Institute of Crop Science and Plant Breeding I

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Investigations on the effects of harvest methods and storage conditions on yield, quality and germination of evening primrose (*Oenothera biennis* L.) seeds

Dissertation

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For my wife

Contents

Abbreviations

A	autumn experiment
ABA	abscisic acid
CF	capsule formation
CMS	number of capsules per main stem
CP	number of capsules per pot
CSS	number of capsules per side shoot
D	days
DAI	days after water imbibing
DL	day length
DM	dry matter content
DT	desiccation time
FB	flower beginning
EPO	evening primrose seed oil
FFA	free fatty acid percentage
GA	gibberellic acid
GLA	γ-linolenic acid
LA	linoleic acid
LDL	low density lipoprotein
LSD	least significant difference
M	harvest method
MO	month of storage after harvest
N	nitrogen
OA	oleic acid
PA	palmetic acid
PDM	plant dry matter content
PV	peroxide value
p-value	probability value
T	harvest time
TEM	temperature
TSW	thousand seed weight
TGP	total growing period
S1	spring experiment 2004
S2	spring experiment 2005
SA	stearic acid
SB	shoot beginning
SDM	seed dry matter content
SG	seed germination
SO	seed oil
SP	seed protein
SST	sowing time
ST	storage time
SSP	number of side shoots per pot
SY	seed yield

1. Introduction

The use of medicinal herbs for curing diseases has been documented in the history of all civilizations. A high percentage of the world's population depends on herbs as their primary source of medicines.

Since the diet in developed as well as the developing countries worldwide has changed, many people suffer from deficiency of many vital compounds such as essential amino- and fatty acids. The current decline of breast-feeding, for example, leads to deficiency in gamma linolenic acid (Zahoor Ahmad and Lapinkase 1998). Gamma linolenic acid (GLA) is the first product in the conversion of linoleic acid (LA) to prostaglandins. Before linoleic acid can be fully utilized by the body, it has to be converted into GLA. In individuals suffering from a number of common diseases this conversion can be very slow and may also be blocked by factors such as aging, high cholesterol levels, stress, high alcohol intake and diabetes. Being a polyunsaturated fatty acid, GLA takes over important functions in the human body such as structural constitution of cell membranes, skin permeability, cholesterol movement and precursors for regulatory molecules (Lapinskas 2000).

The evening primrose is one of the medicinal plants with high potential for the production of GLA. In recent years, it has made the transition from being a wild flower and garden plant to an established agricultural crop (Zahoor Ahmad and Lapinkase 1998). Despite the presence of higher levels of GLA in seeds of plants like black current (*Ribes nigrum*, *Rosaceae*), borage (*Borago officinalis*, *Boraginaceae*) and in the oil produced by some species of the fungus *Mucor* (Lapinkase 1993), evening primrose oil appears to have the most biologically active form of GLA (Wolf et al. 1983, Shewry et al. 1997, Barre 2001, Stuchlik and Zak 2001, Peschel et al. 2007). A special composition of fatty acids in triacylglycerol molecules makes the GLA of evening primrose oil easily accessible to hydrolysis by pancreatic lipase in the small intestines (Rahmatullah et al. 1994). The biological activity may be due to the fact that most of the evening primrose GLA is in the form of “Enotherol”, a particular triacylglycerol consisting of two molecules of LA and one molecule GLA on a glycerol backbone (Horrobin 1992). Another reason for evening primrose oil remaining the major source of the GLA sold worldwide may be seen in the low seed productivity of borage and the high costs of GLA extraction from fungus (El Hafid et al. 2002).

Although evening primrose has a good potential to become a commercial agricultural crop for the production of GLA, but some disadvantages, such as indeterminate inflorescence, high seed shattering during ripeness and a long life cycle (biennial plant) could present significant

impediments. Despite all attempts to eliminate the seed-shattering characteristic, it is still a major problem in the production of evening primrose (Simpson and Fieldsend 1993).

Indeterminate plants, especially with high seed shattering, make it difficult for the farmer to determine the best time for harvest. This is due to the fact that new flowers continue to be produced at the top while those at the base are long overripe. If the farmer waits until the plant produces the final capsules, he will not have enough seeds from the lower part of the plant, as they will have been long shattered. Breeding new cultivars with determinate growth behavior and low shattering is one strategy to solve this problem. Until new cultivars become available, the farmer has to adapt his cultivation methods using present cultivars. In order to obtain high seed yields as well as good seed oil quality in evening primrose, it is essential to know exactly when the exact time of harvest would be.

Defoliation is a standard practice in the harvest of many combinable crops (Growley and Fröhlich 1998, Spain and Hodgen 1994, Guerena and Sullivan 2003). Defoliation is used as a means of acceleration of ripening process or removing weeds and other green materials. Seed water content, seed size, seed weight, seed protein, oil and starch content are the most important parameters that are influenced by pre-harvest defoliation (Growley and Fröhlich 1998, Wilson and Smith 2002). The effect of defoliation can be modified by cultivation methods like nitrogen application and harvest time because of their influence on the plant development. Before this study was carried out, there was no information about the interaction of cultivation techniques like harvest time and nitrogen application with pre-harvest defoliation of evening primrose.

Evening primrose is a biennial plant with relatively long life cycle. Recently breeders introduced some new cultivars like “Anothera” having reduced vernalization demand. A cultivar with low vernalization demand gives us the chance sowing in spring. Spring sowing leads to better crop rotation and will help farmers to produce a pre-crop with longer growing period and late harvest time in autumn. Compared with autumn plants, spring plants are characterized by a shorter growing period, which leads to lower cost of production (Fieldsend and Morison 2000 b). Some studies established the seed yield as well as the GLA percentage of autumn and spring evening primrose are similar (Simpson and Fieldsend 1993, Honermeier et al. 2005). Generally, there are not enough published results about the performance of evening primrose in spring.

The susceptibility of evening primrose oil to oxidation has been proven to be due to the presence of high levels of polyunsaturated fatty acids, especially GLA (a fatty acid characterized with three double bounds). The results of previous studies showed that there is a reverse relationship between the concentrations of polyunsaturated fatty acids and oil stability (Ahmadkhan and Shahidi 2000, Morello et al. 2004). Although some studies about the effect of storage time and temperature on oil seed plants exist (Growley and Fröhlich 1998, Ahmadkhan and Shahidi 2000, Morello et al. 2004, Martini et al. 2005), no information about the effect of storage conditions (time and temperature) on the quality of evening primrose seed oil is available as yet.

Evening primrose seeds have poor and erratic germination potential (Nightingale and Baker 1995). In order to ensure a good crop stand evening primrose is usually cultivated by seedling transplantation that increases the costs of its production (Hall et al. 1988). Investigations with some other plants showed that harvest techniques such as harvest time and pre-harvest defoliation influence the seed germination capability (Bennett and Shaw 1998, Samarah and Abou-Zanat 2005). Since harvest time and pre-harvest defoliation have influence on seed maturity, it seems that seed germination potential of evening primrose is affected by different methods of harvest.

Evening primrose (*Oenothera biennis* L.) is a relatively new and highly valued oil seed crop. Little information about evening primrose and the problems that were mentioned above are the basis for the fallowing hypotheses:

- Defoliation of the plants affects seed yield and seed quality of evening primrose.
- The effect of defoliation depends on harvest time and nitrogen fertilization.
- The performance of spring evening primrose is equal to those sown in autumn.
- Storage time and temperature influence the quality of evening primrose seeds.
- Harvest conditions of the plants and storage conditions of the seeds affect the seed germination.

2. Review of literature

2.1 History

Oenothera biennis L., commonly called evening primrose, is a member of evening primrose family (Onagraceae). The generic name of *Oenothera* is derived from *oinos* (wine) and *thera* (a hunt), and is an old Greek name given by Theophrastus to some plant probably an Epilobium, the roots of which were eaten to provoke a relish for wine, as olives are now; others say it dispelled the effects of wine. Evening primrose originated in Mexico and Central America around 70000 years ago. This plant has a long history as a medicinal plant (Hall et al. 1988). In nature, evening primrose acts as a primary coloniser (Lapinkase 1982). This means that it tends to be found in poorer environments such as dunes, roadsides and railway embankments. It often occurs as a casual, eventually being out-competed by other species (Deng et al. 2001).

Evening primrose has always been considered only as a wild flower until 1970 when research on the species *Oenothera biennis* began in Germany, England and the Netherlands paving the way for its domestication (Zahoor Ahmad and Lapinkase, 1998). Since then research in several countries has produced improved varieties and cultivation techniques that have substantially improved yield, quality and reliability (Lapinkase 1997). Production of seed was started in western countries, but seed production for export began in China in 1980 (Deng et al. 2001). Cultivation as a crop started in earnest in 1986 and in 1993 China had become the world's dominant supplier and now probably accounts for around ninety percent of world evening primrose seed production (Lapinkase 1997). Recently *Oenothera biennis* is commercially cultivated in over 15 countries for its oil (Kemper 1999).

2.2 Botany

The Onagraceae family is a cosmopolitan family whose members are most abundant in temperate areas especially in North America and Mexico (Punt et al. 2003). The family consists of 18 genera and about 640 species. Evening primrose (*Oenothera biennis* L.) has n = 7 chromosomes. Taxonomists disagree strongly on the classification of the genus *Oenothera*, and the number of recognized species (Levin et al. 1972). All cultivars in agricultural production are drawn from the subsection Euoenothera and for this reason agricultural evening primrose is described as *Oenothera biennis* (Fieldsend 1996).

Although *Oenothera* has self-pollination with clystogamic mechanism, the progeny fails to appear in a homozygous state because of a system of saprophytic and gametophytic lethals

(Levin et al. 1972). Otherwise breeding of evening primrose is especially difficult since the plant is, in fact, a true breeding F_1 hybrid (Dodd and Scarisbrick 1989).

Evening primrose is a biennial, herbaceous plant. The plant is called evening primrose because the flowers are partially close during the day and open in the evening (Duncan et al. 1975). The bright yellow to gold corolla is 2-5 cm wide, with four sepals, four petals, eight stamens and one stigma that is divided in to four parts. The erect stem, which branches near the top, can be covered with hairs.

The height of the plant normally varies from 1.0 to 1.5 m but can reach 1.75 m. Basal leaves that form a rosette are about 10-30 cm long. The stem has alternate, lanceolate-shaped leaves, 2.5-5 cm long, that are shallowly toothed and waved at the edge and the leaves are usually hairy (Martin et al. 1951). When the plant is flowering, flowers open at the rate of 2-4 per day, and there is a zone of flowering which gradually moves up the stem as the season progresses. Each flower develops into a capsule (pod) containing about 200-650 seeds. Since flowering can last for two to three months (Indeterminate inflorescence), this means that at one time plant has all stages of development from small buds, through flowers, to mature capsules.

In nature, its life cycle needs two growing seasons (Lapinkase 1982). Depending on growing region and climatic condition there are two sowing times for evening primrose, one in autumn and the next in spring. In china dependent on location, the seeds are sown between middle of March and middle of April in spring and middle of August and middle of September in autumn (Deng et al. 2001). In a study with evening primrose in Germany, Honermeier et al. (2005) showed that end of July till middle of August is the recommended seed sowing time for autumn evening primrose. They also indicated that during the middle to the end of March the best seed sowing time for spring evening primrose would be achieved. According to Honermeier et al. (2005), dependent on sowing-time the seedlings emerge after 19 to 40 days and 5-15 days in spring and autumn, respectively. Although evening primrose flowers from June to September, those sown in autumn have proven to flower earlier than sown in spring (Dodd and Scarisbrick 1989). In China, if spring evening primrose is sown after the middle of May mostly become rosettes (Deng et al. 2001) and neither bolt nor flower during that year. In Germany dependent on sowing time total growth period of spring-sown plant is in the range of 160-200 days. Contrary to that, in autumn-sown plants increases to 390-400 days (Honermeier et al. 2005). Compared to the autumn crop the spring-sown evening primrose more readily fits into the crop rotation and input costs are lower (Fieldsend and Morison 2000 a).

2.3 Uses of evening primrose

2.3.1 Evening primrose as a garden flower

Oenothera biennis L. as other genera of Onagraceae family (Clarkia and Fuchsia) because of its golden yellow flower can be used as a garden flower (as it is very common in Germany). From a horticultural point of view, the species *O. biennis*, *O. grandiflora* and *O. lamarkiana* should always be preferred to the ordinary kind, as the flowers are larger and of a finer color, having a fine effect in large masses, and being well suited for the garden (Grieve 1984). Although in *Oenothera* genus there are many species that are specially bred as an ornamental plant i.e. *O. biennis*, *O. kunthiana, O. speciosa, O. versicolor* and *O. pallida.* Present day encyclopaedia of garden plant lists several species of evening primrose and provide device on cultivation and propagation (Fieldsend 1996).

2.3.2 Traditional uses

Since early times, several native American tribes have used common evening primrose for both food and for medicine purposes (Anderson 2001). Evening primrose root has been used as a vegetable with a peppery flavor (McPherson 1977). The roots were boiled and eaten like potatoes and salsify (Grieve 1984). The bark and the leaves of evening primrose are astringent and sedative. It was considered to be effective in healing asthmatic coughs, gastro-intestinal disorders, and whooping cough (Grieve 1984). The plant was used to treat pain associated with menstruation as well as bowel pain. One of the common names for *Oenothera* "King's cure-all" reflects the wide range of healing powers ascribed to this plant (Anderson 2001). The oil from the seed is added to skin preparations and cosmetics. It is often combined with vitamin E to prevent oxidation (Bown 1995). A yellow dye is obtained from the flowers. A finely ground powder made from the flowering stems is used cosmetically in facemasks to counteract reddened skins. The seeds of *Oenothera biennis* appear to be a good food source for birds.

2.4 Characterization of evening primrose oil

The oil content of evening primrose seeds varies with factors such as cultivar, growing conditions and the age of seed from 20 % to 30 %. The oil consists of 98 % triacylglycerols, with small amount of other lipids (free fatty acids, diacylglycerols and phospholipids) and about 1-2 % unsaponifiable matter, of which sterols and tocopherols are of some importance (Christie 1999). The sterol fraction, as determined by GC, is comprised of 90 % β-sitosterol.

With the reminder being 4- methyl sterols included, citrastadienol 5 %, obtusifoliol 1 %, and gramisterol 1.5 %. γ-tocopherol (18 μ/g of oil) and α-tocopherol (76 μ/g of oil) are the natural tocopherol components of evening primrose seed oil (Hudson 1984). δ-tocopherol (15 μ/g oil) also was found in evening primrose oil (Christie 1999).

Table 2-1. Percentage of main fatty acids of evening primrose (*Oenothera biennis* L.) seed oil (Court et al. 1993)

Fatty acid	short formula	%
Palmitic acid	(C 16:0)	7.0 - 9.0
Stearic acid	(C 18:0)	1.5 - 3.0
Oleic acid	(C 18:1)	8.0 - 12.0
Linoleic acid	(C 18:2)	70.0 - 74.0
γ-linolenic acid	(C 18:3)	7.0 - 10.0

The main fatty acid components of evening primrose oil are palmitic acid, stearic acid, oleic acid, linoleic acid and γ-linolenic acid. They are normally present in the relative proportion listed in Table 2-1. According to Court et al. (1993) a number of minor components including myristic acid (C14:0), palmitioleic acid (C16: 1), vaccenic acid (18:1(n-7)), linolenic acid, (C18: 3(n-3)) and ecosanoic acid (C20:0) at the low levels are presented in the oil of evening primrose. The seed of evening primrose contains 15 % protein, 43 % cellulose + lignin, 20-30 % oil, minerals such as potassium, calcium, phosphorus, magnesium and vitamins A, B, C and E also were detected in evening primrose seed (Anthony et al. 1993).

2.4.1 γ-linolenic acid

Fat is made up of carbon-based molecules known as fatty acids and glycerin. Fatty acids are categorized as saturated and unsaturated according to the presence of double bond in their carbon chain. Whereas saturated fatty acids have no double bonds and monounsaturated fatty acids have one, polyunsaturated fatty acids have multiple double bonds in their carbon chain. γ-linolenic acid (GLA) is a long-chain polyunsaturated fatty acid with 18 carbon atoms and three double bounds in the molecule.

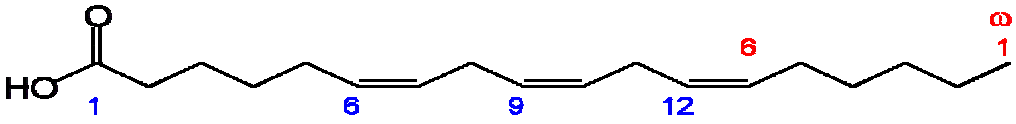


Fig 2-1. γ- linolenic acid structure

Generally, there are two different ways of defining the position of double bond in GLA. First Δ-6 which is based on the first double bond from the carboxyl end (in Fig 2-1 from left to right) and the next is ω-6 which is based on the first double bond from the ω carbon of fatty acid (ω is the last carbon of the chain).

In mammals, GLA is synthesized from dietary linoleic acid (18:2 n-6) by a desaturation reaction catalyzed by the enzyme delta 6-desaturase (Huang et al. 2001). GLA is in turn converted to dihomo-gamma linolenic acid (DGLA, or 20:3 n-6) by elongation and then into a range of other fatty acids, prostaglandins and leukotrienes (Fig 2-2) that have wide-ranging regulatory functions, especially in the mediation of inflammation. GLA therefore has an important function as a precursor in the synthesis of these molecules (Horrobin 1990).

The delta-6-desaturase reaction is the rate-limiting step in the conversion of linolenic acid to long-chain omega-6 fatty acids and is inhibited by a number of factors including excessive alcohol consumption, high intakes of other types of fatty acids, stress, aging, high cholesterol levels, diabetes, smoking and deficiencies in zinc, calcium, magnesium, Vitamin C and B, and other minerals (Horrobin 1992).

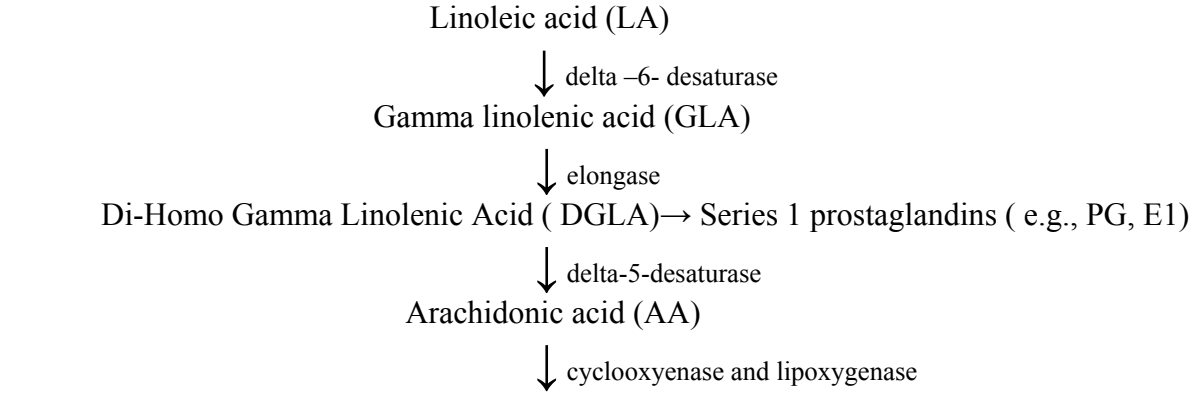


Fig 2-2. Metabolism of γ-linolenic acid (modified from Kember et al. 1999)

2.5 Evening primrose oil and human health

Many studies have demonstrated that evening primrose oil (EPO) has beneficial therapeutic effects on atopic eczema (Wright and Burton 1982, Fiocchi et al. 1994, Yoon et al. 2002). In contrast to some steroidal drugs that are used for eczema, evening primrose oil does not cause side effects (Horrobin and Stewart 1990). Clinical studies showed that EPO has positive effect on rheumatoid arthritis (Leventhal et al. 1993, DeLuca et al. 1995).

