample_type	Area	c in HPLC-Vial [µg/L]	c in microwells [µg/L]	Average	%Degradation
plate 1 rep 1	plate 1	control/uninoculated	1289.4	2832.9	3541.1	2886.3	
plate 1 rep 2	plate 1	control/uninoculated	963.5	2116.9	2646.1		
plate 1 rep 3	plate 1	control/uninoculated	900	1977.4	2471.7		
plate 1 rep 1	plate 1	treatment	726.1	1595.3	1994.1	1972.8	31.6
plate 1 rep 2	plate 1	treatment	797.8	1752.8	2191.0		
plate 1 rep 3	plate 1	treatment	631.1	1386.6	1733.2		
plate 2 rep 1	plate 2	control/uninoculated	935	2054.2	2567.8	2485.9	
plate 2 rep 2	plate 2	control/uninoculated	874.4	1921.1	2401.4		
plate 2 rep 3	plate 2	control/uninoculated	906.1	1990.8	2488.4		
plate 2 rep 1	plate 2	treatment	500.1	1098.7	1373.4	1326.7	46.6
plate 2 rep 2	plate 2	treatment	555.2	1219.8	1524.8		
plate 2 rep 3	plate 2	treatment	394	865.6	1082.1		
plate 3 rep 1	plate 3	control/uninoculated	1026.2	2262.9	2828.7	2784.7	
plate 3 rep 2	plate 3	control/uninoculated	1064.7	2339.2	2924.0		
plate 3 rep 3	plate 3	control/uninoculated	943.8	2081.2	2601.6		
plate 3 rep 1	plate 3	treatment	223.4	492.6	615.8	694.6	75.1
plate 3 rep 2	plate 3	treatment	259.7	570.6	713.2		
plate 3 rep 3	plate 3	treatment	273.8	603.8	754.7		
plate 4 rep 1	plate 4	control/uninoculated	1520.6	3353.2	4191.5	3128.4	
plate 4 rep 2	plate 4	control/uninoculated	787.5	1730.2	2162.7		
plate 4 rep 3	plate 4	control/uninoculated	1099.6	2424.8	3031.0		
plate 4 rep 1	plate 4	treatment	41.8	92.2	115.2	160.7	94.9
plate 4 rep 2	plate 4	treatment	50.1	110.1	137.6		
plate 4 rep 3	plate 4	treatment	83.2	183.5	229.3		

Table 3.314. The initial (Cotr.) and final (Treat.) concentration (µg/L) of the fungicide boscalid after 50 hours in microplates 1,2,3, and 4. In each micro plate there were three rows of 5 wells as replicates for the control and three rows for the control. The area represents the peak of each sample in the HPLC and "c in HPLC-vial" and "c in microwell" represent the concentration [µg/L] of boscalid in the HPLC instrument and the microtiter plate respectively.

Table 3.314 and figure 3.144 Show the final concentration of boscalid in the samples from the four microtiter plates (plate 1,2,3,4), each of which contained two treatments (uninoculated and inoculated) and each treatment contained three replicates (rep.1,2,3). The concentration of boscalid remaining is estimated from the peak height. The concentration of boscalid per liter in the sample is thus determined and corrected for the volume within the plate well to give an amount per well. From this the amount of degradation of the boscalid can be calculated by

subtracting the remainder from the initial amount and expressing the amount degraded as a percentage of the total present at the beginning.

5 rates of boscalid 10, 50, 100, 500, 1000, and 5000 ppm were used to establish a correlation curve on three separate occasions and regression coefficients (R^2) of between 0.99 and 1.0 (Table 3.312) were obtained (Table 3.313).

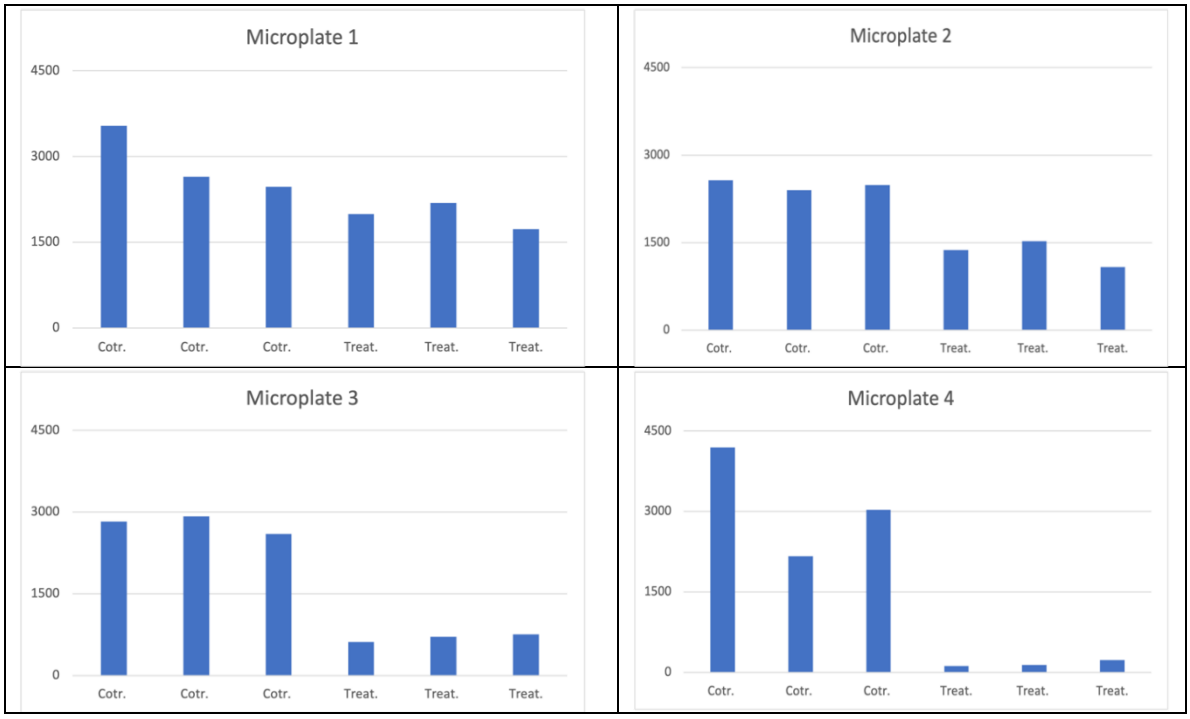


Figure 3.144. The initial (Cotr.) and final (Treat.) concentration (µg/L) of the fungicide boscalid after three days in Microplates 1,2,3, and 4.

Plate	"Growth" of boscalid treatment (% control)	"Growth" of boscalid treatment	% Degradation
1	137.2	39.274	31.6
2	116.8	34.697	46.6
3	112.8	35.088	75.1
4	119.7	38.474	94.9

Table 3.315. The "growth" of isolate 53 in the boscalid treatments and the level of boscalid degradation in each microtiter plate in the boscalid degradation experiment.

According to the data, the 32, 47, 75, and 95% degradation of the boscalid 5 ppm was confirmed in the microtiter plates 1, 2, 3, and 4 respectively. The degradation data obtained from the HPLC

analysis is in agreement with the OD observations where the 5ppm boscalid treatment showed significantly enhanced “growth” compared to the treatment with the 0 ppm in all 4 microplates. This strongly supports the hypothesis that isolate 53 has degraded the boscalid fungicide and most likely utilised the fungicide as a nutrition source.

Considering the “growth” divergence between the control and the treatment with the 5 ppm treatment in each microtiter plate, the greatest enhanced “growth” compared to the 0 ppm occurred in the first plate (Table 3.315). Although, the highest percent of boscalid degradation was achieved from Plate 4 but removing this plate revealed a linear relationship between "growth" rate and degree of degradation.

Accordingly, isolate 53 *(B. megaterium)*, one of selected strains, exhibited an enhanced increase in "growth" in the presence of the fungicide. This promising evidence suggests that *Bacillus* species may be capable of degrading fungicides, as there was a good correlation between "growth" and degree of degradation.

However, many factors can affect bacterial growth and metabolism (Pharm, 2019).Therefore, further research is needed to investigate other strains and fungicides.

3.4. Bioassay

In the course of this experiment, the strain MBI600 played the role of control. This was designed to acquire a respectable observation of fungal activity of selected strains. Because in general, MBI600 registered in the market has the fungicidal character. Besides, it’s confirmed that the strain MBI600 inhibited the growth of all three fungi very significantly at the 0.1 % level (Table 3.316 and 3.317).

All isolates from the Revysol® field trials except the isolates 96 and 124 inhibited very significantly the growth of *Cercospora* at the 0.1 % level (61.a, 61.b, and 80) or the 1% level (53). Moreover, the evaluation of the strains 61.b and 80 indicated a very significant inhibitory effect on the growth of all three fungi at the 0.1% level with the exception of 61.b against *Botrytis* where the result was only statistically significant at the 5% level. Nevertheless, 4 out of 6 strains in the Revysol® group (53, 61.a, 61.b, and 80) inhibited very significantly the growth of *Cercospora* at 0.1 % level with the exception of strain 53 at the 5% level (Table 3.316).

Revysol®	Isolate	*Botrytis*	*Cercospora*	*Sclerotinia*
B. megaterium	53		**	
B. megaterium	61.a		***	
B. velenzensis	61.b	*	***	***
B. velenzensis	80	***	***	***
B. megaterium	96			
B. licheniformis	124			
B. amyloliquefaciens	MBI600	**(*)	***	***

Table 3.316. The results of the biofungicide assays with the leading isolates from the Revysol® field trials with the commercial biofungicide strain MBI 600 as a control. (*) represents statistically significant inhibition of the pathogen at the 5% level, (**) at the 1% level, and (***) at the 0.1% level.

Boscalid	Isolate	*Botrytis*	*Cercospora*	*Sclerotinia*
B. megaterium	180			
Not Identified	184		***	
B. megaterium	195			
B. simplex	196			
B. megaterium	201		***	
B. megaterium	250			
B. megaterium	260			
B. subtilis	280	***	***	
B. licheniformis	284			
B. licheniformis	285		***	
B. amyloliquefaciens	MBI600	***	***	***

Table 3.317. The results of the biofungicide assays with the leading isolates from the boscalid field trials with the commercial biofungicide strain MBI 600 as a control. (*) represents statistically significant inhibition of the pathogen at the 5% level, (**) at the 1% level, and (***) at the 0.1% level.

Most of the isolates from the boscalid field trials, showed antifungal activity against *Cercospora*; the strains 184 (unknown), 201 (*Bacillus megaterium*), and 285 (*Bacillus licheniformis*) inhibited markedly the growth of *Cercospora* at the 0.1 % level. The strain 280 (*Bacillus subtilis*) inhibited very significantly the growth of both *Cercospora* and *Botrytis* at the 0.1% level. On the other hand, none of isolates from boscalid group showed the fungicidal activity against any of the three fungi (Table 3.317).

When comparing the results of two groups, the selected strains inhibited mostly the growth of *Cercospora*. The fungicidal activity occurred primarily by *B. megaterium* followed by *B. velenzensis*, and a few of *B. licheniformis* and *subtilis* strains respectively. To less extend, the growth of *Botrytis* was inhibited by *B. velenzensis* and *B. subtilis*. But the growth of *Sclerotinia* was inhibited only by isolates identified as *B. velenzensis*.

4. Discussion

4.1. Correlation between Primary and Secondary Screens

182 isolates were evaluated in the primary screens, and the integrated OD value of each treatment over the experimental period (the area under the OD curve for the Revysol® isolates and "growth" for the boscalid isolates) was used to select the best strains for secondary screening in more detail.

In secondary screening the selected isolates were evaluated with a broader range of fungicides doses based on the "growth". Observing the increased "growth" again throughout the secondary screening tests in the treatments with fungicides confirmed the reliability of the screening metho d for selecting potential biological strains. Moreover, the enhanced "growth" of the strains selected for identification after the secondary screen compared with the others selected for secondary screening but not subsequently selected for identification, indicated a higher increase of growth with the identified strains than the none identified ones, which supported the efficiency of the method (Table 4.1).

However, the average "growth" across a number of doses was not an appropriate indicator to assess the growth enhancement caused by different treatments since bacterial isolates behave differently to different dose rates of fungicide. For example, with Revysol® at rates above 10 ppm we usually see growth inhibition and thus the effects at the higher dose rates would tend to negate the promotion occurring at lower doses. Accordingly, the highest level of "growth" enhancement at any one dose was considered to be a more appropriate indicator on which to assess the growth enhancement (Table 4.1).

	Primary Screening		Secondary Screening	
	"Growth" Ave. %	Best "Growth" %	"Growth" Ave. %	Best "Growth" %
Mean (Identified Strains)	108.1	111.0	110.9	119.4
Mean (not Identified Strains)	94.9	96.1	99.5	98.7

Table 4.1. The average "growth" and the highest positive growth in the primary and secondary screening test (in percent) for identified and none identified strains.

Eventually 6 isolates 53, 61.a, 61.b, 80, 96, and 124 from the "Revysol® group" and 9 isolates 180, 195, 196, 201, 250, 260, 280, 284, and 285 from the "boscalid group" were selected for identification. These 15 isolates are all members of the *Bacillus* genus. Eight were identified as

Bacillus megaterium, three as *Bacillus licheniformis*, two as *Bacillus subtilis/velenzensis*, one as *Bacillus simplex* and one as *Bacillus subtilis*.

During the development of the screening method used here two types of liquid culture were used: minimal salt medium with and without the glucose supplement using a laboratory strain of *B. subtilis* (Soltani 2019). The results appeared to suggest that the presence of glucose was necessary for the bacterial degradation of the fungicide, suggesting that the degradation occurred through co-metabolism. In other words, the presence of glucose in the medium causes enzyme activities to catalyse the degradation of not only growth-substrates to derive energy and carbon but also additional compounds which are the fungicides in this case. Thereby, all experiments have been carried out in the salt medium supplemented with the glucose.