Excessive low-density lipoprotein (LDL) cholesterol is major risk factor for atherosclerosis that causes cardiovascular disease. Several studies showed that people with cardiovascular

disease may have a reduced ability to convert linolenic acid to γ-linolenic acid and its metabolism (Horrobin 1993). EPO has been shown to reduce the level of harmful LDL cholesterol in the blood of animals (Sugano et al. 1986).

A number of clinical studies have demonstrated that GLA supplementation with evening primrose oil reduces the symptoms of premenstrual syndrome (Puolakka et al. 1985, Budeiri et al. 1996). In addition, results from clinical studies showed that evening primrose oil has positive effect on some diseases such as breast pain (Pye et al. 1985, Cheung 1999), alcohol-related disorders (Horrobin 1987), psoriatic arthritis (Veale et al. 1994), ulcers (Al-Shabanah 1997), cancer (Menendez et al. 2001) and migrine (Wagner and Nootbaar-Wagner 1997).

Short-term side effects of supplementation with evening primrose oil may include loose stools and minor stomach complications (belching, abdominal bloating). Consuming evening primrose oil with food may minimize side effects (De Luca et al. 1995). An optimal dose and duration of treatment for evening primrose oil has not been established. Manufacturers usually recommend a dose of 3 g evening primrose oil per day (2 ×500 mg capsules or 1 ×1000 mg capsule 3 times per day), providing approximately 240-560 mg of GLA (Fan et al. 1998).

Presently there is a widespread healthfood market for the evening primrose oil, and two pharmaceutical products based on the EPO (Epogam® for atopic eczema and Efamast® for mastalgia) have been researched, licensed and marketed (Lapinskas 2000). Generally, due to the fact that there is not enough evidence to support the use of evening primrose oil for human health conditions, more investigations are necessary (Shahidi and Miraliakbari 2006).

2.6 Breeding of evening primrose

Cytogenetic analysis of the population of *Oenothera biennis* showed that the species has arisen through the hybridization of *O. villosa* and *O. grandiflora* (Cleland 1985). The breeding of evening primrose to develop both better cultivar and improved husbandry techniques started since 1970 (Fieldsend 1996). As previously mentioned, even though evening primrose is normally self-pollinated, due to special genetic characters (some saprophytic and gametophytic incompatibility) the progeny of self-pollination is not identical to the parents (Levin et al. 1972). Because of that, the breeding of evening primrose is difficult (Dodd and Scarisbrick 1989). Using the seeds is the best way for evening primrose propagation.

Scientists have screened over 2000 different races of evening primrose. Several improved cultivars, with names such as Cossack, Constable, Juno, Orino, Merlin, Starfire and Anothera have been produced using selection and intercrossing methods (Fieldsend 1996).

2.7 Cultivation of evening primrose

Evening primrose will thrive in almost any soil or situation, being perfectly hardy. It flourishes best in good sandy soil and in a warm sunny position (Fieldsend and Morison 2000). Soil structure from sandy clays to sand is the best for evening primrose (Granic 1988). Although evening primrose can grow in the most conditions, rich soil and a minimum of 400 mm precipitation per year enhance its seed yield and especially seed oil content. It is highly sensitive to soil acidity and pH lower than 6.0 strongly inhibited its growth (Granic 1988).

In China, the row spacing used is in the range of 33 - 60 cm. Under dry conditions the drill is irrigated before sowing and the seed covered with soil to the depth of 0.4-0.5 cm (Deng et al. 2001). According to Granic (1988) 8°C is minimum soil temperature for seed germination in spring sown-evening primrose. Under low soil temperature, plant development generally slow down and maturity will be late (Fieldsend and Morison 2000). Early autumn and early spring are the best sowing times for winter and spring evening primrose, respectively (Honermeier et al. 2005). The sowing rate used is between 3.0 and 9.5 kg ha^{-1} which is dependent on winter or spring cultivation. The TSW is 0.3-0.5 g, and the optimum field germination percentage is 80 – 98 %.

In China before sowing, the seeds are normally soaked in water at 40°-50°C for 24-48 h, or put into oven at 25°C to hasten germination (Deng et al. 2001). Dependent on location, the plants are thinned 2 to 3 times in June to give a density of 75000–210000 plants/ha. Densities that are too high result in over-tall crops with weak stems that are prone to disease and lodging. Normally the plants are harvested when two-thirds of capsules have become ripe, and stacked in sheaves for threshing. After threshing (by hand, or mechanically), the seeds are dried in the sun and stored in a dry well-ventilated warm house (Deng et al. 2001).

Several studies with evening primrose showed that there exists an interaction between seed yield, seed oil content and plant growth conditions (Reiner et al. 1988, Yaniv et al. 1989, Court et al. 1993, Levy et al. 1993, Fieldsend 1996, Fieldsend and Morison 2000 a and b, Simpson and Fieldsend 1993). As well as other oil seed crops, the degree of desaturation of the fatty acid composition of the evening primrose seed oil is inversely related to temperature prevailing during seed maturation (Levy et al. 1993, Sekeroglu and Özgüven 2006).

Harvesting is a critical operation especially in plants with indeterminate inflorescence and high seed shattering during ripeness. Results showed that *Oenothera biennis*, which contains 75 % brown pods, produced the highest seed yield. After this point in time little additional

yield will be obtain and plants become prone to seed losses by shattering and bird damage (Simpson and Fieldsend 1993).

In a study with evening primrose, it has been showed that the seed oil content and γ-linolenic acid percentage were not influenced by harvest times (Simpson and Fieldsend 1993).

Desiccation is a method used for crops stands with heterogeneous ripeness and high weed density also for accelerating maturity. It can be a way of limiting seed losses and improving seed quality (Bowerman 1984). Some results showed that chemical desiccation did not significantly influence seed yield and fatty acid composition of rapeseed (Kimber and Mac Gregor 1995) and cuphea (Johnson et al. 2005). In a field experiment, Simpson and Fieldsend (1993) showed that harvest method as a cultivation technique has significant effect on seed yield and oil percentage of *Oenothera biennis*.

Little information about the effect of fertilizer on *Oenothera biennis* has been presented. Reiner et al. (1988) and Granic (1988) in a field experiment showed that 80 kg of N/ha at the early time of plant growth period has increased seed yield of evening primrose. Recently, Sekeroglu and Özgüven (2006) demonstrated that when compared with 60 and 180 kg of N/ha the plants, which received 120 kg of N/ha, produced the highest seed yield. The oil accumulation of *Oenothera biennis* was significantly influenced by nitrogen (Reiner et al. 1988, Sekeroglu and Özgüven 2006). Sekeroglu and Özgüven (2006) showed that in comparison with control and the plants that received 60 and 120 kg of N/ha, plants that were supplied with 180 kg of N/ha produced the lowest seed oil percentage.

Weed control

As evening primrose is a wild plant it has good competition against weed, for reducing weed competition and obtaining high seed yield in commercial productions the crop is weeded two to four times during the growing season by hand hoeing (Deng et al. 2001). Investigations showed that evening primrose has susceptiblity to dicamba, 2,4-D mixture (Stringer et al. 1985).

A result of an experiment on the activity of some 25 herbicides on *Oenothera biennis* indicates that isoxaben at 0.075 kg/ha, trifluralin and tri-allate at 1.0 kg/ha were non-toxic. Krusche (2006) demonstrated that Basta (Glufosinate 3.0l/ha) is the best herbicide for annual monocotyledon and dicotyledonous weeds of evening primrose in Germany. Target super (Quizalofop-p 1.25 l/ha) is used for annual monocotyledon weeds except blue grass (*Poa annua* L.).

Pests and diseases

Evening primrose has good resistance to drought stress and pest. On the other hand, wet and hot conditions in the rain season, long-term accumulation of water may increase root rot and leaf blight diseases in evening primrose (Deng et al., 2001). Sometimes insect pests such as *Agrotis ypsilon*, *Acherontia lachesis, Cryptothela pryeri* and *Gryllotalpa unispina* (Deng et al. 2001), *Aphis oenothera, Pipolia japonica* and *Macrosiphum gaurae* (Hall et al. 1988) appear on evening primrose. The following fungi *Erysiphe chicoracearum, E. polygoni, Gnomonia misella, Micosphearella tassiana, Pronospora arthuri, Pleospora herbarum, Puccinia diocia, P. oenothera* and *Septoria oenothera* were observed on evening primrose (Hall et al. 1988). Although some pests and diseases were identified on evening primrose, no serious diseases or pests were reported (Deng et al. 2001).

3. Materials and Methods

Table 3-1 gives an overview of the experiments carried out with evening primrose in this project.

Table 3-1. Overview of the executed experiments

Experiment	Year	Treatment
Field experiments		
1. spring 2004	2004	Harvest time and harvest method
2. autumn 2004	2004 - 2005	Harvest time and harvest method
3. spring 2005	2005	Harvest time and harvest method
Pot experiments		
1. spring 2004	2004	Nitrogen, harvest time and harvest method
2. autumn 2004	2004 - 2005	Nitrogen, harvest time and harvest method
3. spring 2005	2005	Nitrogen, harvest time and harvest method
Post harvest experiment	2005	Storage time and storage temperature
Seed germination		
1. experiment 1	2004	Temperature and photoperiodic conditions
2. experiment 2	2005	Storage time and storage temperature
3. experiment 3	2005	Seed age

3.1 Field experiments

3.1.1 Soil and climate conditions

During the years 2004 and 2005, three field experiments were conducted with *Oenothera biennis* L. cv. "Anothera" at the research station of Groß-Gerau (Latitude 49° 45 N: longitude 8° 29E) near Frankfurt/Main. "Anothera" is a cultivar, which was bred by the breeding company Pharma Plant GmbH Arten. The soil was sandy with low clay content (< 5 %, 0-60 cm) and had a pH value of 6.0- 6.5. Groß-Gerau is a dry location due to the prevalent sandy soil as well as relatively low precipitation. For good seed germination, irrigation was done during experiments. Based on soil analysis (Table 3-2), P and K fertilizations were carried out during soil preparation (Table 3-3). The mineral nitrogen of soil (0-90 cm) was determined using N_{min} method (Hoffmann 1991). The available P and K of soil (0-30 cm) were determined according to CAL method (Hoffman 1991). The available Mg of the soil (0-30 cm) was measured using 0.0125 M $CaCl_2$ extraction solution (Hoffmann 1991). The available B of the soil (0-30 cm) was determined using photometric method (Hoffman 1991).

Table 3-2. Soil characterization in Groß-Gerau 2004-2005

Characteristics	2004	2005	Depth
pH	6.6	6.1	-
P (mg/100g) [1)]	11	10	0-30 cm
K (mg/100g) [1)]	7	6	0-30 cm
Mg (mg/100g) [2)]	1.3	1.7	0-30 cm
B (mg/100g) [3)]	0.2	0.3	0-30 cm
N-min (kg/ha) [4)]	20	34	0-90 cm

1) CAL method, 2) 0.0125 M $CaCl_2$ extraction solution method, 3) Photometric method, 4) N_{min} method

Table 3-3. NPK fertilizations in field experiments, Groß-Gerau 2004-2005

Experiment	kg/ha	application time	date	fertilizer
Spring 2004				
N	30	after germination	25.04.04	[NH_4NO_3, $CaCO_3$]
P	31	soil preparing time	20.02.04	Thomaskali®
K	115	soil preparing time	20.02.04	Thomaskali®
Autumn 2004				
N_1	30	after germination	17.03.05	[NH_4NO_3, $CaCO_3$]
N_2	31	shoot beginning	12.05.05	[NH_4NO_3, $CaCO_3$]
P	24	soil preparing time	16.08.04	Thomaskali®
K	90	soil preparing time	16.08.04	Thomaskali®
Spring 2005				
N	30	after germination	31.05.05	[NH_4NO_3, $CaCO_3$]
P	31	soil preparing time	26.01.05	Thomaskali®
K	115	soil preparing time	26.01.05	Thomaskali®

*In autumn experiment N fertilizer (KAS) was used after germination

3.1.2 Weather conditions

Means of air temperature and precipitation during the experiments are presented in Figs 3-1 and 3-2, respectively. The temperature sums were calculated by adding daily air temperatures above 5°C (Table 3-4). In 2004, August was the wettest month and corresponded with seed ripening and harvest time. Contrary to the year 2004, the highest precipitation occurred during the seedling growing period and rosette stage of evening primrose in April of 2005 (Fig 3-2). Generally, compared with 2004 the plants in spring experiment 2005 received higher amount of precipitation during their vegetative phases.

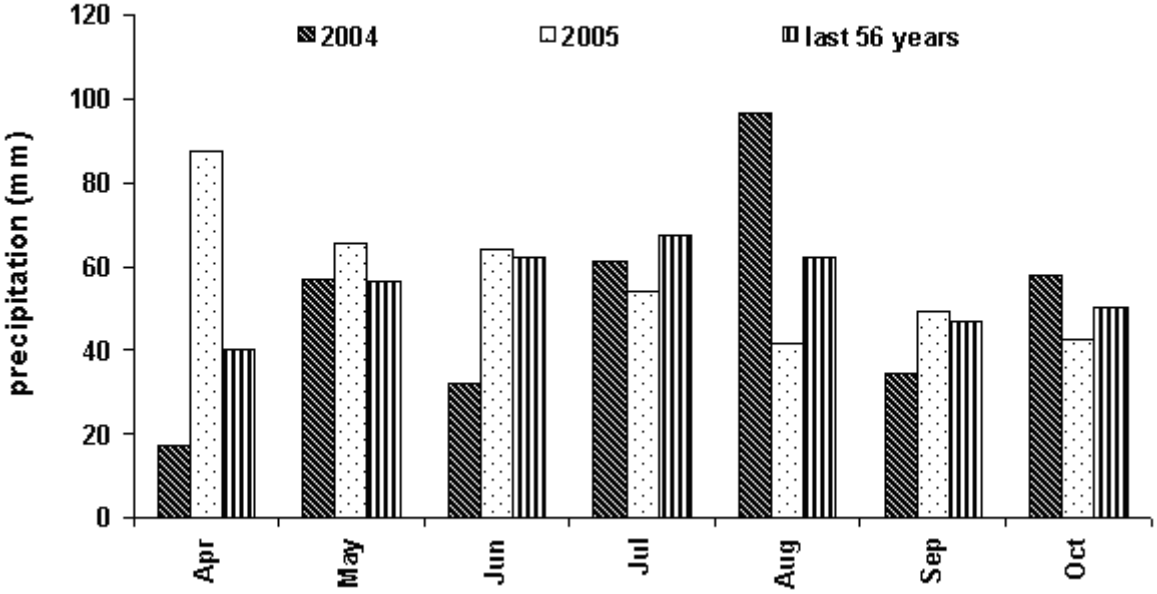


Fig 3-1. Precipitation (mm) per month, Gross-Gerau 2004- 2005

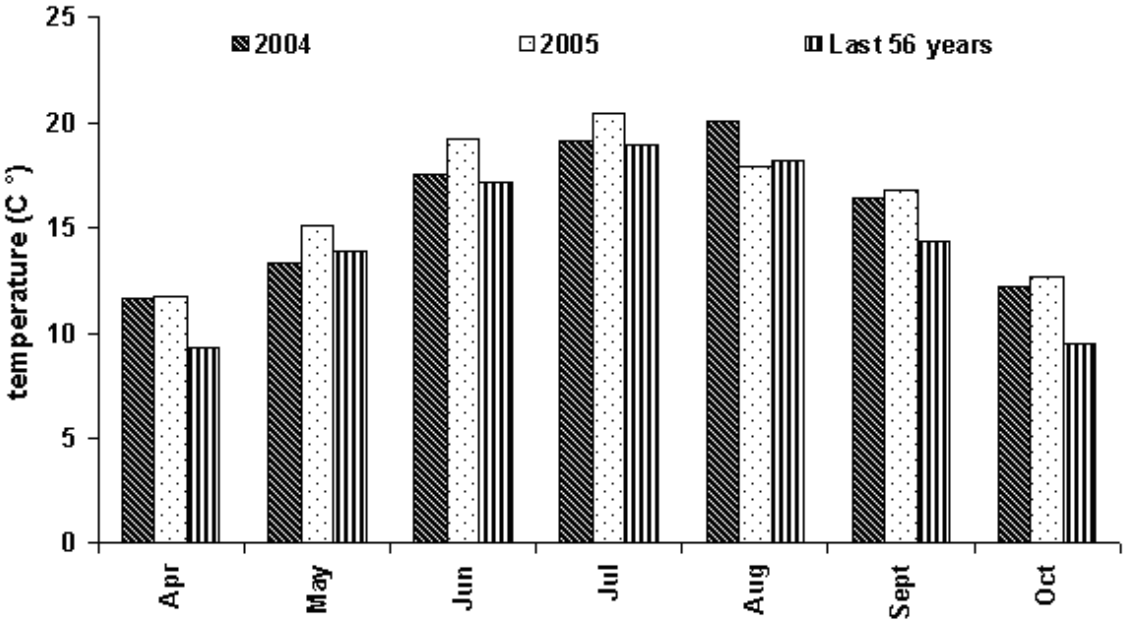


Fig 3-2. Average of air temperature (°C) per month, Gross-Gerau 2004-2005

Table 3-4. Temperature sums during the growing stages of *O. biennis* (Temperature sums > 5°C), field experiments in spring 2004 (S1), autumn 2004 (A) and spring 2005 (S2), respectively

Experiment	S1 (2004)			A (2004/2005)			S2 (2005)		
Growth periods	Early	Middle	Late	Early	Middle	Late	Early	Middle	Late
ST- SG	198	198	198	260	260	260	299	299	299
SG- SBT	445	445	445	882	882	882	295	295	295
SBT-FB	411	411	411	475	475	475	427	427	427
FB-T	1035	1191	1260	1157	1409	1524	1051	1556	1556
Total °C	2089	2245	2314	2774	3026	3141	2072	2577	2577

Early = early harvest time, Middle = middle harvest time, Late = late harvest time, ST = sowing time, SG = seed germination time, SBT = shoot beginning time, FB = flower beginning, T = harvest time

Table 3-5. Average of precipitation (mm) during field experiments, field experiment in spring 2004 (S1), autumn 2004 (A) and spring 2005 (S2), respectively

Experiment	S1 (2004)			A (2004/2005)			S2 (2005)		
Growth periods	Early	Middle	Late	Early	Middle	Late	Early	Middle	Late
ST- SG	21	21	21	65	65	65	66	66	66
SG- SBT	66	66	66	338	338	338	60	60	60
SBT-FB	56	56	56	47	47	47	76	76	76
FB-T	131	173	189	147	173	192	149	158	158
Total (mm)	247	316	332	647	673	692	351	360	360

Early = early harvest time, Middle = middle harvest time, Late = late harvest time, ST = sowing time, SG = seed germination time, SBT = shoot beginning time, FB = flower beginning, T = harvest time

As is presented in Figure 3-2, the year 2005 was warmer than 2004 and the last 56 years. In year 2004, plants received the highest temperature during August (20.1°C). In contrast to that, July was the hottest (20.4 °C) month in the growing period in 2005. The total temperature and precipitation in 2005 was higher than that of year 2004 (Table 4-6).