4.2. Effect of Different Dose Rates

The extended range of doses in the secondary screens didn't result in the identification of rates that consistently showed better "growth" effects than those used in the primary screen. In most cases, 5 ppm was the optimum dose rate. However, the isolate 61.a is as an exception showing the best "growth" with the dose rates 0.625 and 2.5 ppm. Indeed, the treatments with Revysol® at 10 ppm and above tended to inhibit the "growth" of the isolates, especially with 25 ppm where marked inhibition tended to be observed. In contrast, the treatments with boscalid, rates of 10 ppm and above didn't show this inhibitory effect but also did not lead to further enhanced "growth" with increasing doses. This inhibitory effect at the higher rates was also observed in the cross-screening tests with Revysol® where all treatments with 25ppm of Revysol® showed a significant reduction in "growth" compared to the control. In addition, the 10 ppm rate of Revysol® did not result in additional growth enhancement compared to the lower rates and often led to the inhibition of "growth" (Table 4.2). This caused the average of the increased growth in treatments with the fungicides to fall.

Treat. / Doses (ppm)	0.625	1	1.25	2.5	5	10	15	25
Boscalid Secondary Screening	102.8		108.4	106.2	110.9	105.7		86.8
Revysol® Secondary Screening	160.0		151.2	136.2	139.1	97.3	47.3	
Boscalid Cross-screening		110.2			112.6			101.3
Revysol® Cross-screening		110.6			111.6			45.1

Table 4.2. The mean "growth of a broad range of doses of boscalid and Revysol® in secondary and cross screening.

		1. Screening	2. Screening	Cross Screening (Bosc.)	Cross Screening (Rev.)	Cross Screening (Pyra.)	Cross Screening (Flux.)
Identification	Strain ID	5ppm	5ppm	5ppm	5ppm	5ppm	5 ppm
B. megaterium	53	65.7	138	114.9	112.5	104.8	109.4
B. megaterium	61	120.1	63.1	108.4	105.9	99.3	98.5
B. megaterium	80	165.7	141.5	97.2	114.4	101.4	102.1
B. velenzensis	96	115.2	135.7	122.5	106.7	105.3	104.1
B. licheniformis	124	123	217.3	133.7	110.1	149.2	92.8
B. megaterium	180	27.4	106.9	107.2	103.0	91.8	106.9
B. megaterium	195	120	109.2	130.8	116.4	105.3	
B. megaterium	196	80.4	105.6	101.5	113.0	100.9	
B. megaterium	201	111.4	117.8	109.2	102.5	93.5	107.9
B. megaterium	250!	87.5	113.0	119.0	121.0	108.3	112.0
B. megaterium	260	144.9	105.9	91.2	84.7	81.8	
B. subtilis	280	107.3	104.0	121.5	114.7	100.1	127.5
B. licheniformis	284	122.4	120.0	109.0	115.4	127.2	108.4
B. licheniformis	285	128.9	110.1	98.4	120.25	125.6	93.4
B. megaterium	250!	115.3	108.12				
B. megaterium	260	142.2					
B. subtilis	280	120.1					
B. licheniformis	284	119.8					
B. licheniformis	285	91.9					

Screened with Revysol®
Screened with boscalid
Screened with pyraclostrobin
screened with fluxapyroxad

Table 4.3. The percent "growth" of Isolates tested in three types of screening (primary, secondary, cross-screening) with the 5ppm of the fungicides Revysol®, boscalid, pyraclostrobin, and fluxapyroxad.

4.3. Cross Screening

In the cross-screening tests most of the identified strains showed a markedly greater increase in the percent "growth" in the presence of boscalid. Furthermore, although it was initially assumed that bacterial strains are more likely to be able to degrade fungicides with which they have been exposed for long periods and that this degradation ability was likely to be fungicide specific, the results from cross-screening tests were generally not consistent with this assumption. The only support that can be drawn from the results for this hypothesis is that all *B. licheniformis* strains tended to show a significant increase in the percent "growth" in treatments with pyraclostrobin. From this standpoint, the enhanced growth in the presence of pyraclostrobin could be seen to be due to the source of the isolate, which is Bellis, a combination of both pyraclostrobin and boscalid. However, it remains unclear to which degree it is attributed to the

source of the isolate. More often, the enhanced "growth" of bacterial isolates in the presence of fungicides occurred not only with the fungicide applied in the trials from which they were isolated, but also with other fungicide classes as well (Table 4.4). It suggests that the ability to degrade fungicides is not fungicide specific.