Table 4-6. Total temperature and precipitation, Groß-Gerau 2004 and 2005

	Temperature (°C)	Precipitation (mm)	
2004	2457.7	503.7	Jan.-Dec.
2005	2518.6	572.4	Jan.-Dec.

3.1.3 Experimental design

All the experiments were randomized in a complete block design with four replications. The dimension of each plot was 7 × 1.5 m and consisted of eight rows with a row spacing of 18.7 cm. Seeds were sown at a rate of 6 kg/ha. As presented in Table 3-3 in spring experiments based on soil basal fertilizer 30 kg/ha N (KAS [NH_4NO_3, CaCO3]) was applied after germination and during the vegetative growth period.

In autumn experiment, nitrogen was applied twice (Table 3-3). The application of phosphorous and potassium were based on the results of soil analysis. Weed control was conducted manually by hacking and with licensed herbicide (Treflan 1.5 l/ha) that was used as a preemergence herbicide. Under dry conditions, irrigation was done to stimulate seed germination and vegetative growth of the plants. Three harvest times were conducted: early harvest time when all capsules were still green, middle harvest time when ⅓ of capsules were brown and late harvest time with more than ⅔ ripened capsules and three harvest methods: conventional harvest, defoliation by chemicals and defoliation by flame.

For chemical defoliation over-ground parts of plants were sprayed with herbicide Reglone (Diquat 1.5 l/ha). Flame defoliation was carried out using Propane gas. In this treatment, the basal part of plants was directly treated with flame.

In all trials, depending on weather conditions experimental plants were harvested 14-20 days after defoliation. The plants were harvested with a plot harvesting machine (type Wintersteiger Classic). To be able to investigate the effects of these factors on seed yield and oil quality both in autumn and spring-sown evening primrose as well as to investigate the performance of spring evening primrose, one experiment was conducted in autumn and two in spring (Table 3-7).

3.1.4 Measurements

Seed yield (seed yield was calculated on the basis of 91 % DM), seed dry matter, plant dry matter and thousand seed weight (91% DM) were measured before laboratory analysis. The thousand seed weight (TSW) was measured by hand using two samples of 500 seeds from each plot. In the laboratory, the oil percentage was determined according to the method of Soxhlet (Court et al. 1993, Christie 1999). The fatty acid composition was analyzed by Varian CP 3800 gas chromatography with a modified method of Court et al. (1993). CHNS elemental analyzer EA1110 measured seed N percentage and the percentage of raw protein was calculated by using protein conversion factor of 6.25 (the methods will be described in chapter 3.4).

Table 3-7. Development stages (dates) of evening primrose, Gross-Gerau 2004-2005

Experiment	ST	SG	SB	FB	DT	T
Spring 2004						
Early	05.04.04	03.05.04	16.06.04	18.07.04	02.09.04	30.09.04
Middle	05.04.04	03.05.04	16.06.04	18.07.04	22.09.04	21.10.04
Late	05.04.04	03.05.04	16.06.04	18.07.04	11.10.04	01.11.04
Autumn 2004						
Early	18.08.04	06.09.04	28.04.05	13.06.05	04.08.05	29.08.05
Middle	18.08.04	06.09.04	28.04.05	13.06.05	26.08.05	14.09.05
Late	18.08.04	06.09.04	28.04.05	13.06.05	02.08.05	27.09.05
Spring 2005						
Early	03.05.05	31.05.05	23.06.05	18.07.05	09.09.05	11.10.05
Middle	03.05.05	31.05.05	23.06.05	18.07.05	04.10.05	31.10.05
Late	03.05.05	31.05.05	23.06.05	18.07.05	17.10.05	31.10.05

ST= sowing time, SG = seed germination, SB = shoot beginning, FB = flower beginning, DT = desiccation time, T = harvest time

3.2 Pot experiments

3.2.1 Soil and climate conditions

During the years 2004 and 2005; three experiments were conducted with *Oenothera biennis* L. cv. "Anothera" at the research station of Justus Liebig University in Rauischholzhausen. For the execution of pot experiments, 72 and 162 small (6.2/l) Mitscherlich pots were prepared for 2004 and 2005 experiments, respectively. The pots were filled with 6 kg soil that consisted of two parts sand and one part local soil (4000 g sand: 2000 g local soil).

During the soil preparation, the minerals such as Phosphorus (0.1 g P/pot) Potassium (0.8 g K/pot) and $CaCO_3$ (0.3 g $CaCO_3$/pot) were inserted to the soil as basal fertilizers. Means of temperature and precipitation during experiments are presented in Figures 3-3 and 3-4, respectively. The highest precipitation was appeared in May in both experimental years. In the aspect of rainfall, 2005 was wetter than 2004. As is presented in Figure 3-4, the year 2005 was warmer than 2004 and the last 56 years. In the year of 2004 as well as 2005, plants received the highest temperature during August.

3.2.2 Maximum soil water-holding capacity

For obtaining the maximum soil water-holding capacity of a pot, water was applied to the pots (containing two parts sand and one part local soil) till saturation. After that, the pots were covered to prevent evaporation and were allowed water percolation for 1day (24 h). Then the weight of pots were determined and the maximum soil water–holding capacity (1423 ml) was calculated by reducing the weight of pot under maximum soil water-holding capacity (8438 g) and the weight of pot (included soil) under dry conditions (7015 g).

3.2.3 Experimental design

Pot experiments were randomized in split plot designs with nitrogen being the main factor and with harvest time and harvest method as the sub main and sub-sub main factors, respectively. The number of replications was six. The description of pot experiments is presented in Table 3-8.

Table 3-8. Treatments in pot experiments with evening primrose, Rauischholzhausen 2004-2005

		2004 (spring)	2005 (autumn)	2005 (spring)
Nitrogen	1	0.3 g N/pot	0.5 g N/pot	0.5 g N/pot
	2	0.6 g N/pot	1.0 g N/pot	1.0 g N/pot
	3	-	2.0 g N/pot	2.0 g N/pot
Harvest time	1	capsules are still green	capsules are still green	
	2	two weeks after (1)	two weeks after (1)	
	3	two weeks after (2)	two weeks after (2)	
Harvest method	1	conventional harvest	drying capsules at 40 °C	
	2	defoliation with herbicide	defoliation with herbicide	
	3	-*	cutting and natural drying	
Pot water supply		75 %	75 %	
Plant density		3 plants/pot	3 plants/pot	

* Two harvest methods

In the 2004 spring experiment, the pots were treated with two amounts of nitrogen (0.3 and 0.6 g of N/pot), and the crops were harvested at three times. During the first harvest, all the capsules were still green. The second harvest then followed two weeks later and the third harvest followed the another two weeks later. Harvest method was divided into two forms, a) conventional harvest and b) defoliation with herbicide.

Because of the significant effect of nitrogen application on most measured parameters, the level of nitrogen was increased in the following. In addition, the method of harvest in next experiments has been changed. The second and the third experiments were designed with three different levels of nitrogen (0.5 g, 1 g and 2 g of N/pot), three harvest times and three harvest methods with a) separating the capsules from plant and drying under 40 °C, b) defoliation before harvest (chemical defoliation) and c) conventional harvest with accelerating the dryness by hanging.

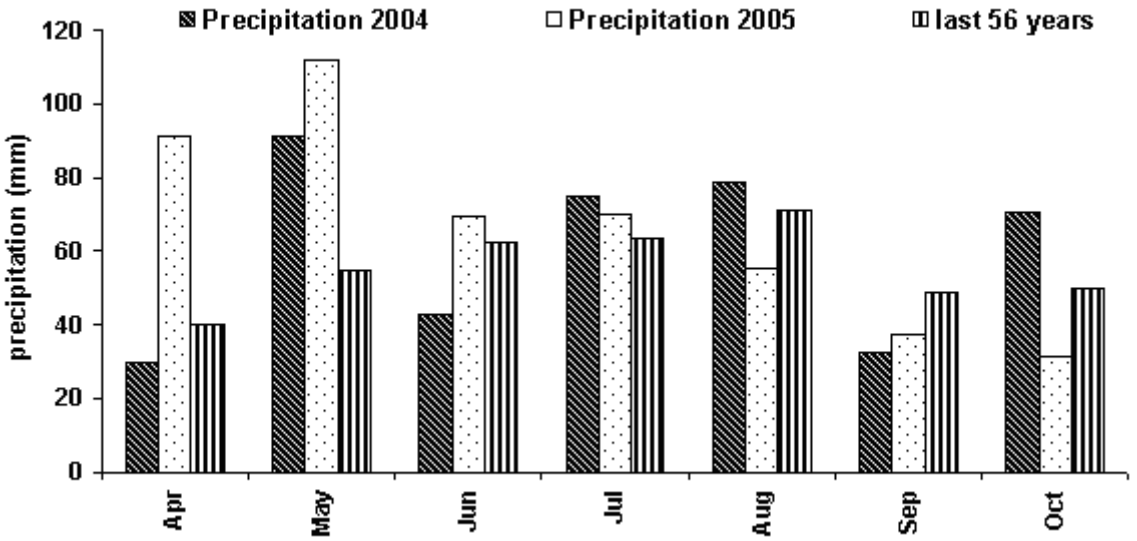


Fig 3-4. Precipitation (mm) per month, Rauischholzhausen 2004-2005

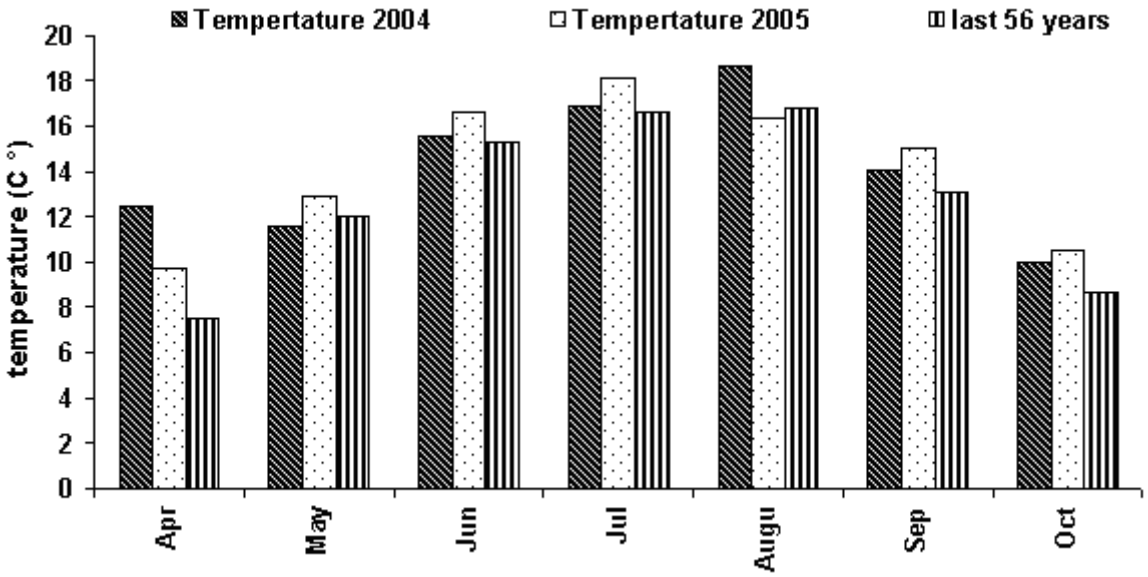


Fig 3-5. Average of air temperature (°C) per month, Rauischholzhausen 2004-2005

Table 3-9. Development stages (dates) of evening primrose in pot experiments, Rauischholzhausen 2004-2005

Experiment	ST	SG	SB	FB	CF	T
Spring 2004						
1st harvest	21.04.04	30.04.04	14.06.04	23.07.04	10.09.04	16.09.04
2nd harvest	21.04.04	30.04.04	14.06.04	23.07.04	10.09.04	30.09.04
3rd harvest	21.04.04	30.04.04	14.06.04	23.07.04	10.09.04	14.10.04
Autumn 2004						
1st harvest	23.09.04	04.10.04	18.03.05	30.06.05	08.08.05	16.08.05
2nd harvest	23.09.04	04.10.04	18.03.05	30.06.05	08.08.05	30.08.05
3rd harvest	23.09.04	04.10.04	18.03.05	30.06.05	08.08.05	13.09.05
Spring 2005						
1st harvest	06.04.05	22.04.05	13.06.05	24.06.05	19.09.05	13.09.05
2nd harvest	06.04.05	22.04.05	13.06.05	24.06.05	19.09.05	27.09.05
3rd harvest	06.04.05	22.04.05	13.06.05	24.06.05	19.09.05	14.10.05

ST = sowing time, SG = seed germination, SB = shoot beginning, FB = flower beginning, CF = beginning of capsule formation, T = harvest time

3.2.4 Measurements

In the pot experiments, different parameters such as seed yield per pot (seed yield was calculated on the basis of 91 % DM), number of side shoots per plant (three plants per pot), number of capsules per side shoot, number of capsules per main stem, number of capsules per pot and thousand seed weight were measured before laboratory analysis. In the laboratory the percentage of seed oil and raw protein as well as fatty acid composition were measured.

Seed oil percentage was determined according to method of Soxhlet (Court et al. 1993, Christie 1999). The fatty acid composition was analyzed by Varian CP 3800 gas chromatography with a modified method of Court et al. (1993). CHNS elemental analyzer EA 1110 measured the percentage of raw protein of seed samples.

3.3 Post-harvest experiment

3.3.1 Effect of storage time and storage temperature on the seed oil quality

From the field experiment (spring 2004) the seeds of *Oenothera biennis* L. cv. Anothera, which were harvested at the late harvest time and under conventional harvest method with 91 % DM, were used as experimental material in this study. The seed samples were packed in brown paper bags and were stored at different temperatures: 4°C, 21°C, and 35°C for a period of four months (Table 3-10). Seeds were taken regularly every month to examine the changes in the percentage of oil, fatty acid composition and some other chemicals that may have occurred during the storage period.

The following results of the first taken samples, the percentage of seed protein, fatty acid composition, acid and peroxide value were used as a control for the rest of the storage times. Stored seed materials were finely ground in a coffee grinder and oil extraction (8 h at 70 °C) was performed in a Soxhlet extractor using n-hexane. Fatty acid composition of seeds was determined as methyl esters by GC (Varian CP-3800) according to Court et al. 1993. The acid value (AV) and peroxide value (PV) were determined by IUPAC (International Union of Pure and Applied Chemistry) methods (Anonymous 1987). EA 1110 Elemental Analyzer was used for measuring the percentage of raw protein of the seed samples (the methods will be described in the chapter 3.4).

Table 3-10 Treatments in the storage experiment with evening primrose

A: storage time (month)	B: storage temperature
1. control	1. 5 °C, refrigerator
2. one month	2. 35 °C, climate chamber
3. two months	3. 21 °C, room temperature
4. three months	
5. four months	

3.4 Study on seed germination

During this project, investigations on the seed germination of evening primrose under different conditions were carried out. Some information is presented in Table 3-11.

3.4.1 First experiment

Seeds of *Oenothera biennis*, which were stored under room temperature conditions, were obtained from the research station in Gross-Gerau. The seed samples were one year old with a 50 % germination rate. Seeds were placed in the containers containing pure sand and were wetted with tap water. The experiments were directed in four replications with 50 seeds per replication.

To determine the effect of temperature and day length on seed germination, containers were placed in climatic chamber with different temperatures and photoperiodic conditions. Experiments were carried out at 5, 10, 15 and 20 °C with day lengths of 0, 8, 16 and 24 h, respectively. The relative humidity of the growth chamber was 75 %. The night temperature of short and long day conditions (8 and 16 h/day lengths) was 4-6 °C lower than that of day temperature.

Table 3-11. Treatments in germination experiments with evening primrose seeds

Experiment	Traetments*
First	A- temperature: 5, 10, 15, 20 °C B- day length: 0, 8, 16, 24 hours
Second	A- storage temperature: 4, 21, 35 °C B- storage period: four months
Third	A- harvest time: early, middle, late B- harvest method: conventional harvest, defoliation with herbicide, defoliation with flame C- seed age

* In all experiments, the number of replications was four

3.4.2 Second experiment

Newly harvested seeds (with 91 % DM) of evening primrose were obtained from a field experiment (conventional harvest and late harvest time). Seed samples were stored under different storage temperatures; 4°C, 21°C, and 35°C for a period of four months and were taken regularly every month to evaluate seed germination percentage. For protecting seeds against light, seed samples were stored in brown paper bags. Germination was carried out in 90 mm diameter plastic petri dishes on filter paper circles with 5 ml water. Four replications of 50 seeds were used for each treatment.

3.4.3 Third experiment

In this experiment, the seeds of evening primrose (91 % DM), which were harvested at different times (early, middle and late harvest times) and with different methods (conventional harvest, defoliation with herbicide and defoliation with flame) were used as experimental samples. One month after harvesting, the seeds were transferred to laboratory and stored in brown paper bags. During the experiment, the seed samples were stored under room temperature conditions. Every month 200 seeds (50 seeds per petridish) were used for germination test. The experiment was done in the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th} and 12^{th} month after harvesting.

3.5 Laboratory analysis

3.5.1 Oil extraction with Soxhlet

The soxhlet method, which was described by Soxhlet in 1879, is the most commonly used example of a semi-continuous method applied to extraction of lipids from plant tissues and foods (Fig 3-6). According to the Soxhlet procedure, oil and fat from solid material are extracted by repeated washing (percolation) with an organic solvent, usually hexane or petroleum ether, under reflux in a special glassware (Leray 2006).