		Boscalid (ppm)			Revysol® (ppm)			Pyraclostrobin (ppm)			Fluxapyroxad (ppm)		
Identity	Strain	25	5	1	25	5	1	25	5	1	25	5	1
Bacillus megaterium	53	104.3	114.2	113.8	75.0	112.1	109.6	106.0	105.8	108.9	111.4	109.4	108.2
Bacillus megaterium	53	109.6	108.8	109.1	6.8	103.4	116.4	94.5	94.5	96.9			
Bacillus megaterium	53	105.8	123.1	116.1	75.4	122.7	118.5	109.4	112.2	113.8			
Bacillus megaterium	61.a	105.0	110.5	108.0	97.5	104.8	110.7	107.4	102.3	109.2			
Bacillus megaterium	61.a	91.3	111.8	109.0	54.8	106.0	96.0	92.8	92.6	93.2	105.0	101.2	106.0
Bacillus megaterium	61.a	97.5	102.8	103.5	58.3	106.8	106.0	106.3	103.0	103.5	99.6	95.8	94.3
Bacillus velenzensis	61.b	120.4	126.1	122.2	-0.7	110.0	115.7	47.3	93.2	104.7	111.0	102.1	107.6
Bacillus velenzensis	61.b	76.6	87.4	96.3	80.8	109.9	106.2	85.5	90.4	101.0			
Bacillus velenzensis	80	79.9	106.6	103.5	98.6	116.0	111.4	100.2	107.7	109.8	105.7	102.9	111.1
Bacillus velenzensis	80	57.0	83.3	101.6	22.4	108.1	102.4	67.5	91.8	97.2			
Bacillus velenzensis	80	98.5	106.9	110.0	54.2	116.0	114.2	96.5	100.1	113.9	98.6	106.3	114.3
Bacillus velenzensis	80	65.5	91.9	103.2	80.0	117.5	114.2	84.2	105.9	112.2	103.3	97.1	105.7
Bacillus megaterium	96	108.6	108.5	106.2	20.4	96.9	101.9	100.5	100.0	106.7	103.0	103.3	110.9
Bacillus megaterium	96	127.5	131.6	121.2	4.8	112.9	122.5	124.6	107.2	120.3			
Bacillus megaterium	96	118.9	127.5	128.6	89.4	110.3	122.7	100.1	108.6	110.7	116.3	104.8	105.5
Bacillus licheniformis	124	129.7	158.2	106.4	21.0	75.9	95.2	152.6	153.0	129.5	91.3	92.8	110.8
Bacillus licheniformis	124	107.6	109.1	89.4	27.7	144.3	111.9	144.9	145.3	119.0			
Bacillus megaterium	180	95.8	107.2	109.9	8.2	103.0	100.9	89.8	91.8	97.2			
Bacillus megaterium	195	105.2	118.4	112.8	64.2	109.6	110.5	100.8	106.4	113.1	100.5	106.9	111.8
Bacillus megaterium	195	126.0	143.1	124.8	6.8	123.2	129.2	102.3	104.1	122.6			
Bacillus simplex	196	99.3	101.5	100.8	46.6	113.0	106.2	95.0	100.9	101.9			
Bacillus megaterium	201	86.4	110.8	112.3	44.2	102.4	98.1	87.1	92.7	96.3			
Bacillus megaterium	201	85.7	103.7	108.3	45.5	92.4	100.6	91.4	94.3	97.3	105.9	106.0	108.8
Bacillus megaterium	201	101.8	113.2	118.3	27.4	112.7	103.5				116.5	109.7	108.9
Bacillus megaterium	250	100.2	115.9	115.7	45.8	121.6	113.7	100.5	105.3	104.0			
Bacillus megaterium	250	91.7	112.7	112.2	96.9	106.7	103.1	103.4	103.8	103.9	104.8	103.5	112.0
Bacillus megaterium	250	119.7	128.7	130.3	45.8	129.4	126.3				129.0	128.2	127.0
Bacillus megaterium	250	115.8	118.5	109.8	97.4	126.2	131.1	114.6	115.7	115.1	96.6	104.3	110.6
Bacillus megaterium	260	85.1	91.2	95.4	5.7	84.7	90.6	86.7	81.8	99.8			
Bacillus subtilis	280	78.2	108.6	102.3	13.9	99.9	99.0	84.9	98.6	111.9			
Bacillus subtilis	280	88.6	110.3	106.6	31.9	107.9	107.3	86.8	101.6	106.3	124.7	107.7	107.3
Bacillus subtilis	280	144.6	145.5	141.9	35.7	136.2	147.2				147.7	147.3	143.5
Bacillus licheniformis	284	108.2	117.7	127.8	31.8	85.7	116.0	151.5	123.7	113.1	125.0	114.4	129.7
Bacillus licheniformis	284	107.6	109.1	89.4	27.7	144.3	111.9	144.9	145.3	119.0			
Bacillus licheniformis	284	108.7	100.3	106.5	27.6	116.3	106.9	89.4	112.7	108.5	99.8	102.3	114.0
Bacillus licheniformis	285	113.4	105.2	102.9	15.1	140.0	128.9	125.1	135.3	77.5			
Bacillus licheniformis	285	77.1	91.6	93.4	23.7	100.5	87.3	117.0	115.9	108.4	101.3	93.4	100.1

		Boscalid (ppm)			Revysol® (ppm)			Pyraclostrobin (ppm)			Fluxapyroxad (ppm)		
Identity	Strain	25	5	1	25	5	1	25	5	1	25	5	1
Bacillus amyloliquefaciens	Serenade®	95.3	108.2	108.8	9.5	115.9	124.0	73.8	79.5	108.5	95.9	104.2	109.3
Bacillus amyloliquefaciens	Serenade®	100.0	110.6	97.2	17.8	120.7	117.4	82.7	87.7	82.9	98.7	101.7	114.4
Bacillus amyloliquefaciens	MBI	90.1	97.4	103.6	19.5	127.5	108.2	82.2	96.5	113.3	119.0	97.4	99.0
Bacillus amyloliquefaciens	MBI	103.7	121.8	123.0	109.6	124.2	143.6	97.1	109.5	118.5	138.1	113.8	113.6

Table 4.4. The mean "growth" of isolates tested in cross screening. LSDs derived from the ANOVA at the 0.1, 1 and 5% significance levels. Significantly increased ODs compared to the control are highlighted in shades of green depending on the level of significance and significantly decreased ODs highlighted in shades of orange according to the degree of significance.

4.4. Growth Dynamics

The general pattern of growth development in the microtiter plate system that formed the basis of this work was examined with selected Revysol® isolates using six doses of Revysol® 100, 50, 25, 12.5, 5, 2.5 ppm with OD readings being made every 30 minutes for the first 48 hours and thereafter 4 measurements were taken per day every 3 to 4 hours between 08:00 and 17:00. From the results, it is clear that peak ODs occurred between 8 and 15 hours in the experiment. The lag occupies the time between inoculation and establishment of the maximum division rate. Since the duration of the lag phase depends on the previous culture, the age of the culture and the composition and suitability of the nutrient medium, an overnight inoculation of isolates was carried out before each test set-up to attempt to standardize conditions and reduce possible variation between isolates and between experiments.

The ODs reach a peak value at the end of the log phase and then start to fall or level off due to nutrients becoming limiting and the stationary phase begins as the cells can no longer reproduce. As the bacterial growth rate depends on, among other factors, the concentration of substrate, and because this decreases during the growth, the growth rate of a culture usually begins to decline even before all substrate has been consumed. Thereby, transition from the exponential to the stationary phase is usually taking place gradually (Schlege, 2018) (Fig. 4.1). Apart from substrate limitation, other factors such as very high cell concentrations, low partial oxygen, and accumulation of toxic metabolic end products can also lead to a decrease in the growth rate and initiation of the stationary phase. These unfavourable metabolic products or exhausted nutrients lead to gradual initiation of the sporulation process. The death phase then

starts when factors like the acid accumulation, such as carboxylic and hydrochloric acids causing the viable count to decline exponentially (Schlege, 2018).