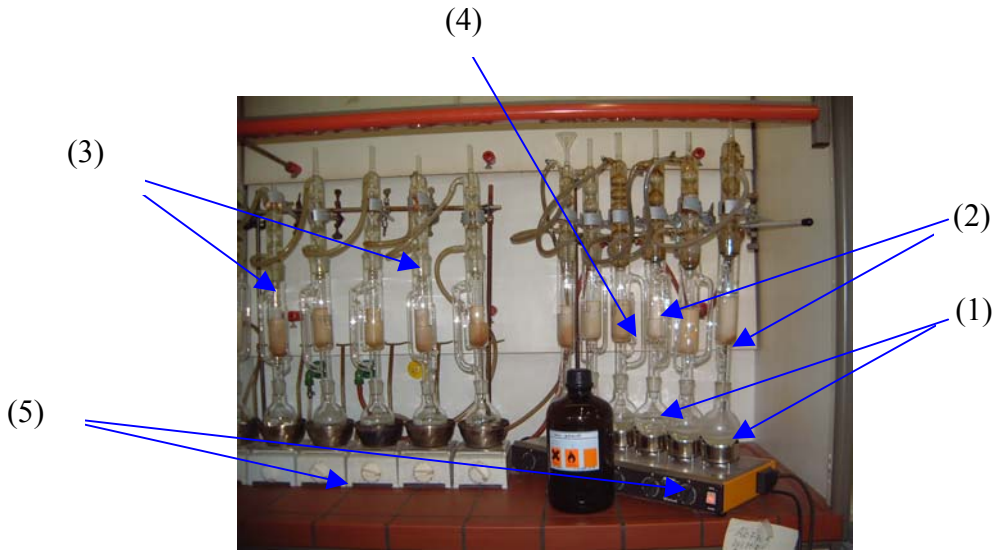


Fig 3-6. Oil extraction with soxhlet apparatus

In this method the seed samples of *Oenothera biennis* were ground into small particles with a coffee grinder machine. The ground seed materials were dried in an oven (105 °C, 3 h). Then 5 g of seed sample were placed in a porous cellulose thimble. For preventing the movement of small sections of ground seed to capillary channels, in thimble the samples were covered by waterproof cotton. The thimble was placed in an extraction chamber (2), which is suspended above a flask containing the solvent (1) and below a condenser (3). The flask was heated (5) and the solvent evaporated and moved up into the condenser where it was converted into a liquid that trickles into the extraction chamber containing the sample. The extraction chamber was designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down (4) into the boiling flask (1). At the end of the extraction process, which lasts 8 hours, the thimble was removed. The flask containing oil and solvent was heated, the solvent in the flask was then evaporated, and the mass of the remaining lipid was measured. The percentage of lipid in the initial sample then was calculated.

3.5.2 Fatty acid analysis with GC

Fats are esters of the trivalent alcohol glycerin and fatty acids. Most of plant natural fats naturally appear in the form of triacylglycerol. In triacylglycerols, the acyl groups substitute the H atoms of glycerol. Fats in the form of triacylglycerol are not volatile. For quantitative determination of the portions of individual fatty acids by gas chromatography (GC) easily volatile substances are necessary. Methyl ester is a volatile form of fatty acid molecule and is easily analyzable by GC.

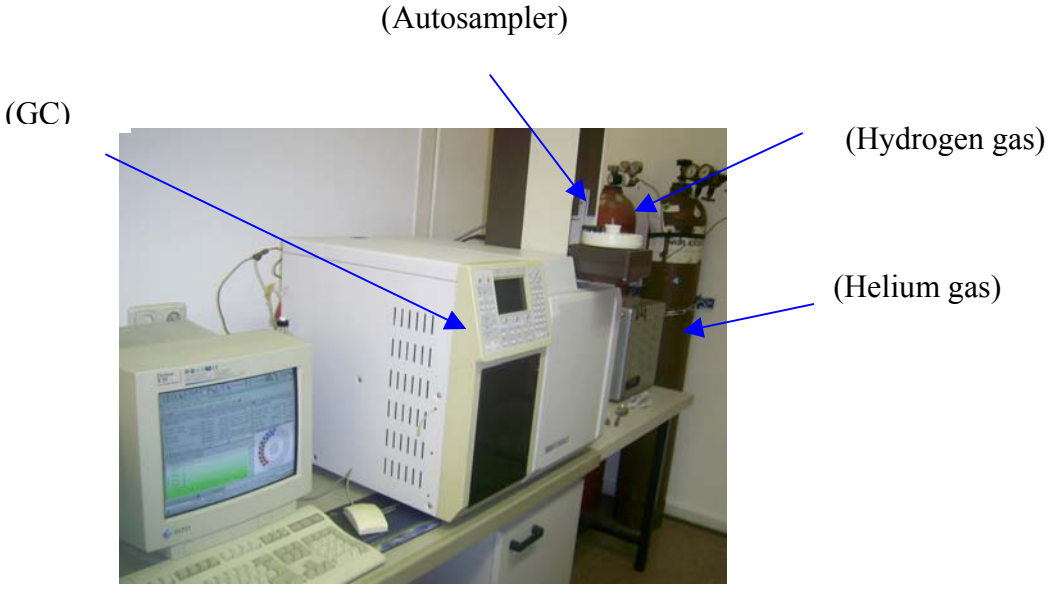


Fig 3-7. Varian CP 3800 gas chromatography

The splitting of the triacylglycerols for preparing fatty acid methyl ester by the following method was done. Evening primrose finely ground seeds (0.3 g) were weighed and were put in to a 10 ml glassy test tube. Then 3 ml petroleum benzene was added to the tube and sample was shaken by a shaker. The mixture was placed in a refrigerator for 30 min during which the fats of the seed samples were dissolved by the petroleum benzene. Two milliliters of the surface liquid of test tube were pipetted and transferred to a 4 ml test tube. The mixture was kept in a desiccator for 24 hours for separation of the oil from organic solution by evaporation. After this period, only the oil remained in the test tube. Sodium methylate (2 ml) was added to the test tube for diesterification. The mixture was kept in the dark for 30 min. Then 1 ml of isooctane was added to the mixture. As isooctane is not soluble in sodium methylate, two liquid phases were formed in the tube and most part of the fatty acid esters were transferred to the isooctane portion. Isooctane phase was pipetted to a glass vial and made ready for GC analysis.

GC is by far the most appropriate equipment for analysis of fatty acids, generally in the form of volatile methyl esters derivatives as mentioned above. In these experiments oil quantitative analysis were performed on a Varian CP 3800 gas chromatograph with duel FID detector (Fig 3-6). A Permabond® FFAP column (25 m × 0.25 mm i.d., film thickness 0.25 µm) with CP-SIL 88 foe FAME stationary phase which was equipped with CS-fused-Silica precolumn was used. One microliter injection was made with Varian 8200 CX autosampler. The column oven was temperature programmed from 200 to 240 at 10 °C/min and held to 220 for 1 min. The injector temperature was 280 °C, detector, 280 °C and the flow rate was 2.0 ml/min.

3.5.3 Protein measurement

In all experiments, the percentage of nitrogen of seeds was measured by EA1110 type Thermo Finnigan. EA1110 (Fig 3-7), a CHNS configuration, is an elemental analyzer for the simultaneous determination of the percentage of carbon, hydrogen, nitrogen and sulphur contained in organic and inorganic chemical compounds.

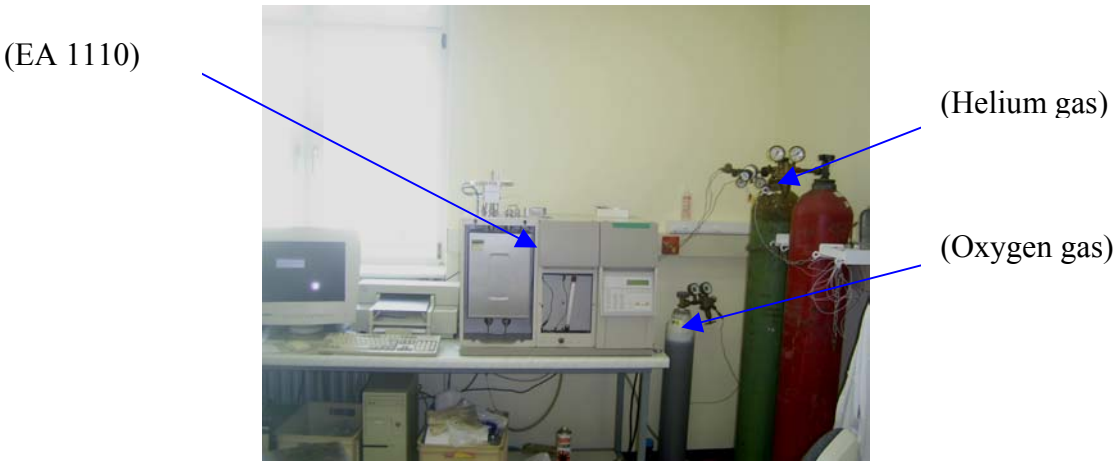


Fig 3-8. EA 1110 elemental analyser

In EA1110, the analytical method used develops on subsequent steps: at first the samples undergo a controlled combustion, immediately followed by catalytic oxidation and reduction. The produced gas is separated by gas chromatography and then passed through a thermal conductivity detector that generates an electrical signal, proportional to the amount of gas produced by combustion gives the percentage of nitrogen in which the percentage of raw protein obtained by multiplication of the percentage of N with factor 6.25.

3.5.4 Peroxide value

The peroxide value (PV) is defined as a number of milliequivalents of active oxygen per kilogram of fat, which oxidizes potassium iodide. About 0.3 g to 5 g of oil samples were carefully weighed into an Erlenmeyer flask, and stirred with a magnetic stirrer. About 50 ml of a mixture of acetic acid/isooctane (2:1 v/v) and 0.5 ml of saturated potassium iodide solution were added to the now well-mixed oil sample and was shaken exactly for 60 s. The color of the solution changed to yellow-orange, and immediately 30 ml of distilled water and 0.5 g starch was added. The mixture was again covered and swirled and the color changed to dark blue. The mixture was now titrated against sodium thiosulfate (0.1 N) until the solution became colorless. Parallel to this a blind experiment was carried out using the solution of the same normality.

The PV in meq of active oxygen was calculated as follows:

$$PV = \frac{(a-b) \times N \times 1000}{E}$$

Where:

a is the volume of sodium thiosulfate solution (ml)

b is the volume of the sodium thiosulfate solution used for the blind test (ml)

N is the concentration of the sodium thiosulfate solution (mol/l)

E is the amount of the oil sample

3.5.5 Free fatty acid percentage

The free fatty acid percentage (FFA) is defined as the number of milligram of potassium hydroxide required to neutralize the acidic constituents in 1 g of oil. For calculating FFA, 2.0 g of oil sample were weighted directly into a 200 ml conical flask. Then 50 ml of ethanol/toloule (1:1 v/v) mixture were added to the sample in the flask, and shaken to dissolve. Once the sample had dissolved, a few drops of phenolphthalein solution were added and titrated against potassium hydrate solution (0.5 mol/l) until the violet color developed and maintained for at least 5 s. The free fatty acid percentage was calculated using the formula below.

The calculated value of FFA is usually expressed as milligram potassium hydroxide per 1 gram of oil sample.

$$FFA = \frac{56.1 \times V \times c}{m}$$

Where:

V is the titration volume of potassium hydroxide solution in ml, c is the molarity of the potassium hydroxide solution in mol/l and m is the oil sample weight in gram.

3.6 Statistic analysis

The statistical analysis was separately done for all experiments by using the SPSS software version 12.1. Before analysis the normal distribution of data was evaluated by One-Sample Kolmogorov-Smirnov Test. Normal distributed data were checked by Split File to find the source of non-normal distribution. Some data that did not have normal distribution were recalculated by the method of Computing Variables for normal distribution. When the recalculated data were not normally distributed, Two-Independent-Samples Tests were used. The GLM univariate Analysis or GLM Multivariate Analysis were used for multifactor variables analysis.

Least significant difference (LSD) at $\alpha = 5\%$ was manually calculated for measurements with p- value < 0.05 by the following formula:

$$LSD = t_{Table} \sqrt{\frac{(SE)2}{r}}$$

Where: LSD = least significant difference, SE = standard error, r = number of replication

4. Results

4.1 Field experiments

4.1.1 Plant development

From Table 4-1 it can be inferred that the first harvest of evening primrose in spring 2004 was done approximately 155 d after germination. The second and third harvest time were done at 176 and 187 d after germination, respectively. The vegetation period of the plants sown in spring 2005 was shorter than sown in the spring of 2004 (Table 4-1). The autumn-sown plants had a longer growing period of the 398 to 427 d during the early and late harvest times, respectively (Table 4-1).

Table 4-1. Development stages of *Oenothera biennis* in field experiments Gross Gerau, spring 2004 (S1), autumn 2004 (A) and spring 2005 (S2)

Experiment	T	SST-SG	SG-SB	SB-FB	FB-DT	FB-T	DT-T	TGP (days)
Spring	T1	28	45	32	22	50	28	155
2004	T2	28	45	32	42	71	29	176
(S1)	T3	28	45	32	61	82	21	187
Autumn	T1	20	234	47	52	77	25	398
2004	T2	20	234	47	73	93	20	414
(A)	T3	20	234	47	81	106	25	427
Spring	T1	28	22	26	53	85	32	161
2005	T2	28	22	26	64	91	27	167
(S2)	T3	28	22	26	77	91	14	167

T: harvest time; T1: early harvest (when all capsules were green), T2: middle harvest time (when 30 % of capsules were brown), T3: late harvest time (when more than 75 % of capsules were brown), SST-SG: sowing time to seed germination, SG-SB: germination to shoot beginning, FB-T: duration between flower beginning to harvest, FB-DT: duration between flower beginning to desiccation time, DT-T: duration between desiccation to harvest, TGP: total growing period.

Autumn sown plants required more than 230 d from germination to shoot beginning. In contrast to that, spring evening primrose took only 22-45 d to achieve the same stage of growth (Table 4-1). The shortest (50 d) and the longest (106 d) duration from flower beginning to harvest were observed at the early harvest of the spring experiment in 2004 and late harvest of the autumn experiment in 2004. The shortest growing periods from flower beginning to desiccation (22 d) and flower beginning to harvest (28 d) were observed in the spring 2004 experiment. In contrast, plants harvested at the late time in autumn 2004 experiment had the longest period from flower beginning to desiccation and harvest (81 and 106 d in flower beginning to desiccation and harvest, respectively).

4.1.2 Seed yield and seed yield components

Seed yield

The seed yield of evening primrose varied from a minimum of 9.1 dt/ha in the spring 2005 experiment to a maximum of 14.6 dt/ha in the autumn 2004 experiment. In both spring experiments, harvest time did not have a significant effect on seed yield (see Table 4-2). Contrary to that, in autumn 2004 experiment the seed yield was significantly influenced by harvest time.

Table 4-2. Effect of harvest time and harvest method on the seed yield and seed yield compounds of *Oenothera biennis*, field experiments Groß-Gerau, in spring 2004 (S1), autumn 2004 (A) and spring 2005 (S2)

T	M	SY(dt/ha)			SDM (%)			PDM (%)			TSW(g)		
		S1	A	S2	S1	A	S2	S1	A	S2	S1	A	S2
T1	M1	13.0	10.9	10.6	90.7	92.6	86.7	18.2	18.7	26.1	0.35	0.43	0.47
	M2	14.0	10.5	9.1	90.8	90.4	83.6	18.2	27.5	25.2	0.33	0.50	0.47
	M3	13.4	11.7	10.4	91.2	91.8	84.6	18.3	27.3	25.5	0.45	0.50	0.49
T2	M1	12.3	10.6	11.1	92.7	91.7	92.1	18.6	18.4	22.2	0.34	0.47	0.42
	M2	14.1	12.0	10.4	92.8	92.7	89.4	18.7	27.9	19.7	0.37	0.47	0.41
	M3	14.1	11.1	11.3	92.4	92.5	89.9	18.5	27.2	21.5	0.36	0.47	0.48
T3	M1	11.6	11.5	9.3	88.9	90.7	88.9	18.1	18.7	19.8	0.38	0.40	0.47
	M2	14.2	13.2	11.0	89.4	90.5	90.1	17.5	27.8	21.0	0.40	0.47	0.47
	M3	13.7	14.6	10.0	87.7	90.8	88.5	16.9	27.3	22.1	0.40	0.47	0.43
T1		13.4	11.0	10.0	90.9	91.7	85.0	18.2	24.5	25.6	0.34	0.48	0.47
T2		13.5	11.2	10.9	92.6	92.3	90.5	18.6	24.5	21.1	0.35	0.47	0.44
T3		13.2	13.1	10.1	88.7	90.7	89.2	17.5	24.6	21.0	0.39	0.44	0.46
	M1	12.3	11.0	10.3	90.8	91.7	89.2	18.3	18.6	22.7	0.35	0.43	0.45
	M2	14.1	11.9	10.2	91.0	91.2	87.7	18.1	27.7	22.0	0.36	0.48	0.45
	M3	13.7	12.5	10.5	90.4	91.7	87.7	17.9	27.6	23.0	0.37	0.48	0.48
LSD 5%	T	ns	1.78	ns	0.77	0.32	1.24	0.81	ns	1.66	0.03	ns	ns
	M	1.53	ns	ns	ns	0.32	1.24	ns	0.15	ns	ns	ns	ns
	T×M	ns	ns	ns	ns	0.57	ns	ns	0.26	ns	ns	ns	ns

T: harvest time; T1: early harvest (when all capsules were green), T2: middle harvest time (when 30 % of capsules were brown), T3: late harvest time (when more than 75 % of capsules were brown), M: harvest method; M1: conventional harvest, M2: defoliation by herbicide, M3: defoliation by flame, SY: seed yield, SDM: seed dry matter content, PDM: plant dry matter content, TSW: thousand seed weight

As presented in Table 4-2, the significant effect of harvest methods on seed yield was observed only during the spring 2004 experiment. In this experiment, the plants that were defoliated using herbicide produced a higher (14.1 dt/ha) seed yield than conventionally harvested plants. No interaction on seed yield was observed between harvest time and harvest method.

Thousand seed weight

A significant influence of different harvest times on TSW was observed only in the spring 2004 experiment (Table 4-2). In this experiment the late harvest time, when more than 75 % of capsules were brown, led to an increase in the TSW from 0.34 g (early harvest) or 0.35 g (middle harvest) to 0.39 g (late harvest).

Seed dry matter content

Table 4-2 shows that the seed dry matter content varied from a minimum of 83.6 % in the spring 2005 experiment to a maximum of 92.8 % in the spring 2004 experiment. In all cases, the seed dry matter content (SDM) was significantly influenced by harvest time. As presented in Table 4-2, in all experiments the highest (92.6 %) SDM was produced by the plants harvested when 30 % of capsules were brown (middle harvest).

A significant effect of harvest method on SDM was observed both in the autumn 2004 experiment and in the spring 2005 experiment. In the autumn experiment, the plants defoliated using herbicide produced a lower SDM (91.2%) than those of flame-defoliated plants (M3) and the plants that were conventionally harvested (M1). As presented in Table 4-2, in the spring 2005 experiment, the plants that were conventionally harvested (M1) produced the highest (89.2%) seed dry matter content.

In the autumn 2004 experiment, an interaction between harvest time and harvest method on seed dry matter content was observed. When all capsules were green the SDM of plants which were defoliated by herbicide (M2) was lower than using the other harvest methods. Contrary to that, when 30 % of capsules were brown the conventional method led to the lowest seed dry matter content. By delaying the harvest time, none of the different harvest method had a significant influence on the dry matter content of seeds (Table 4-2).