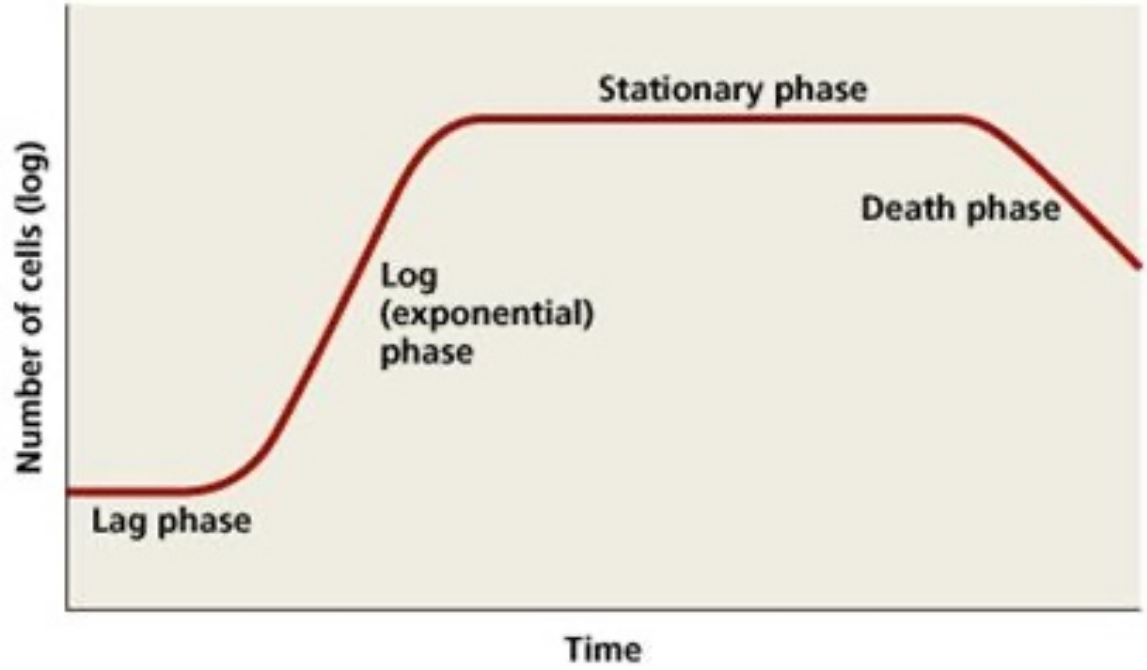


Figure 4.1. A typical growth curve for bacterial cultures (Schlege, 2018).

Following the selection of isolates for identification, the growth dynamics of the selected isolates in the absence of any fungicide were examined, and the growth curves obtained could be attributed to one typical of the three curves identified with the Revysol® isolates consistent with the species identification. The differences between the species predominantly occur after the attainment of the peak value. Type 1 *(B. megaterium)* showed a continuous slow decrease, Type 2 *(B. velenzensis)* a steep decline followed by a gentle increase before plateauing out, and Type 3 *(B. licheniformis)* a more marked decrease followed by a plateauing out (Fig. 4.2).

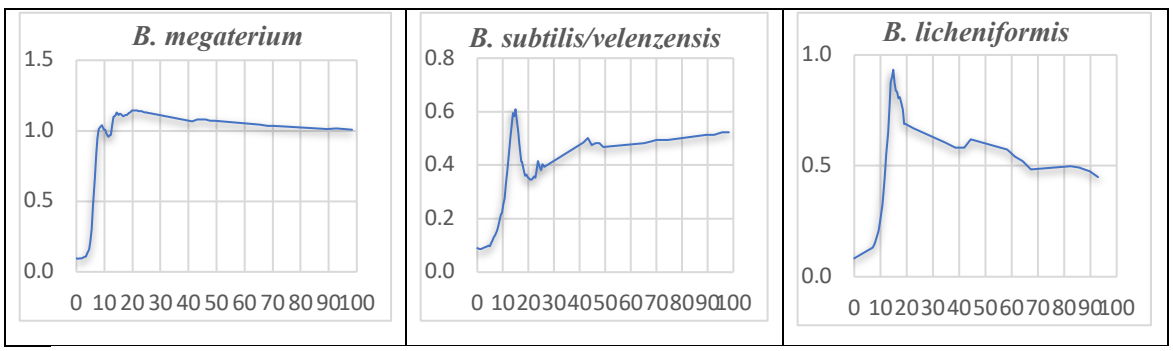


Figure 4.2. OD curves of different *Bacillus* species isolated from boscalid and Revysol® trials in the absence of fungicides.

Comparing the general bacterial growth behaviour mentioned above to the treatments in screening tests with different rates of fungicides, the presence of the fungicides didn't modify the general growth curve of the bacterial isolates, there was only a vertical shift in the position of the growth curve (Fig. 4.3). The exception to this was with the higher rates of Revysol® where strong inhibition occurred (above 10ppm) (Fig. 4.4-6).

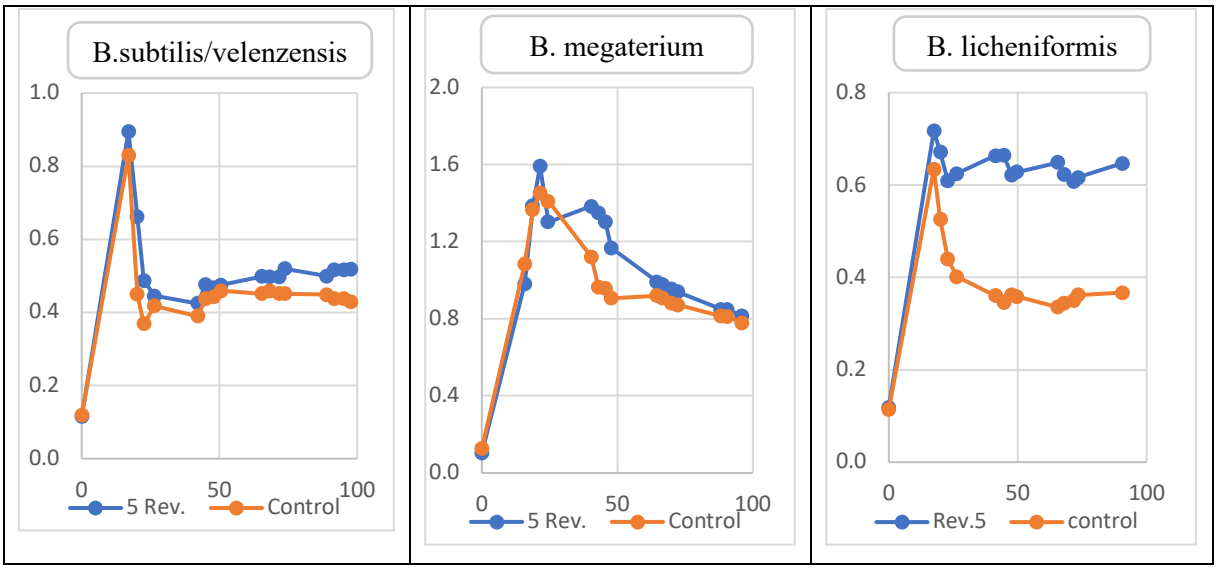


Figure 4.3. OD responses of different *Bacillus* species to Revysol®.

Bacillus megaterium

Figure 4.4. The OD curves of the selected *Bacillus megaterium* isolates.