Plant dry matter content

The plant dry matter content (PDM) was significantly influenced by harvest time in both spring experiments. The lowest PDM was observed at the late harvest time. In the spring experiment of 2004, a significant difference in the plant dry matter was observed at the middle and late harvest times. In the spring 2005 experiment, significantly a higher dry matter content was recorded in the early harvest time relative to the middle and late harvests (Table 4-2).

In the experiments carried out in spring no significant effect of harvest method on the plant dry matter was observed. On the other hand, the plants sown in autumn were significantly

influenced by the various harvest methods used. Overall treatments the lowest plant dry matter content (18.7 %) was produced by using the conventional harvest method.

4.1.3 Oil and protein percentage

Oil percentage

In the field experiments, the seed oil percentage varied from a minimum of 23.4 % in the spring experiment of 2004 to a maximum of 29.8 % in the spring experiment of 2005. An effect of harvest time on the percentage of oil was observed only at the second spring experiment (S2). Delayed harvest increased the percentage of oil from 24.9 % when all capsules were green to 29.0 % when more than 75 % of capsules were brown.

A significant interaction was observed between harvest time and harvest method on the oil percentage. This interaction led to a lower oil percentage in defoliated plants at first harvest time (T_1) as compared to the non-defoliated. When all capsules were green (early harvest), defoliated plants produced a lower seed oil percentage than non-defoliated plants (Table 4-3). A similar interaction between harvest time and harvest method was observed in the spring experiment of 2004 (S1).

Protein percentage

The percentage of seed raw protein was not significantly influenced by harvest time and harvest method except in the spring experiment of 2005. In this experiment, a delayed harvest reduced the percentage of raw protein from 16.2 % (all capsules were green) to 14.8 % (30 % of capsules were brown) and 15.1 % (75 % of capsules were brown). In all the experiments, between harvest time and harvest method no interaction on the seed protein percentage was observed.

4.1.4 Fatty acid composition

Palmetic acid

The results of fatty acid composition are presented in Tables 4-3 and 4-4. Table 4-3 shows a significant influence of harvest time on the percentage of palmetic acid (PA) in at least two of three experiments. The autumn sowing times produced the lowest percentage of palmetic acid as compared to the spring sowing times. The percentage of palmetic acid in all the experiments was not significantly influenced by any of the harvest methods (Table 4-3). Between treatments, no interaction on PA was observed in these experiments.

Table 4-3. Oil percentage, raw protein percentage and the percentage of saturated fatty acids (PA, SA) in the seeds of *Oenothera biennis*, field experiments Groß-Gerau, in spring 2004 (S1), autumn 2004 (A) and spring 2005 (S2)

T	M	SO (%)			SP (%)			PA (%)			SA (%)		
		S1	A	S2	S1	A	S2	S1	A	S2	S1	A	S2
T1	M1	27.6	26.4	26.5	15.4	16.3	17.1	7.6	5.8	6.3	1.6	2.3	2.1
	M2	24.6	26.7	23.4	15.4	16.0	15.9	7.6	5.8	6.7	1.8	2.2	2.3
	M3	26.5	27.2	24.9	15.5	15.3	15.7	7.7	5.7	6.5	1.8	2.2	2.2
T2	M1	25.5	26.0	27.2	15.4	16.5	14.0	7.7	5.7	6.1	1.6	2.2	2.0
	M2	26.9	27.6	27.7	15.1	16.0	14.3	7.8	5.7	6.0	1.7	2.2	2.1
	M3	27.5	27.4	27.3	14.2	16.3	15.2	7.5	5.8	6.0	1.8	2.2	2.2
T3	M1	25.8	26.4	28.2	14.7	16.2	15.2	7.7	5.8	5.8	1.5	2.3	2.1
	M2	26.3	27.8	28.9	15.0	15.6	15.2	7.7	5.9	5.7	1.8	2.2	2.2
	M3	26.7	26.9	29.8	16.1	16.9	14.7	7.7	6.0	5.8	1.8	2.2	2.1
T1		26.2	26.8	24.9	15.4	16.3	16.2	7.8	5.8	6.5	1.6	2.2	2.2
T2		26.6	27.0	27.4	14.9	16.3	14.8	7.7	5.7	6.0	1.7	2.2	2.1
T3		26.3	27.0	29.0	15.2	16.2	15.1	7.8	5.9	5.8	1.8	2.2	2.1
	M1	26.3	26.3	27.3	15.2	16.3	15.4	7.7	5.8	6.0	1.8	2.3	2.1
	M2	25.9	27.4	26.7	15.1	15.9	15.2	7.8	5.8	6.1	1.7	2.2	2.2
	M3	26.9	27.1	27.3	15.3	16.2	15.4	8.1	5.8	6.1	1.7	2.2	2.2
LSD $_{5\%}$ T		ns	ns	0.82	ns	ns	0.91	ns	0.10	0.17	ns	ns	ns
M		ns	0.20	ns	ns	ns	ns	ns	ns	ns	ns	0.05	0.08
M ×T		1.90	ns	1.43	ns	ns	ns	ns	ns	ns	ns	ns	ns

T: harvest time; T1: early harvest (when all capsules are green), T2: middle harvest time (when 30 % of capsules were brown), T3: late harvest time (when more than 75 % of capsules were brown), M: harvest method; M1: conventional harvest, M2: defoliation by herbicide, M3: defoliation by flame, SO: seed oil, SP: seed protein, PA: palmitic acid, SA: stearic acid

Oleic acid

Plants that were harvested at the early harvest time by flame defoliation in the spring 2004 experiment, produced 8.4 % of oleic acid as compared to 13.2 % obtained in the spring 2005 experiment used herbicide defoliation. The oleic acid (OA) percentage was influenced by harvest time in all experiments (Table 4-4). In the spring experiment of 2004 (S1), plants harvested at the early harvest produced the lowest oleic acid percentage. Contrary to that, both in the autumn experiment of 2004 and the spring experiment of 2005 a reduction in the oleic acid percentage was observed by delayed harvest. In the spring experiment of 2005, the percentage of OA was significantly influenced by harvest method. Results showed that plants which were defoliated before harvest produced a higher OA percentage than non-defoliated plants. In this experiment, there was no difference in the oleic acid percentage of plants defoliated with flame and with herbicide.

Table 4-4. Effects of different harvest times and harvest methods on the percentage of unsaturated fatty acids in seeds of *Oenothera biennis*. Field experiments Groß-Gerau, in spring 2004 (S1), autumn 2004 (A) and spring 2005 (S2)

T	M	OA (%)			LA (%)			GLA (%)		
		S1	A	S2	S1	A	S2	S1	A	S2
T1	M1	8.4	11.0	12.5	73.2	72.7	70.4	9.0	7.7	8.9
	M2	8.6	11.2	13.2	73.1	72.6	70.0	9.3	7.8	9.0
	M3	8.3	11.3	12.9	73.7	72.5	70.3	9.1	8.2	8.1
T2	M1	9.2	10.2	12.0	73.4	73.2	72.2	8.0	8.6	7.7
	M2	8.8	10.1	12.0	73.6	73.7	71.7	8.1	8.3	8.2
	M3	9.2	10.2	12.9	73.3	72.6	71.0	7.8	9.0	7.7
T3	M1	9.0	10.2	11.7	73.4	72.5	72.3	8.3	8.7	7.6
	M2	8.9	10.3	12.3	72.2	73.2	71.5	8.0	8.4	7.7
	M3	9.4	10.1	12.7	73.2	73.8	71.7	8.0	7.9	7.5
T1		8.2	11.2	12.9	73.4	72.6	70.2	9.3	7.9	8.7
T2		9.3	10.2	12.3	73.5	73.2	71.6	8.0	8.6	7.9
T3		9.2	10.2	12.2	73.1	73.2	71.8	7.8	8.3	7.6
	M1	9.2	10.5	12.0	73.4	72.8	71.6	8.3	8.4	8.1
	M2	8.8	10.5	12.5	73.1	73.2	70.1	8.4	8.1	8.3
	M3	8.7	10.5	12.8	73.5	73.0	71.0	8.2	8.3	8.7
LSD $_{5\%}$ T		0.37	0.52	0.45	ns	ns	0.28	0.40	0.41	0.47
M		ns	ns	0.45	ns	ns	0.28	ns	ns	ns
M × T		ns	ns	ns	ns	ns	ns	ns	ns	ns

T: harvest time; T1: early harvest (when all capsules are green), T2: middle harvest time (when 30 % of capsules were brown), T3: late harvest time (when more than 75 % of capsules were brown), M: harvest method; M1: conventional harvest, M2: defoliation by herbicide, M3: defoliation by flame, OA: oleic acid, LA: linoleic acid, GLA: y-linolenic acid, Treatments in harvest method; 1: conventional harvest, 2: defoliation by herbicide, 3: defoliation by flame

Linoleic acid

The percentage of linoleic acid varied from 70.0 % in the spring experiment of 2005 to 73.8 % in the autumn experiment of 2004. Linoleic acid (LA) was significantly affected by both harvest time and harvest method in the spring experiment of 2005. Although the highest LA percentage was observed in plants that were harvested when more than 75 % of capsules were brown (late harvest), no significant difference between middle and late harvest was observed. The results of this experiment show that plants defoliated using herbicide produced the lowest oleic acid percentage (Table 4-4).

Gamma linolenic acid

The results of the field experiments recorded a minimum of 7.5 % GLA in the spring experiment of 2005 and a maximum of 9.3% in the spring experiment of 2004 (Table 4-4). In these experiments, harvest time significantly influenced the GLA percentage of evening

primrose seed oil. In the spring experiments, the highest GLA percentage was recorded in plants that were harvested when all capsules were green. In both spring experiments a delay in harvest date led to a reduction in the percentage of GLA (Table 4-4). In the autumn 2004 experiment, the situation was different. Here it was observed that harvesting when all the capsules were green produced the lowest percentage of GLA. In the same experiment, harvesting when 30 % of capsules were brown produced the maximum (8.6 %) GLA percentage. Despite all these differences, none of the harvest methods had a significant influence on the GLA percentage (Table 4-4).

4.2 Pot experiments

4.2.1 Plant development in pot experiments

In the spring 2004 experiment, the plants were harvested at 148, 164 and 178 d from the emergence respectively. This corresponded to the three different harvest times mentioned in the text under material and methods (Table 4-5).

Contrary to the spring 2004 experiment, the harvest times in the spring 2005 experiment were carried out at longer maturity stages (i.e. 178 d from emergence in S1 and 196 d from maturity in S2). While plants sown in autumn took more than 230 d from germination to the shoot beginning, those sown in spring took only 22-45 d to reach the same stage of maturity. By comparing all the experiments in both spring and autumn, it can be observed that the time from flowering to harvest was shortest (47 d) at the first harvest in the autumn 2004 experiment and longest (91 d) at the third harvest time in the spring 2005 experiment.

Table 4-5. Development stages of *Oenothera biennis* in pot experiments spring 2004 (S1), autumn 2004 (A), spring 2005 (S2)

Experiment	Harvest time	ST	FB	T	FB-T $_{(d)}$	TGP$_{(d)}$
Spring 2004	1st harvest	21.04.04	23.07.04	16.09.04	56	148
	2nd harvest	21.04.04	23.07.04	30.09.04	70	164
	3rd harvest	21.04.04	23.07.04	14.10.04	84	178
Autumn 2004	1st harvest	23.09.04	30.06.05	16.08.05	47	328
	2nd harvest	23.09.04	30.06.05	30.08.05	63	344
	3rd harvest	23.09.04	30.06.05	13.08.05	76	357
Spring 2005	1st harvest	06.04.05	13.07.05	13.09.05	62	167
	2nd harvest	06.04.05	13.07.05	27.09.05	76	181
	3rd harvest	06.04.05	13.07.05	11.10.05	91	196

ST: sowing time, FB: flower beginning, T: harvest time, FB-T: flower beginning to harvest, TGP: total growing period

4.2.2 Experiment S1 (spring 2004)

4.2.2. 1 Seed yield and seed yield components

Seed yield

From Table 4-6 it can be seen that seed yield was strongly influenced by different levels of nitrogen. The pots received 0.3 g of N, produced 8.8 g seed per pot. Contrary to that, 14.8 g seed/pot was produced by pots that were given 0.6 g of N. Harvest time and harvest method had no significant effect on the seed yield in this experiment.

Interaction between nitrogen and harvest time on seed yield indicated that, under a low level of nitrogen, seed yield was not influenced by different harvest times. Contrary to this under a high level of nitrogen (0.6 g) the seed yield of evening primrose significantly increased from a minimum of 13.7 g/pot at the first harvest time to a maximum of 15.2 g/pot and 15.5 g/pot in second and third harvest times (Fig 4-1).

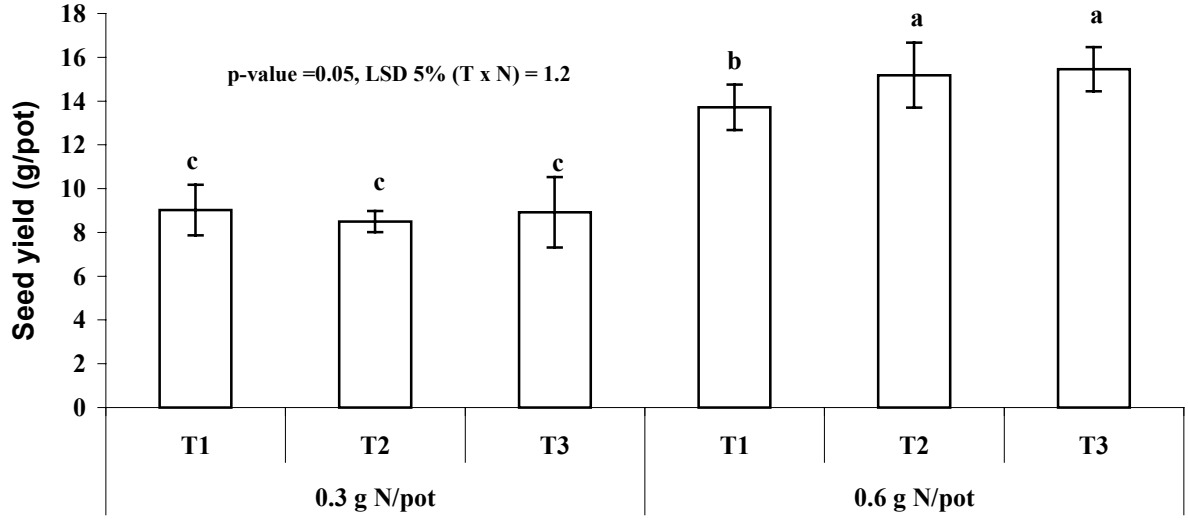
Table 4-6. Effect of nitrogen fertilization, harvest time and harvest method on seed yield and seed yield components of *Oenothera biennis*. Pot experiment spring 2004 (S1)

N	T	M	SY (g/pot)	SSP	CMS	CSS	CP	TSW (g)
0.3 g			8.8	8.1	82.0	18.9	100.9	0.37
0.6 g			14.8	16.0	93.4	59.4	152.8	0.38
	T1		11.4	12.3	90.2	41.1	131.3	0.38
	T2		11.8	11.6	88.8	37.9	126.7	0.37
	T3		12.2	12.3	84.1	38.4	122.5	0.38
		M1	12.0	12.5	88.6	39.8	128.4	0.38
		M2	11.6	11.7	86.8	38.5	125.3	0.37
LSD 5%	N		0.71	1.8	5.5	9.8	9.6	ns
	T		ns	ns	ns	ns	ns	ns
	M		ns	ns	ns	ns	ns	ns
	N × T		1.2	ns	ns	ns	ns	ns
	N× M		ns	ns	ns	ns	ns	ns
	T × M		ns	ns	ns	ns	ns	ns
	N× T × M		ns	ns	ns	ns	ns	ns

N: Nitrogen, T: harvest time; T1: first harvest, T2: second harvest, T3: third harvest, M: harvest method; M1: conventional harvest, M2: defoliation by herbicide, SY: seed yield, SSP: side shoots per pot, CMS: capsules per main stem, CSS: capsules per side stem, CP: capsules per pot, TSW: thousand seed weight

Seed yield components

Nitrogen had a strong influence on most of the measured seed yield components. It was observed that the pots which were given 0.3 g of nitrogen produced 18.9 CSS and the pots that received 0.6 g of nitrogen produced 59.4 CSS per pot. There was neither a harvest time and harvest method effect nor any interactions in this experiment (Table 4-6).



* bars at the top of the columns mean standard deviation

Fig 4-1. Interaction between nitrogen (N) and harvest time (T1: first harvest, T2: second harvest, T3: third harvest) on seed yield of *Oenothera biennis*, pot experiment spring 2004 (S1),

4.2.2.1 Oil and protein percentage

Oil percentage

It can be inferred from Table 4-7 that, the seed oil percentage varied from a minimum of 28.4 % in $N_1T_1M_2$ to a maximum of 30.7 % in $N_1T_3M_1$. Compared with the effect of nitrogen, different harvest times significantly influenced the seed oil percentage in the spring experiment of 2004. There was a significantly lower seed oil percentage in plants which were harvested at the first harvest date as compared to those harvested at the second and third harvest dates (Table 4-7).

Although the seed oil percentage was not influenced by different methods of harvest, there was an interaction between the harvest time and harvest method. It can be observed that, with early harvest, harvest method had no effect on seed oil accumulation. Contrary to this, during the second harvest time defoliated plants produced a higher oil percentage than non-defoliated plants. With delaying in harvest (third harvest time), defoliation decreased the oil percentage (Fig 4-2).

Protein percentage

The percentage of raw protein of seed varied from 11.5 % in $N_1T_1M_1$ to 13.6 % in $N_2T_2M_2$. From all the treatments, only nitrogen significantly influenced the percentage of seed raw

protein. The pots containing 0.6 g of N produced 12.8 % raw protein. In contrast to that 12.2 %, raw protein was obtained from the pots, which were supplied with 0.3 g of N (Table 4-7). The observed interaction of nitrogen and harvest time on seed raw protein show that, under a low level of nitrogen (0.3 g of N/pot), the plants which were defoliated before harvest, reached a higher percentage of raw protein in the seeds than conventionally harvested plants. On the other hand, 0.6 g of N/pot combined with the conventional harvest method produced a higher percentage of protein than those with 0.6 g of N/pot combined with defoliation (Fig 4-3). The N×T×M interaction on the raw protein percentage indicated that, under low level of nitrogen and different harvest times, defoliated plants produced a higher percentage of raw protein than non-defoliated plants.

Table 4-7. Effects of different harvest times and harvest methods on the percentage of oil, raw protein and fatty acid composition of *Oenothera biennis*. Pot experiment, spring 2004 (S1)

N	T	M	SO (%)	SP (%)	PA (%)	SA (%)	OA (%)	LA (%)	GLA (%)
0.3 g N/pot	T1	M1	29.5	11.5	7.3	1.4	10.4	72.2	8.5
	T1	M2	28.4	12.7	7.4	1.7	11.5	71.4	7.9
	T2	M1	29.5	12.1	7.2	1.6	11.6	71.7	7.8
	T2	M2	30.3	12.3	7.1	1.5	11.8	71.0	8.6
	T3	M1	30.7	12.2	7.2	1.7	11.4	71.5	7.5
	T3	M2	28.9	12.3	7.6	1.9	12.0	71.5	7.1
0.6 g N/pot	T1	M1	29.3	13.2	7.5	2.0	11.7	72.1	7.4
	T1	M2	28.8	12.6	6.7	1.6	10.6	72.5	9.1
	T2	M1	28.8	13.6	7.4	1.9	9.7	72.6	7.3
	T2	M2	30.1	12.4	7.4	1.5	10.6	71.7	9.6
	T3	M1	30.1	12.2	7.6	1.6	9.9	72.5	8.5
	T3	M2	30.1	12.9	7.2	1.8	10.1	72.0	7.3
0.3 g N/pot			29.5	12.2	7.3	1.6	11.4	71.6	7.9
0.6 g N/pot			29.5	12.8	7.3	1.8	10.4	72.2	8.2
	T1		29.0	12.5	7.2	1.7	10.6	72.1	8.2
	T2		29.7	12.6	7.3	1.7	11.0	71.8	8.3
	T3		29.9	12.4	7.4	1.8	11.3	71.9	7.6
		M1	29.6	12.5	7.4	1.7	10.8	72.1	7.8
		M2	29.4	12.5	7.2	1.7	11.1	71.7	8.2
LSD 5%	N		ns	0.44	ns	0.06	0.37	0.37	ns
	T		0.69	ns	ns	0.08	0.52	ns	0.38
	M		ns	ns	ns	ns	ns	0.37	0.54
	N×T		ns	ns	ns	0.12	ns	ns	ns
	N×M		ns	0.46	0.32	0.08	ns	ns	0.38
	T×M		0.98	ns	ns	0.12	0.52	ns	0.54
	N×T×M		ns	0.92	ns	0.17	1.0	ns	0.76

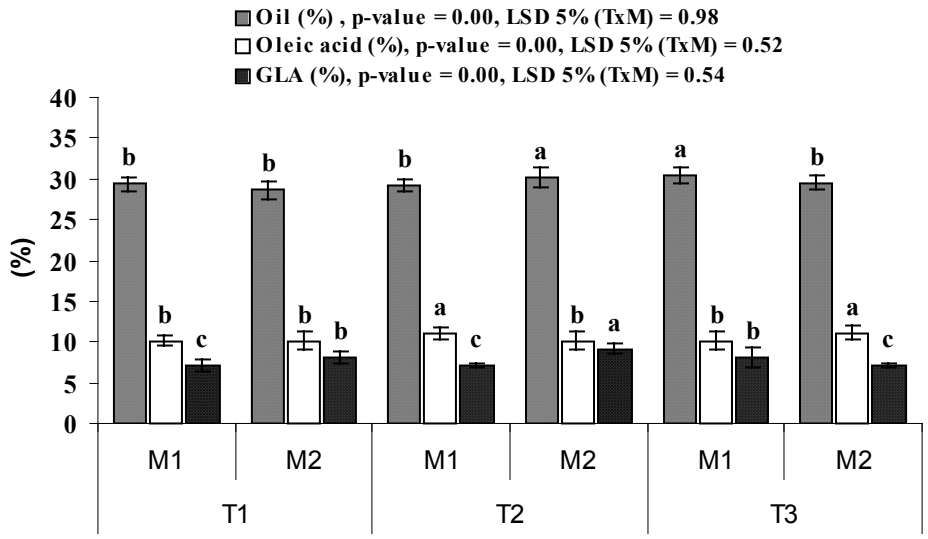
N: Nitrogen, T: harvest time; T1: first harvest, T2: second harvest, T3: third harvest, M: harvest method; M1: conventional harvest, M2: defoliation by herbicide, SO: seed oil, SP: seed protein, PA; palmitic acid, SA; stearic acid, OA: oleic acid, LA; linolenic acid, GLA; γ- linolenic acid.

In contrast, under the influence of high doses of nitrogen and at the first and second harvest times, conventionally harvested plants produced the highest percentage of raw protein (Table 4-7).

4.2.2.2 Fatty acid composition

Palmetic acid

From the Table 4-7 it can be inferred that the percentage of palmitic acid (PA) varied from 6.7 % in $N_2T_1M_2$ to 7.6 % both in $N_1T_3M_2$ and in $N_2T_3M_1$. The percentage of PA was not influenced by treatments in this experiment. Statistical analysis showed that there is an interaction between nitrogen and harvest method on the percentage of PA. The interaction showed that under low level of nitrogen the PA percentage of defoliated and non-defoliated plants was similar. In contrast, under high level of nitrogen defoliation before harvest produced a lower percentage of PA than the non-defoliated plants (Fig 4-4).



T: harvest time; T1: first harvest, T2: second harvest, T3: third harvest, M: harvest method; M1: conventional harvest, M2: defoliation by herbicide, bars at the top of the columns mean standard deviation, * different letters (a, b) indicate significant differences between oil, oleic acid and γ-linolenic acid percentages, respectively,

Fig 4-2. Interaction between harvest time and harvest method on the percentage of oil, oleic acid and γ-linolenic acid, pot experiment (S1) 2004

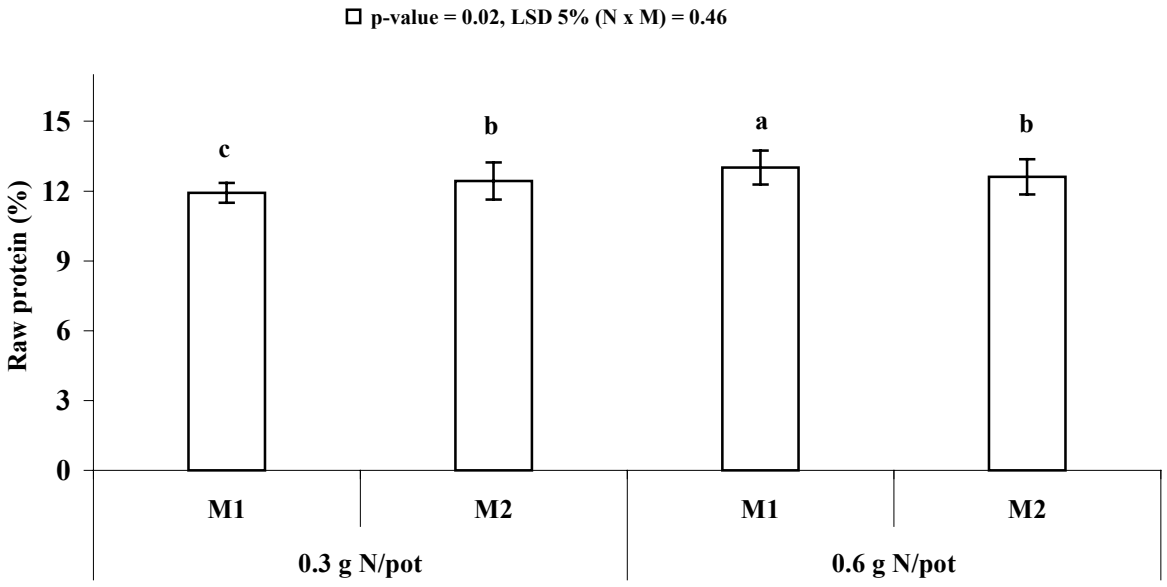
Oleic acid

In the spring experiment of 2004, the percentage of oleic acid varied from 9.7 % to 12.0 % in $N_2T_2M_1$ and $N_1T_3M_2$, respectively. From Table 4-7 the significant influence of nitrogen on the

percentage of oleic acid can be inferred. The nitrogen dose of 0.3 g was observed to produce higher oleic acid percentage (11.4 %) as compared to the 0.6 g of N dose, which gave 10.4 % of oleic acid. The percentage of oleic acid was influenced by different harvest times. The plants which were harvested at the last harvest date, produced a higher OA (11.3 %) than those of the remaining two harvest times that produced 10.6, 11.0 % of oleic acid.

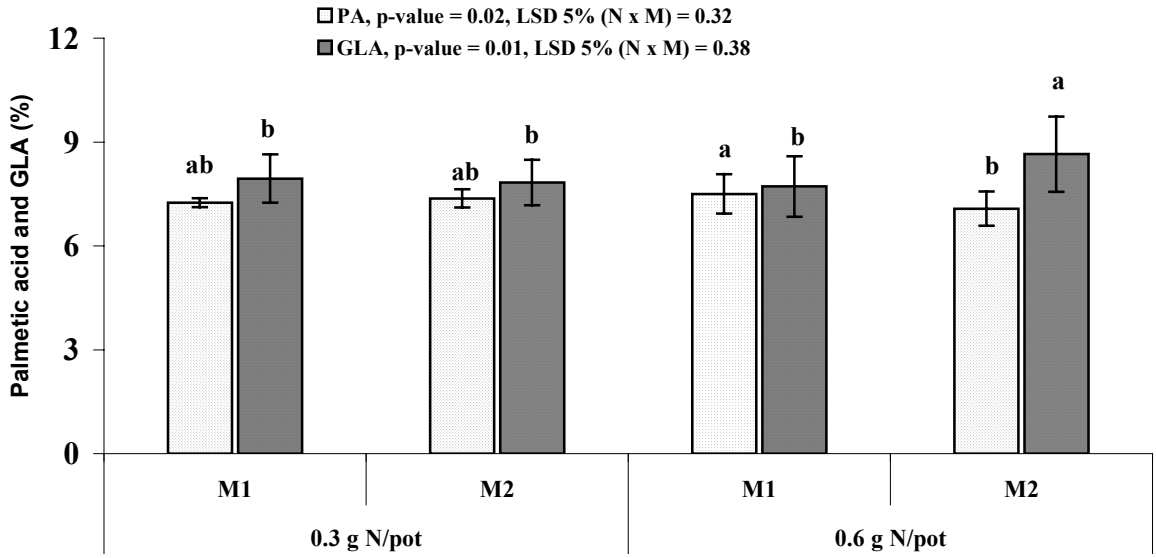
The observed interaction between harvest time and harvest method on the percentage of oleic acid indicated that at the early harvest time, the OA percentage of conventionally harvested plants and defoliated plants were similar. At the second harvest time, defoliation gave rise to lower percentage of oleic acid when compared with the conventional harvested plants. In contrast, during the third harvest date it was observed that defoliation gave rise to higher percentages of oleic acid as compared with conventionally harvested plants (Fig 4-2).

The above mentioned two times interaction was overlapped by an interaction of N×T×M (Table 4-7). The percentage of oleic acid in the seed oil varied from a minimum of 9.7 % ($N_2T_2M_1$) to a maximum of 12.0 % ($N_1T_3M_2$). Under low levels of nitrogen and all harvest



N: nitrogen; N1: 0.5 g of N/pot, N2: 1.0 g of N/pot, N3: 2.0 g of N/pot, M: harvest method; M1: conventional harvest, M2: defoliation by herbicide, bars at the top of the columns mean standard deviation

Fig 4-3. Interaction between nitrogen and harvest method on the percentage of raw protein, pot experiment spring 2004(S1)



N: nitrogen; N1: 0.5 g of N/pot, N2: 1.0 g of N/pot, N3: 2.0 g of N/pot, M: harvest method; M1: conventional harvest, M2: defoliation by herbicide, bars at the top of the columns mean standard deviation, * different letters (a, b) indicate significant differences between palmetic acid and γ-linolenic acid percentages, respectively

Fig 4-4. Interaction between nitrogen and harvest method on the percentage of palmitic acid and γ-linolenic acid, pot experiment (S1) 2004

times, defoliated plants produced higher OA than those of conventionally harvested plants. By increasing the nitrogen dose from 0.3 g to 0.6 g of N/pot, the conventionally harvested plants produced higher oleic acid percentage than defoliated plants at the first harvest time. At the later harvest times, the percentage of OA of conventionally harvested plants strongly decreased and was lower than that of defoliated plants.

Linoleic acid

The percentage of linoleic acid (LA) varied from a minimum of 71.0 % to maximum of 72.6 % in $N_1T_2M_2$ and $N_2T_2M_1$, respectively. As presented in Table 4-7, the percentage of linoleic acid was significantly influenced by nitrogen and harvest method in this experiment (Table 4-7). The pots contained 0.6 g of N produced the highest LA percentage (71.6 %).

Different harvest time did not have significant effect on the LA percentage. In contrast, the LA percentage was significantly influenced by different methods of harvest. In this experiment, conventionally harvested plants produced a higher percentage of OA than defoliated plants (Table 4-7). No interaction on LA percentage was observed in this experiment.

Gamma linolenic acid

As can be inferred from Table 4-7 the percentage of gamma linolenic acid (GLA) varied in this experiment. The lowest GLA percentage (7.1 %) was recorded in the plants that were supplied with 0.3 g of N/pot, harvested at the late harvest and defoliated before harvest. In contrast, pots containing 0.6 g of N and whose plants were harvested at the second harvest time with defoliation were observed to produce the highest GLA percentage. The percentage of GLA was strongly influenced by harvest time and harvest method. The highest percentage of GLA was recorded at the first and second harvest times. The last harvest date reduced the GLA percentage from 8.3 to 7.6% (Table 4-7). The defoliated plants produced a higher percentage of the GLA (8.2 %) than conventionally harvested plants (7.7 %).

The interaction of harvest time and harvest method on the GLA percentage indicated that, at the early and middle harvest times the GLA percentage of defoliated plants was higher than that of non-defoliated plants. In contrast, a higher GLA percentage was recorded in the defoliated plants harvested at the late harvest date (Fig 4-2). Although there was no main effect of nitrogen application on the percentage of GLA, interactions between nitrogen and harvest method (Fig 4-4) as well as nitrogen, harvest time and harvest method (Table 4-7) on the GLA percentage were observed. The interaction indicated that defoliation did not have significant influence on the GLA percentage in plants that were supplied with 0.3 g of N. As nitrogen increased from 0.3 g to 0.6 g per pot, defoliated plants produced a higher percentage of GLA than non-defoliated plants (Fig 4-4). Three times interaction on GLA revealed that, under low level of nitrogen defoliated plants produced the highest GLA percentage at the second harvest time. On the other hand, at the first and third harvest times, the GLA percentage in conventionally harvested plants was higher than defoliated plants.

4.2.3 Experiment A (autumn 2004)

4.2.3.1 Seed yield and yield components

Seed yield

In the autumn sown plants, the seed yield varied from a minimum of 11.3 g/pot in $N_1T_2M_2$ to a maximum of 28.8 g/pot in $N_3T_3M_3$ (Table ap.1). In this experiment, the seed yield was significantly influenced by different levels of nitrogen (Table 4-8). The pots that were given 2.0 g of N produced the highest seed yield (24.7 g seed/pot).

Table 4-8. Effect of different levels of nitrogen, harvest times and harvest methods on seed yield and yield components of *Oenothera biennis*. Pot experiment, autumn 2004 (A)

N	T	M	SY (g/pot)	SSP	CMS	CSS	CP	TSW (g)
0.5 g N			12.8	10.0	71.4	65.3	136.6	0.40
1.0 g N			22.2	10.6	113.4	137.5	250.9	0.41
2.0 g N			24.7	11.3	137.9	192.9	330.7	0.40
	T1		19.5	11.3	104.7	130.4	235.2	0.39
	T2		19.7	9.8	106.5	121.7	228.2	0.41
	T3		20.6	10.8	111.4	143.4	254.9	0.40
		M1	19.8	11.3	116.6	141.1	257.7	0.39
		M2	19.6	10.5	103.7	129.1	232.9	0.41
		M3	20.4	10.1	102.3	125.4	227.7	0.41
LSD 5%	N		2.0	1.2	13.3	14.0	17.9	ns
	T		ns	1.2	ns	14.0	17.9	0.02
	M		ns	ns	ns	ns	17.9	ns
	N×T		ns	ns	ns	24.2	30.1	ns
	N×M		ns	ns	ns	24.2	30.1	ns
	T×M		ns	ns	ns	ns	ns	ns
	N×T×M		ns	ns	ns	ns	ns	ns

N: Nitrogen, T: harvest time; T1: first harvest, T2: second harvest, T3: third harvest, M: harvest method; M1: drying the capsules under 40°C, M2: defoliation by herbicide, M3: conventional harvest with accelerating the dryness by hanging, SY: seed yield, SSP: side shoots per pot, CMS: capsules of main stem, CSS: capsules of side shoot, CP: capsules per pot

In contrast to that, the pots received 0.5 g of N/pot produced significantly lower seed yield than plants that were given 1.0 and 2.0 g of N/pot. Although 2.0 g of N/pot produced a significantly higher seed yield than 0.5 g of N/pot, the difference between 1.0 and 2.0 g of N/pot was not significant. In this experiment the seed yield was not influenced by harvest time and harvest method.

Thousand seed weight

In the autumn 2004 experiment (A), the thousand seed weight (TSW) of evening primrose was significantly influenced by harvest time. Under different harvest times, second harvest time (T2) produced the highest TSW. No interaction between treatments on the seed yield and TSW was observed in this experiment (Table 4-8). As it can be inferred from Table 4-8, all the measured seed yield components except TSW were significantly influenced by nitrogen.

Number of side shoots per pot

The effect of nitrogen on the number of side shoots per pot (SSP) was significant. Plants that were fertilized with 2.0 g of N produced the highest (11.3) number of side shoots per pot.

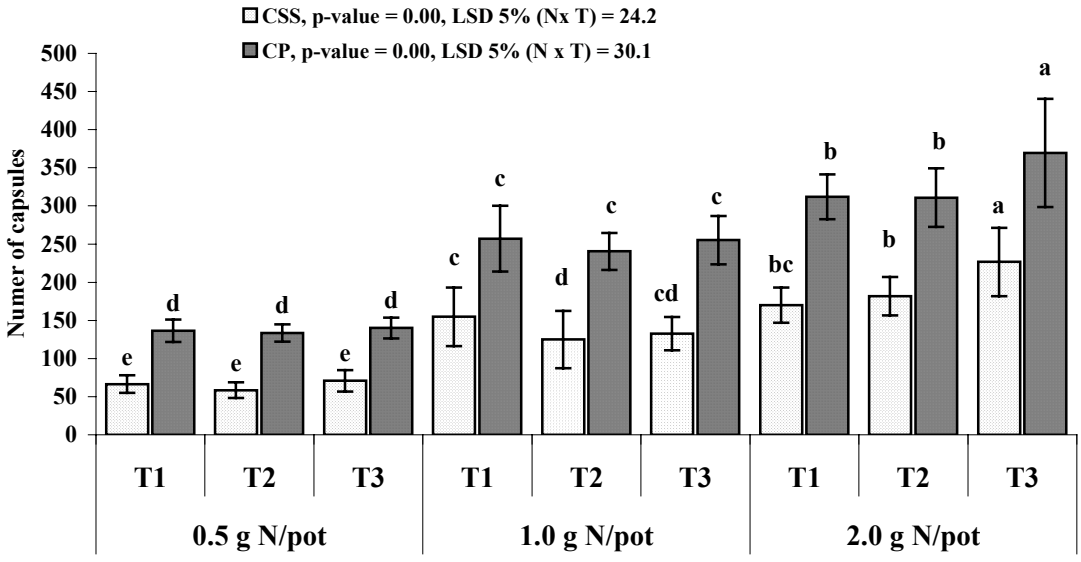
Although the harvest time had significant effect on the number of side shoots per pot, this effect did not produce a clear result. As presented in Table 4-8, SSP varied from a minimum of 9.8 at the second harvest (T2) to a maximum of 11.3 in the first harvest (T1). It can be supposed that the significant influence of harvest time interacted with other factors like plant density or weeds.