Bacillus subtilis/velenzensis

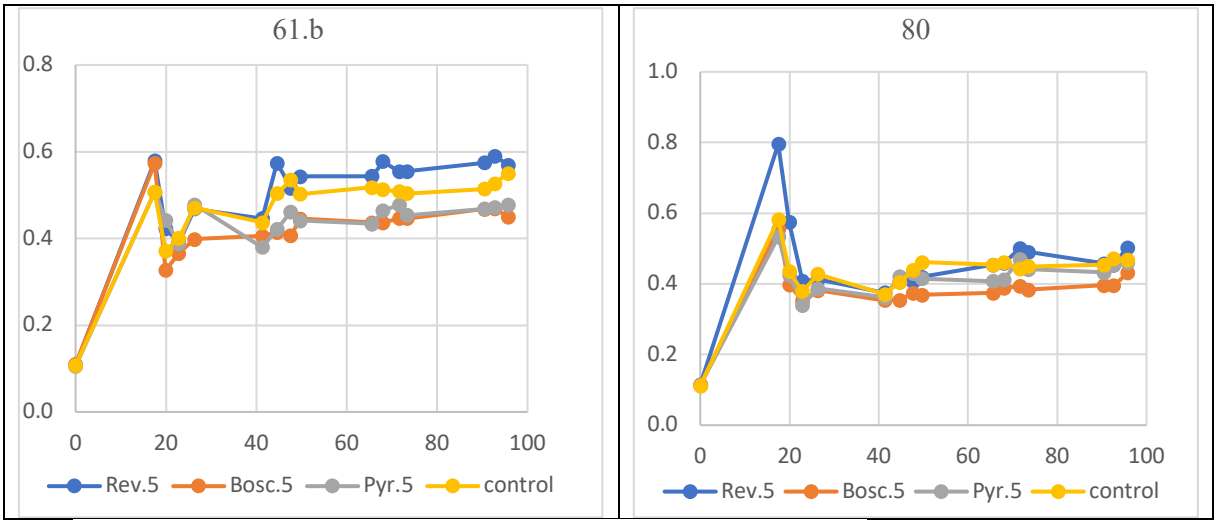


Figure 4.5. The OD curves of the selected *Bacillus subtilis / Velenzensis* isolates.

Bacillus licheniformis

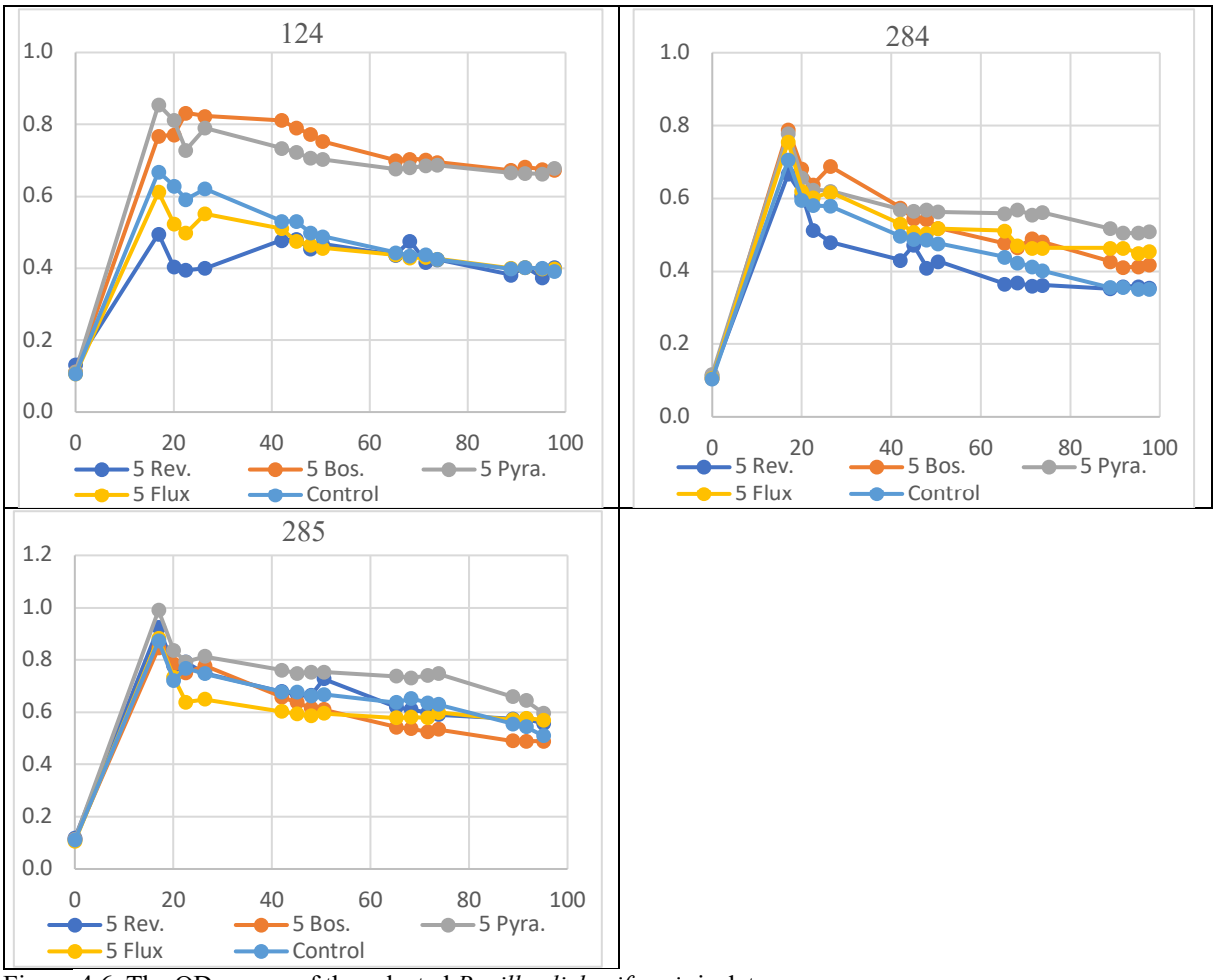


Figure 4.6. The OD curves of the selected *Bacillus licheniformis* isolates.

4.5. Relationship between Enhanced Growth and Fungicide Degradation

The hypothesis used as the basis for testing of the isolates for their ability to degrade the fungicides was that if the fungicides were being degraded and used as a nutrient source for the bacterial isolates, this would lead to higher "growth" occurring with the fungicide treatments compared to the control where no fungicide is present. The degradation analysis showed degradation of between 30 and 95% in treatments where the isolate had significantly higher "growth" (Fig. 4.7). These results provide promising evidence in support of the hypothesis.
In plates 2,3 and 4 there is a good correlation between the "growth" observed and the amount of degradation that has occurred. With plate 1 however the high growth value is not associated with a high level of degradation. Clearly with only four examples only limited conclusions can be drawn about this possible correlation and further experimentation is necessary in order to determine whether a good quantitative correlation between "growth" and degradation exists or not or indeed whether other parameters measured in the microtiter test (e.g., peak OD value) provide a better prediction of the degree of degradation.

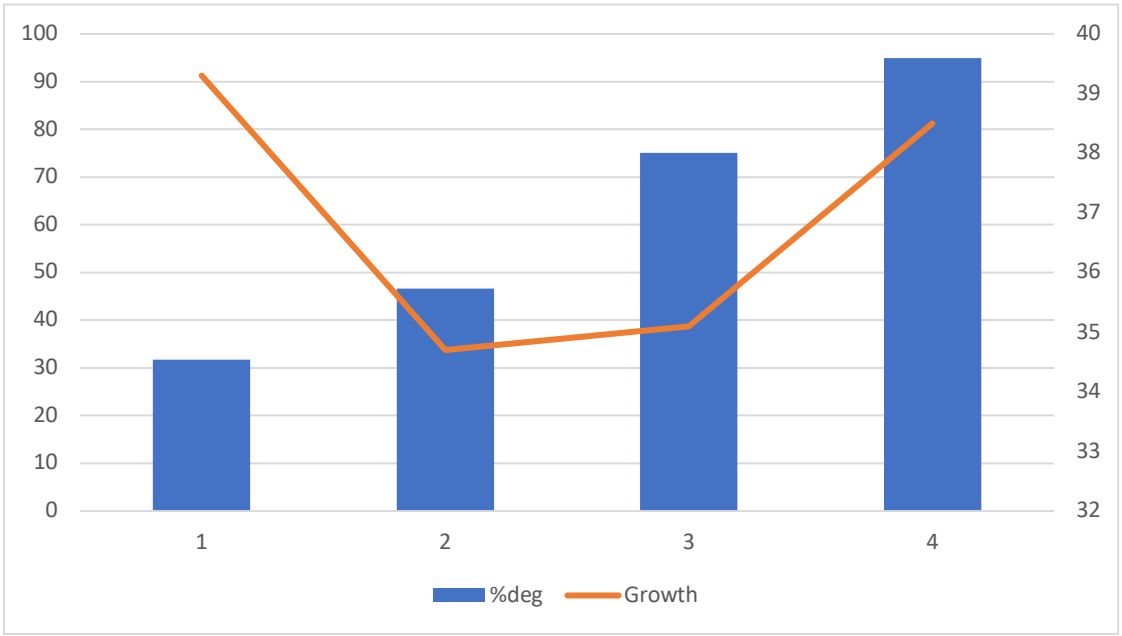


Figure 4.7. The relationship between the percent degradation and "growth" for the four microtiter plates in the boscalid degradation experiment.

Further investigations are necessary to further substantiate the conclusions drawn from this work. Repeats of the HPLC degradation studies with other isolates drawn from the *Bacillus* strains identified in this study. It might be even a better approach to use the commercial strain

MBI600 (*Bacillus velenzensis*), which has indicated the capability to degrade multiple fungicides in the cross-screening test. Establishing products using the strains already commercialised avoids many costs of the development process that would result from using the new isolates.

This method can also be utilised where there is interest in quantifying the potential of microorganisms and in particular bacteria to degrade a diverse range of substances including other crop protection chemicals such as herbicides and insecticides. Indeed, this method has been used in a study to investigate the ability of *B. subtilis* to degrade ivermectin where there was interest from a bioremediation perspective (Loew and Taghibeiglou, 2022 unpublished data).

Moreover, another study on the ability of the *Bacillus subtilis* for degrading the chemical fungicide boscalid led to promising results confirming findings in the present work. In fact, this time the data was collected from the growth comparison of *Botrytis cinerea* in the presence of boscalid remaining in the culture medium after 48 hours incubation following inoculation *B. subtilis* DMS indicating the significant reduction of boscalid by bacteria (Taghibeiglou, 2023 unpublished data).

4.6. Biofungicidal Activity Correlation with Degradability, Feasibility of Finding One Strain to do both

The main objective of the study was to try and find an isolate that could not only function as a biofungicide but also degrade the chemical fungicide residues that are present in the crop following an integrated chemical and biological fungicide spray program. In particular the *B. velenzensis* isolates functioned well as biofungicides, significantly inhibiting the growth of all three fungal pathogens (*Botrytis*, *Cercospora*, and *Sclerotinia*) tested. This suggests that the concept of a biofungicide strain that can also degrade chemical fungicides is feasible.

Another promising finding is that the commercial biofungicide MBI600, *B. velenzensis* illustrated not only the strong fungicidal activity against all three fungi but also indicated a good potential to degrade fungicides due to its significantly enhanced ‘‘growth’’ in the presence of fungicides particularly Revysol®, pyraclostrobin, and boscalid. To a lesser extent, the enhanced growth of Serenade®, another commercial *B. velenzensis* biofungicide was also observed to show enhanced “growth” in the presence of fungicides, although to a slightly lesser extent than

MBI600. Accordingly, if a commercial strain can deliver both performance (MBI600), then it might be better to focus on optimizing the biodegradation properties of the commercial product.

4.7. Assessment of Progress against Objectives

In the frame of this study, the screening method developed was found to be efficient. Over the primary screening tests the isolates were selected based on their "growth" and then the increased "growth" was observed again throughout the secondary screening tests in the treatments with fungicides, supporting the reliability of the method. Applying this method led to detecting isolates that could degrade the fungicides and also function as biofungicides.

However, it was shown that contrary to initial expectations, the degradation ability is not fungicide specific. Following that, since two commercial *Bacillus* biofungicide strains were used in this study and both showed they have the potential to degrade fungicides and biofungicidal activity, this led us to question whether these already commercialised strains could be optimised for a dual function (biofungicide and chemical fungicide degradation) and thereby save costs rather than developing a product based on a new isolate.

4.8. The Practical Implications of this Work

These results found clear support for the potential of *Bacillus* species as microbial fungicides that can degrade the residue of chemical crop protection products. Since biofungicides are not currently financially attractive in terms of cost performance, such additional properties (the residue degradation) as unique selling points (USP) may justify their costs and thereby markedly increase their attractiveness.

4.9. Next Steps

The ultimate aim of this novel work is to establish strains that show potential in the dual role as biological fungicides and "degraders" of one or more classes of chemical fungicides. This necessitates that the bacterium ultimately carries out the dual function in vivo, on the leaf and this is the next logical step in developing this concept further. This could begin with studies on plant tissue such as detached leaves to which fungicides will be applied and the leaf will be treated with the bacterial strains of interest. The next step afterwards would be the scaled-up application to whole plants in glass house conditions and eventually following successful outcomes, transition into field grown plants.

One critical point that future research should also address is that the final degradation products of the fungicides should not cause adverse toxicological or environmental effects and whether the quantities involved after the degradation are within the legally allowed limits. The breakdown products of chemical fungicides have been well studied prior to registration and information from these studies may also be useful in this context. This research could also be extended to explore the potential of the bacteria to degrade other chemical crop protection products such as herbicides, insecticides, etc.

6. References

(EFSA), T. E. (2005). Report From The Commision To The council And The European Parlament. The EFSA Journal 256, 1-25.

Ákos Mesterházy, J. O. (2020). Losses in the Grain Supply Chain: Causes and Solutions. Sustainability, 12(6), 2342.

Andow, D. A. (1998). F2 Screen for Rare Resistance Alleles. Journal of Economic Entomology 91 (3): 572-578.

André Luiz Meleiro Porto, G. Z. (2011). *Pesticides in the Modern World - Pesticides Use and Management.* chapter 20, Biodegradation of Pesticides.