Number of capsules per main stem

Nitrogen had significant influence on the number of capsules per main stem (CMS). The pots that received 2.0 g of nitrogen produced a maximum of 138 capsules per main stem. In contrast, the minimum number of 71 capsules per main stem was observed in the pots in which nitrogen was applied at a low level (0.5 g of N/pot). In this experiment, different harvest times and harvest methods did not have any influence on the number of capsules per main stem.

Number of capsules per side shoot

The numbers of capsules per side shoot varied from 65 to 193 in the pots with 0.5 g of N and 2.0 g of N, respectively. The number of capsules per side shoot (CSS) was affected by both nitrogen and harvest time. Harvest time did not have clear effect on the number of capsules per side shoot. As Table 4-8 shows, between the firs and third harvest times no significant difference on the number of capsules per side shoot was observed (T1 and T2).



N1: 0.5 g of N/pot, N2: 1.0 g of N/pot, N3: 2.0 g of N/pot, T: Harvest time; 1: first harvest, 2: second harvest, 3: third harvest, bars at the top of the columns mean standard deviation, * different letters (a, b) indicate significant differences between CSS and CP, respectively

Fig 4-5. Interaction between nitrogen (N) and harvest time (T) on the number of CSS and CP, pot experiment (A) autumn 2004-2005

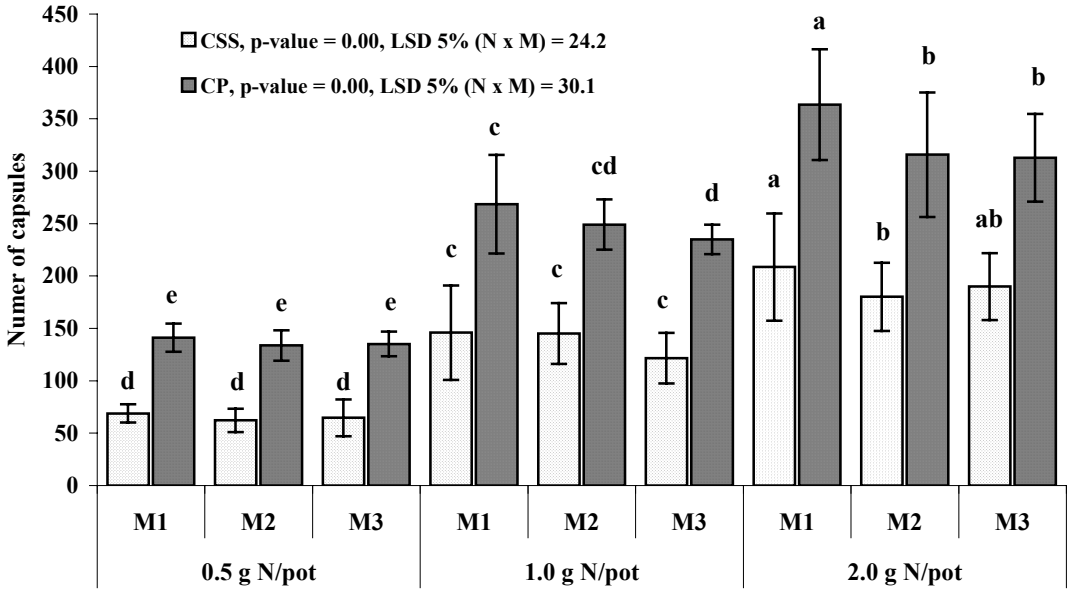
On the other hand, the difference of the number of capsules per side shoot of second and third harvest times was significant. As it has been described before a similar effect of harvest time was observed on the number of side shoot per pots. Low number of side shoots per pot might be the reason why the numbers of capsules per side shoot in second harvest time (T2) was lower than the first and the third harvest dates.

In Fig 4-5 the interaction between nitrogen and harvest time on the number of CSS indicates that the number of CSS was not influenced by different harvest times under low level of nitrogen (0.5 g of N/pot). Under moderate level of nitrogen (1.0 g of N/pot) the highest number of CSS (155) was recorded in the plants which were harvested at the first harvest time (T1). On the other hand, the plants that were given 2.0 g of N/pot produced the highest CSS (227) at the third harvest time (T3). There was also an interaction between nitrogen and harvest method on the number of capsules per side shoot (Fig 4-6). This interaction showed that, under low amount of nitrogen no significant difference was observed between different harvest methods. In contrast, under both moderate and high doses of nitrogen the highest number of CSS was recorded in the plants which capsules were dried at 40 °C (M1).

Number of capsules per pot

The number of capsules per pot (CP) was influenced by all treatments in this experiment. Number of CP varied depending on different harvest times, whereby the highest number of CP (255) was recorded in the plants which were harvested at the third harvest time (T3). Different harvest methods had significant influence on the number of CP. The plants which their capsules were dried at 40 °C (M1) produced the highest number of capsules per pot. The number of CP of the plants that were defoliated with herbicide (M2) and the plants which were conventionally harvested (M3) were similar. The interaction of nitrogen and harvest time on CP (Fig 4-5) indicated that, under low and moderate levels of nitrogen (0.5g and 1.0 g of N/pot) harvest time did not have significant difference. Contrary to that, under high level of nitrogen (2.0 g of N/pot) plants that were harvested at the third harvest time (T3) produced the highest number of capsules per pot (369 CP).

Interaction of nitrogen and harvest method (Fig 4-6) on CP revealed that, under low level of nitrogen the number of capsules per pot (CP) was not significantly influenced by different methods of harvest (Fig 4-5). In contrast, with additional nitrogen application plants whose capsules were dried at 40 °C (M1) produced the highest number of capsules per pot.



N1: 0.5 g of N/pot, N2: 1.0 g of N/pot, N3: 2.0 g of N/pot, M1: drying capsules under 40 °C, M2: defoliation by herbicide, M3: conventional harvest with accelerating the dryness by hanging, bars at the top of the columns mean standard deviation * different letters (a, b) indicate significant differences between CSS and CP, respectively

Fig 4-6. Interaction between nitrogen (N) and harvest method (M) on CSS and CP, pot experiment (A) autumn 2004-2005

4.2.3.2 Oil and protein percentage

Oil percentage

The seed oil percentage varied from a minimum of 25.2 % in $N_1T_1M_1$ to a maximum of 28.9 % in $N_1T_2M_1$ (see Table ap.2). In this experiment both nitrogen and harvest time had significant influence on the percentage of seed oil (Table 4-9). The highest percentage (28.1 %) of oil was recorded in the pots contained 0.5 g of N. On the other hand, the lowest percentage of oil (26.7 %) was recorded in the pots which were supplied with 2.0 g of N. The pots which contained 1.0 g of N produced 27.9 % oil. As presented in Table 4-9 it can be inferred that; there was no significant difference between the pots which contained 0.5 g and 1.0 g of N. Only the percentage of oil of the pots that were supplied with 2.0 g of N showed a significant difference.

Harvest time had significant influence on the seed oil percentage. The highest percentage of oil (28.0 %) was recorded in the pots harvested at the second harvest time (T2). The seed oil percentage of plants harvested at the first harvest time (T1) was significantly lower than those of plants which were harvested at the second and third harvest times. Harvest method did not have significant influence on the seed oil percentage in this experiment (Table 4-9).

Protein percentage

The raw protein percentage of seeds varied from 12.1 % in $N_1T_3M_1$ to 16.7 % in $N_3T_3M_3$ (Table ap.2). The percentage of seed raw protein was significantly influenced by nitrogen in this experiment (Table 4-9). There was a positive relationship between the nitrogen application and the percentage of seed raw protein i.e. the higher the amount of N/pot the higher the percentage of raw protein and vice versa. The pots that were given 0.5 g of N produced the lowest percentage of raw protein (12.6 %). In contrast, the highest percentage of raw protein was observed in the pots containing 2.0 g of N. There was a significant difference between all levels of nitrogen. No interaction between different treatments on the percentage of raw protein was observed in this experiment (Table 4-9).

4.2.3.3 Fatty acid composition

Palmetic acid

Nitrogen influenced the percentage of all the measured fatty acids of evening primrose seed oil except stearic acid (Table 4-9). The highest percentage of palmitic acid (PA) was produced in the pots which were supplied with 0.5 g of N. Harvest time had significant effect on the percentage of PA in this experiment. The lowest percentage of PA was produced in the pots which were harvested at the third harvest time. There was no significant difference between the first and the second harvest times. Harvest method had no significant influence on the PA percentage.

Oleic acid

The data in Table 4-9 show that, the application of different doses of N/pot had a significant effect on the oleic acid (OA) accumulation. Plants that were given 2.0 g of N/pot produced the lowest percentage of OA (9.7 %). On the other hand, plants that were supplied with 0.5 g of N/pot produced the highest percentage of OA. The results of statistical analysis show that there is a significant difference between all levels of nitrogen. Different harvest times and harvest methods had no effect on the percentage of OA in this experiment.

Linoleic acid

Nitrogen had significant influence on the percentage of linoleic acid (LA). The pots that received 2.0 g of N produced a LA percentage of 73.4 %. It was significantly higher than the values of the LA in the plants that were supplied with 0.5 g and 1.0 g of N. The LSD value

shows that there is no significant difference between 0.5 g of N/pot and 1.0 g of N/pot. No interaction between three different treatments on the LA was observed in this experiment.

Table 4-9. Effect of different levels of nitrogen, harvest times and harvest methods on the percentage of oil, raw protein and fatty acid composition of *Oenothera biennis*, pot experiment (A) autumn 2004-2005

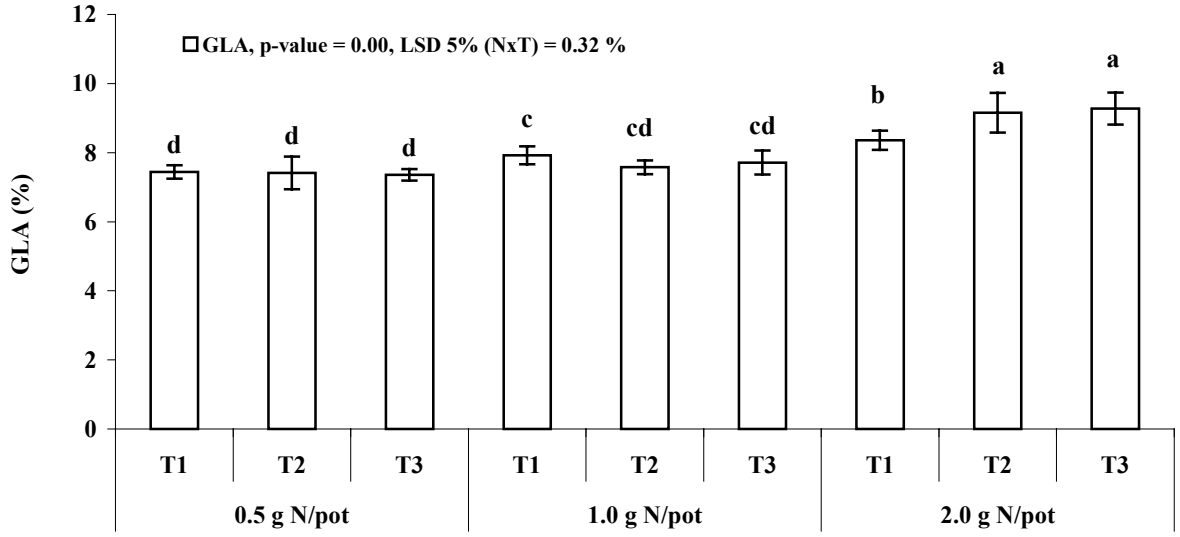
N	T	M	SO (%)	SP (%)	PA (%)	SA (%)	OA (%)	LA (%)	GLA (%)
0.5 g N			28.1	12.6	5.7	2.2	11.5	72.2	7.4
1.0 g N			27.9	13.9	5.5	2.2	10.8	72.3	7.7
2.0 g N			26.7	15.7	5.4	2.2	9.7	73.4	8.9
	T1		27.0	13.8	5.7	2.2	10.7	72.4	7.9
	T2		28.0	14.1	5.7	2.2	10.8	72.7	8.0
	T3		27.7	14.2	5.3	2.2	10.5	72.8	8.1
		M1	27.7	13.9	5.6	2.1	10.5	72.7	8.0
		M2	27.9	14.1	5.6	2.3	10.6	72.6	8.1
		M3	27.7	14.1	5.4	2.2	10.9	72.6	7.9
LSD $_{5\%}$	N		0.58	0.41	0.15	ns	0.47	0.55	0.19
	T		0.58	ns	0.15	ns	ns	ns	ns
	M		ns	ns	ns	0.10	ns	ns	ns
	N×T		ns	ns	ns	ns	ns	ns	0.32
	N×M		ns	ns	ns	ns	ns	ns	ns
	T× M		ns	ns	ns	ns	ns	ns	ns
	N×T×M		ns	ns	ns	ns	ns	ns	ns

N: Nitrogen, T: harvest time; T1: first harvest, T2: second harvest, T3: third harvest, M: harvest method; M1: drying the capsules under 40°C, M2: defoliation by herbicide, M3: conventional harvest with accelerating the dryness by hanging, SO: seed oil, SP: seed protein, PA; palmitic acid, SA; stearic acid, OA: oleic acid, LA; linolenic acid, GLA; γ- linolenic acid

Gamma linolenic acid

As presented in Table 4-9, it seems that additional nitrogen application increases the GLA percentage of the seed oil. The highest percentage of GLA (8.9 %) was recorded in the pots that were given 2.0 g of N. The increase in the percentage of GLA was found to exhibit a direct proportional relationship with increasing amount of nitrogen. For example, it was observed that when the nitrogen amount was increased from 0.5 to 1.0 g/pot the increase in GLA was less than when it was increased from 1.0 to 2.0 g of N/pot.

Interaction between nitrogen and harvest time indicated that, with low amount of nitrogen, no change was observed in the percentage of GLA of the seeds which were harvested in different harvest times. In contrast, in the pots that were supplied with high level of nitrogen the GLA percentage was increased by delayed harvest (Fig. 4-7).



N: Nitrogen, T: harvest time; T1: first harvest, T2: second harvest, T3: third harvest, bars at the top of the columns mean standard deviation

Fig 4-7. Interaction between nitrogen (N) and harvest time (T) on GLA percentage of evening primrose, pot experiment (A) autumn 2004-2005

4.2.4 Experiment S2 (spring 2005)

4.2.4.1 Seed yield and seed yield components

Seed yield

In the spring 2005 experiment, the seed yield of evening primrose varied from a minimum of 14.3 g/pot in $N_1T_2M_1$ to a maximum of 33.6 g/pot in $N_3T_2M_1$ (Table 4-10). In this experiment the seed yield was significantly affected by nitrogen. Plants that were given 0.5 g of N/pot produced a lower seed yield (16.5 g/pot) than plants supplied with 1.0 and 2.0 g of N/pot. Increasing the amount of nitrogen led to a significant increase in the seed yield (25.1 g/pot in 1.0 g of N and 29.5 g/pot in 2.0 g of N).

The seed yield was significantly influenced by different harvest times. This means that the pots which were harvested at the third harvest date produced the highest seed yield (25.4 g of N/pot). Looking at Table 4-10 it can be seen that, there was no significant difference between the second and the third harvest times. Interaction between nitrogen and harvest time on seed yield indicated that, under low level of nitrogen different harvest times did not make significant influence. On the other hand, under the moderate and the high levels of nitrogen the seed yield was increased by delayed harvest (Fig 4-8).

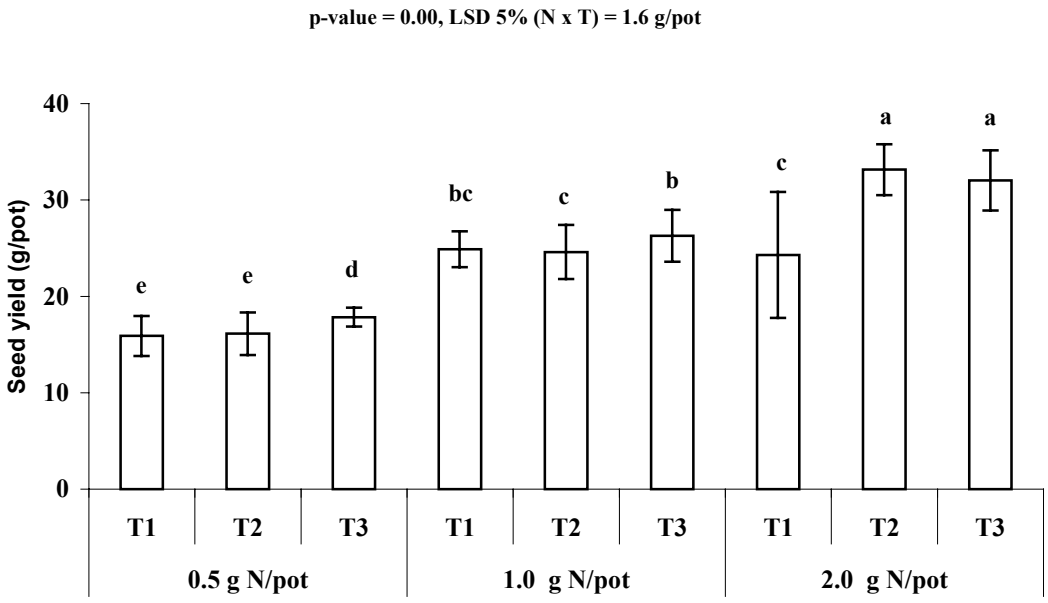
Thousand seed weight

Nitrogen did not influence the thousand seed weight (TSW) of evening primrose in this experiment. On the other hand, a significant difference of TSW depending on different harvest times was observed in this experiment. From Table 4-10 it can be inferred that the plants which were harvested at the first harvest time produced the lowest TSW. There was not any significant difference between the second and the third harvest times. Harvest method did not have significant influence on TSW.

Significant interactions on TSW was observed between nitrogen, harvest time and harvest method (Table 4-10). It can be supposed that this three times interaction is due to low TSW of the plants which were supplied with 2.0 g of nitrogen and were conventionally harvested at the first harvest date (see Table 4-10).