Anusha Delanthabettu, N. S. (2022). Molecular Characterization of Native *Bacillus* thuringiensis Strains from Root Nodules with Toxicity Against the Fall Armyworm (FAW, Spodoptera frugiperda) and Brinjal Ash Weevil (Myllocerus subfasciatus). Current Microbiology 79(9):274.

Barbara S Schuwirth, J. M.-S. (2006). Structural analysis of kasugamycin inhibition of translation. Nature Structural & Molecular Biology volume 13, pages 879–886.

BASF. (1998). Analytical Method CP-No. 327 . BASF AKtiengesellschaft Reg. Doc. BASF 98/11549.

Bruinsma, N. A. (2012). World agriculture towards 2030/2050: the 2012 revision. ESA Working Paper No. 12-03.

Cesare Pacini, A. W. (2003). Evaluation of sustainability of organic, integrated and conventional farming systems: a farm and field-scale analysis. Agriculture, Ecosystems and Environment 95, 273–288.

Charu Verma, S. N. (2011). A Review on Impacts of Genetically Modified Food on Human Health. The Open Nutraceuticals Journal, 4, 3-11.

Danny A. P. Hooftman, J. G. (2005). Demographic Vital Rates Determine the Performance Advantage of Crop-Wild Hybrids in Lettuce. Journal of Applied Ecology, Vol. 42, No. 6 .

Dimitrios T. Karalis, T. K. (2020). Genetically Modified Products, Perspectives and Challenges. cureus, v.12(3).

Efraín Escudero-Leyva, P. A.-V.-A.-A.-M.-M.-V.-R.-R. (2022). Tolerance and Biological Removal of Fungicides by Trichoderma Species Isolated From the Endosphere of Wild Rubiaceae Plants. Frontiers in Agronomy Volume 3, Article 772170.

EFSA. (2004). Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants. The EFSA Journal 48, 1-18.

Elżbieta Wołejko, B. Ł. (2016). The influence of effective microorganisms (EM) and yeast on the degradation of strobilurins and carboxamides in leafy vegetables monitored by LC-MS/MS and health risk assessment. Environ Monit Assess 188: 64.

Elkhouly, A. (2021). Survey of Global Crop Loss. Balance journal - in Applied and Humanities - Issue 2 .

Food and Agriculture Organization(FAO). (kein Datum). Land use statistics and indicators Global, regional and country trends 1990–2019. FAOSTAT Analytical Brief 28.

Frankenhuyzen, K. v. (2009). Insecticidal activity of *Bacillus* thuringiensis crystal proteins. Journal of Invertebrate Pathology, 101, 1–16.

Herring, R. (2013). *Transgenics and the poor : biotechnology in development studies.* Routledge.

J. D. Arthington, S. D. (2003). Effect of transportation and commingling on the acute-phase protein response, growth, and feed intake of newly weaned beef calves. Journal of Animal Science, Volume 81, Issue 5, Pages 1120–1125.

Janette V. Pham, M. A. (2019). A Review of the Microbial Production of Bioactive Natural Products and Biologics. Frontiers in Microbiology Volume 10 | Article 1404.

Jitendra Kumar, A. R. (2021). An Overview of Some Biopesticides and Their Importance in Plant Protection for Commercial Acceptance. Plants, 10,1185.

Jitendra Kumar, A. R. (2021). An Overview of Some Biopesticides and Their Importance in Plant Protection for Commercial Acceptance. Plants, 10,1185.

Jose M. Gonzalez, J. a. (1980). Patterns of plasmid DNA in crystalliferous and acrystalliferous strains of *Bacillus* thuringiensis. Plasmid Volume 3, Issue 1.

Junaid Ahmad Malik, M. R. (2023). *Bioremediation and Phytoremediation Technologies in Sustainable Soil Management, Microbial Degradation of Organophosphorus Pesticides, Degradation of Pesticides and Polychlorinated Biphenyls.* Apple Academic Press, Volume 4.

Kefi, A. (2015). Characterization of endophytic *Bacillus* strains from tomato plants (Lycopersicon esculentum) displaying antifungal activity against *Botrytis cinerea* Pers.

Kimberly M.Parker, V. B. (2019). Environmental Fate of RNA Interference Pesticides: Adsorption and Degradation of Double-Stranded RNA Molecules in Agricultural Soils. Environmental Science Technology. 53, 3027−3036.

Lane, P. (2009). *Innovation in crop production for productivity and biodiversity.*

Luca Ruio, A. S. (2008). Immature house fly (Musca domestica) control in breeding sites with a new Brevibacillus laterosporus formulation.- Environmental Entomology 37: 505-509. Environmental Entomology, Volume 37, Issue 2.

Morris, R. M. (2001). Food safety risk. British Food Journal, Vol. 103 No. 3.

Nahila Anjum, Q. R. (2022). *Microbial Degradation of Organophosphorus Pesticide.* Bioremediation and Phytoremediation Technologies in Sustainable Soil Management, Megh R. Goyal, volume 4.

Nikos Alexandratos and Jelle Bruinsma. (2012). WORLD AGRICULTURE TOWARDS 2030/2050.

Nitschke, M. (2011). *Pesticides in the Modern World - Pesticides Use and Management.*

Oerke. (2006). Crop losses to pests. Agricultural Science, 144, 31–43.

P.J. Jones, I. M. (2017). Assessing the potential economic benefits to farmers from various GM crops becoming available in the European Union by 2025: Results from an expert survey. Agricultural Systems 155 158–167, 159.

Phillips McDougall. (2018). Evolution of the Crop Protection Industry since 1960.

Rowe, G. (2004). How can genetically modified foods be made publicly acceptable? TRENDS in Biotechnology Volume 22, Issue 3.

S.R.Joshi. (2006). *Biopesticides, Biotechnological Approach.* New Age International.

Sabine Grundmann, R. F. (2004). Application of microbial hot spots enhances pesticide degradation in soils. Chemosphere Volume 68, Issue 3.

Schein, Z. a. (1979). Comparative epidemiology. Centre for Agricultural Publishing and Documentation Wageninge.

Schlege, H. G. (2018). General microbiology. Cambridge University Press.

Steier, G. (2017). *Advancing Food Integrity: GMO Regulation, Agroecology, and Urban Agriculture, UK, New York, NY: CRC Press Inc. Tayloy & Francis Group; 2018. 250 pages. ISBN 9781138305250.*

Teng et al. ; Oerke et al. : Oerke . (1987;1994;2006).

Unzue, A. (2022). Multifunctional Properties of a *Bacillus* thuringiensis Strain (BST-122): Beyond the Parasporal Crystal. Toxin, 14,768.

Yanmei Feng, Y. H. (2020). An Overview of strobilurin Fungicide Degradation:Current Status and Future Perspective. Frontiers in Microbiology Volume 11 | Article 389.

Yann Devos, D. R. (2004). Management of herbicide-tolerant oilseed rape in Europe: A case study on minimizing vertical gene flow. Environmental Biosafety Research 3, 135–148.