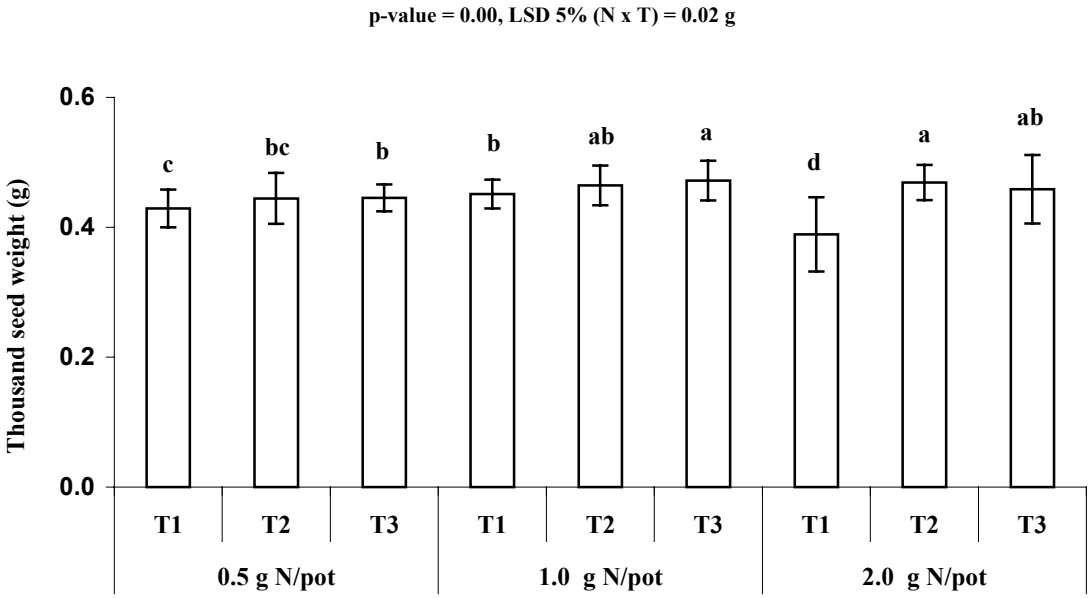
Side shoots per pot

In all the measured yield components (SSP, CSS, CP and TSW) interactions between the treatments were found. The number of side shoots per pot (SSP) ranged from 5.7 ($N_1T_2M_3$) to 13.3 ($N_3T_2M_3$). Table 4-10 shows that, there is a positive effect of nitrogen on the number of side shoots per pot (SSP). In contrast to that, no main effect of harvest time as well as harvest method on SSP was observed in this experiment.



N: Nitrogen, T: harvest time; T1: first harvest, T2: second harvest, T3: third harvest, bars at the top of the columns mean standard deviation

Fig 4-8. Effect of nitrogen and harvest time on seed yield per pot of evening primrose, pot experiment (S2) spring 2005



N: Nitrogen, T: harvest time; T1: first harvest, T2: second harvest, T3: third harvest, bars at the top of the columns mean standard deviation

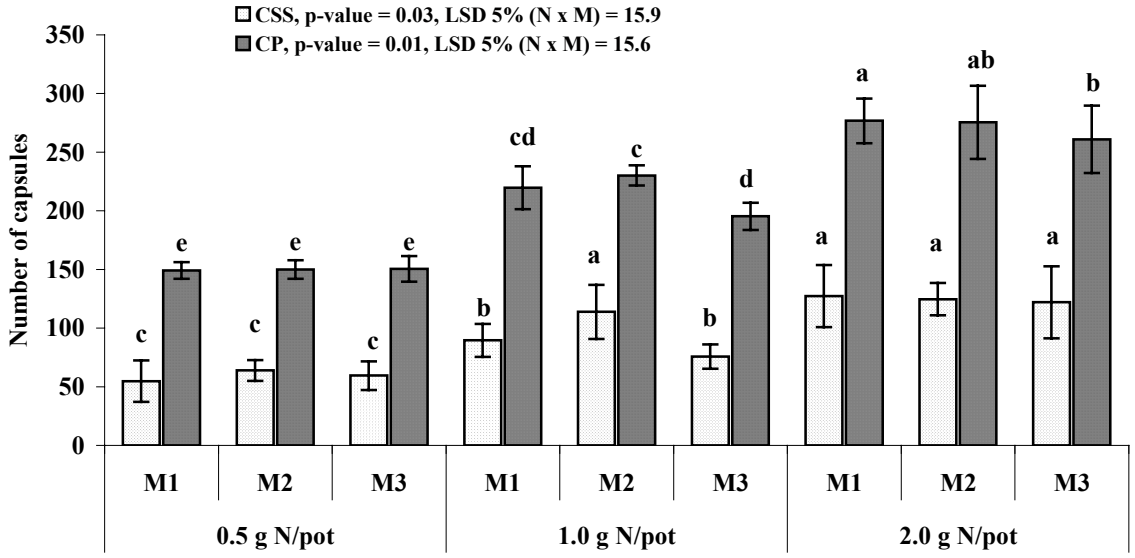
Fig 4-9. The effect of nitrogen and harvest time (T) on TSW, pot experiment (S2) spring 2005

Number of capsules per main stem

Nitrogen significantly influenced the number of capsules per main stem (CMS). The pots that were given 2.0 g of N produced the highest number of capsules per main stem (145 CMS). On the contrary, the lowest CMS (91) was observed in the pots containing the low level of nitrogen (0.5 g of N/pot). Different harvest times and harvest methods did not have any influence on CMS. Between different factors, no interaction on CMS was observed (see Table 4-10).

Number of capsules per side shoot

The number of capsules per side shoots (CSS) varied from 46 to 155 and was significantly influenced by different doses of nitrogen. There were no significant differences between different harvest times. The plants which were harvested at the third harvest date produced more capsules per side shoots than other harvest times. Harvest method had a significant influence on the number of capsules per side shoot (Table 4-10). Plants which were defoliated using herbicide (M2) produced the highest CSS (100 CSS).



N1: 0.5 g of N/pot, N2: 1.0 g of N/pot, N3: 2.0 g of N/pot, M1: drying capsules under 40 °C, M2: defoliation by herbicide, M3: conventional harvest with accelerating the dryness by hanging, bars at the top of the columns mean standard deviation, * different letters (a, b) indicate significant differences between CSS and CP, respectively

Fig 4-10. Interaction between nitrogen and harvest method on the number of CSS and CP, pot experiment (S2) spring 2005

Interactions were observed between the levels of nitrogen and harvest method (Fig 4-10), harvest time and harvest method (Fig 4-11), as well as three times interaction of all treatments (Table 4-10) on CSS. The interaction of nitrogen and harvest method indicated that, with low and moderate levels of nitrogen, plants which were defoliated using herbicide produced the highest number of CSS. In contrast, under high level of nitrogen no significant difference between different harvest methods was observed (Fig 4-10).

The interaction of harvest time and harvest method on CSS reveals that, at first harvest time, plants which were conventionally harvested (method M3) produced a lower CSS than plants which were defoliated using herbicide (method M2). Contrary to that, a delaying harvest did not show the above mentioned difference (Fig 4-11).

Number of capsules per pot

In the spring 2005 experiment, the number of capsules per pot (CP) varied from 144 in $N_1T_3M_3$ to 278 in $N_3T_3M_1$ (Table 4-10). In this experiment, CP was significantly influenced by nitrogen and harvest method. As it has been shown in Table 4-10, pots which were given 0.5 g of N produced the lowest CP of 150.

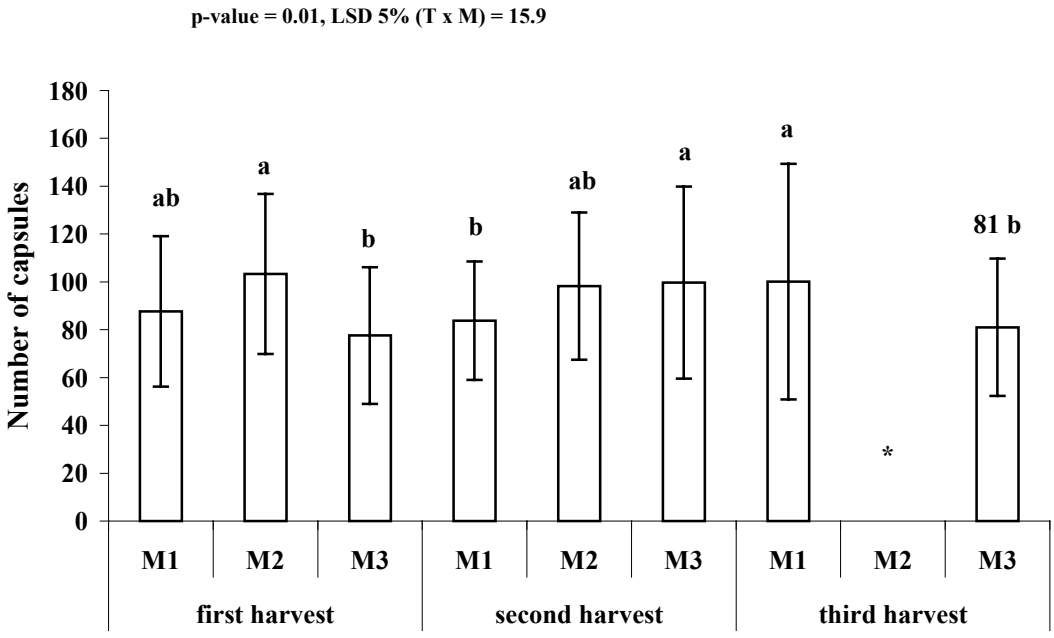
Table 4-10. Effect of nitrogen application, harvest times and harvest methods on seed yield and seed yield components of *Oenothera biennis*. Pot experiment, spring 2005(S2)

N	T	M	SY (g/pot)	SSP	CMS	CSS	CP	TSW (g)
0.5 g N	T1	M1	15.2	8.7	84.0	64.7	148.7	0.42
		M2	16.5	7.7	86.7	65.7	152.3	0.44
		M3	16.0	7.3	95.3	58.7	154.0	0.43
	T2	M1	14.3	6.0	97.0	53.7	150.7	0.42
		M2	16.3	6.3	85.7	62.0	147.7	0.46
		M3	17.8	5.7	86.7	66.7	153.3	0.45
	T3	M1	18.5	6.7	102.7	45.7	148.3	0.46
		M2	-	-	-	-	-	-*
		M3	17.2	7.0	91.3	53.0	144.3	0.43
1.0 g N	T1	M1	25.9	9.0	132.3	74.3	206.7	0.44
		M2	24.8	9.7	110.0	122.3	232.3	0.45
		M3	24.0	6.7	123.3	67.3	190.7	0.47
	T2	M1	26.5	9.0	139.0	94.3	233.3	0.48
		M2	24.8	7.7	122.7	105.3	228.0	0.46
		M3	22.5	6.0	107.3	85.3	192.7	0.46
	T3	M1	27.6	7.3	119.0	100.0	219.0	0.48
		M2	-	-	-	-	-	-*
		M3	24.9	7.3	128.0	74.3	202.3	0.47
2.0 g N	T1	M1	26.6	9.7	171.3	124.0	295.0	0.44
		M2	27.6	9.7	169.0	122.0	291.0	0.39
		M3	18.7	8.0	135.0	106.7	241.7	0.34
	T2	M1	33.6	9.3	153.7	103.3	257.0	0.46
		M2	33.5	7.3	132.3	127.3	259.7	0.50
		M3	32.6	13.3	123.0	147.7	273.7	0.45
	T3	M1	30.6	11.3	123.0	154.7	277.7	0.44
		M2	-	-	-	-	-	-*
		M3	32.3	8.3	154.7	112.7	267.3	0.48
0.5 g N			16.5	6.9	91.2	58.8	149.9	0.44
1.0 g N			25.1	7.8	122.7	90.4	213.1	0.46
2.0 g N			29.5	9.6	145.4	124.5	270.0	0.43
	T1		21.7	8.5	123.0	89.5	212.5	0.42
	T2		24.7	7.2	115.5	87.3	202.8	0.46
	T3		25.4	8.0	119.8	90.1	209.8	0.46
		M1	24.3	8.6	124.7	90.5	215.2	0.45
		M2	23.9	8.1	117.7	100.8	218.5	0.45
		M3	22.9	7.7	116.5	85.7	202.2	0.44
LSD 5%	N		1.6	0.95	11.9	9.2	9.0	0.02
	T		1.6	ns	ns	ns	ns	0.02
	M		ns	ns	ns	9.2	9.0	ns
	N×T		2.8	ns	ns	ns	ns	0.03
	N×M		ns	ns	ns	15.9	15.6	ns
	T× M		ns	ns	ns	15.9	ns	ns
	N×T×M		ns	2.9	ns	27.6	27.1	0.05

N: Nitrogen, T: harvest time; T1: first harvest, T2: second harvest, T3: third harvest, M: harvest method; M1: drying the capsules under 40°C, M2: defoliation by herbicide, M3: conventional harvest with accelerating the dryness by hanging, SY: seed yield, SSP: side shoot per pot, CMS: capsules of main stem, CSS: capsules of side shoot, CP: capsules per pot, * Siccation was not applied

Additional nitrogen application increased the CP to 270 capsules per pot. All levels of nitrogen supplied produced significant difference in CP. Different harvest methods significantly influenced the number of CP. The pots that were defoliated before harvest produced a higher number of capsules than other harvest methods (Table 4-10).

In this experiment, an interaction between nitrogen, harvest time and harvest method on CP was observed (Table 4-10). This interaction indicated that, under the low level of nitrogen (0.5 g of N/pot) CP was not influenced by different methods of harvest. Under moderate level of nitrogen, the highest CP was observed in the plants which were defoliated using herbicide (M2). In contrast to that, in pots with 2.0 g of nitrogen, the highest CP was observed in the plants whose capsules were dried at the 40 °C (Fig 4-10).



N1: 0.5 g of N/pot, N2: 1.0 g of N/pot, N3: 2.0 g of N/pot, M1: drying capsules under 40 °C, M2: defoliation by herbicide, M3: conventional harvest with accelerating the dryness by hanging, bars at the top of the columns mean standard deviation, * Siccation at the third harvest time was not applied

Fig 4-11. Interaction between harvest time and harvest method on the number of CSS of evening primrose, pot experiment (S2) spring 2005

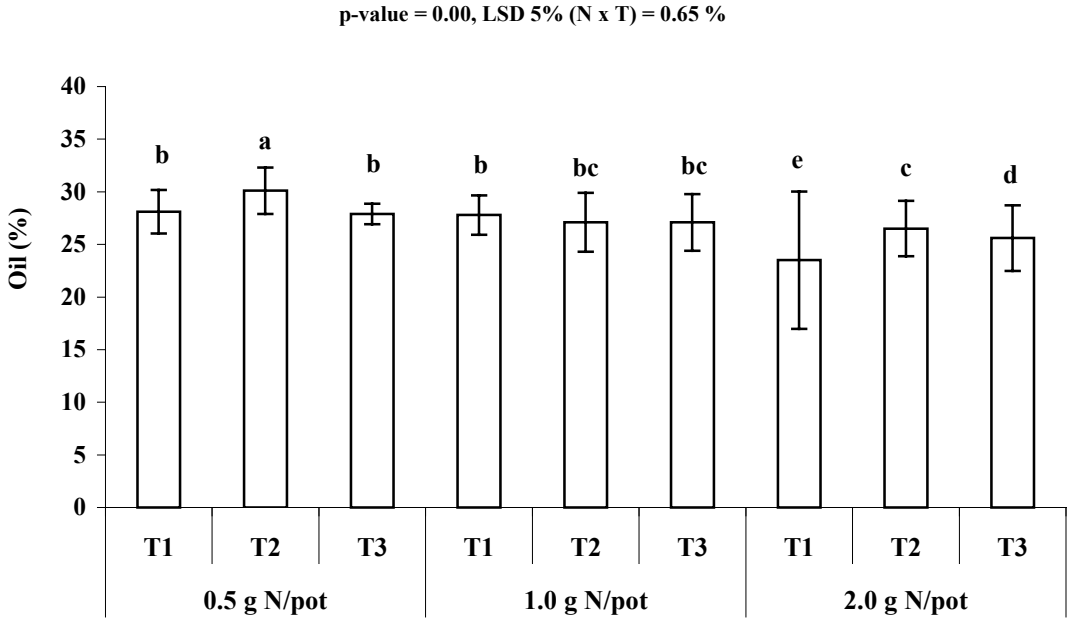
4.2.4.2 Oil and protein percentage

Oil percentage

As showed in Table 4-11, the percentage of seed oil was significantly influenced by the interaction of nitrogen fertilization, harvest time and harvest method.

The percentage of oil ranged from a minimum of 22.3 % ($N_3T_1M_1$) to a maximum of 30.5 % (N_1 T_1 M_1). Depending on the harvest time the percentage of oil varied from a minimum of 26.5 % to a maximum of 28.1 %. The highest percentage of oil was recorded at the second harvest time (28.1 %).

Interaction of nitrogen and harvest time observed in this experiment indicated that, under low level nitrogen (0.5 g of N/pot) the highest percentage of oil was observed at the second harvest time. In contrast, in the pots with moderate level of nitrogen no significant difference between different harvest times was observed. Under the high level of nitrogen, the seed oil percentage of the plants which were harvested at the early harvest time was significantly low and delayed harvest induced the oil accumulation.



N1: 0.5 g of N/pot, N2: 1.0 g of N/pot, N3: 2.0 g of N/pot, T1: first harvest time, T2: second harvest time, T3: third harvest time, bars at the top of the columns mean standard deviation

Fig 4-12. Interaction between nitrogen fertilization and harvest method on the percentage of oil of evening primrose seed, pot experiment (S2) spring 2005

Protein percentage

It can be inferred from Table 4-11 that the percentage of raw protein was significantly influenced by nitrogen. There was a positive effect of nitrogen on the percentage of raw protein. The higher the amount of nitrogen per pots, the higher the percentage of raw protein in the corresponding seeds and vice versa. The pots that contained 0.5 g of N produced the lowest percentage of raw protein (12.6 %). Contrary to that the highest percentage of raw

protein (15.6 %) was observed in the pots which received 2.0 g of N. Different harvest times and harvest methods had no significant difference on the percentage of raw protein.

4.2.4.3 Fatty acid composition

Palmetic acid

The measured fatty acids were significantly influenced by nitrogen in this experiment. Interactions of N×T and T×M on PA were observed in this experiment (Table 4-11). The interaction of nitrogen and harvest time indicated that, pots which received 2.0 g of N showed a significantly higher percentage of PA at the early time. With 0.5 and 1.0 grams nitrogen per pot, no significant differences between different harvest times were observed. With the T×M interaction, conventionally harvest method induced higher PA percentage after early harvest (T1). With delayed harvest, no effect of harvest method on PA was observed.

Oleic acid

The percentage of oleic acid varied from a minimum of 9.3 % in $N_3T_2M_2$ to a maximum of 13.1 % in $N_1T_1M_3$. The data in Table 4-11 show that the application of different doses of nitrogen had a significant effect on oleic acid (OA) accumulation. This effect indicated that, the pots which were given 2.0 g of nitrogen produced the lowest percentage of OA (10.3 %). Contrary to that pots with 0.5 g of N produced the highest percentage of OA (11.2 %). Apart from this observation, there were no significant differences between the 0.5 g of N and 1.0 g of N. The percentage of oleic acid was affected by several interactions between the treatments (N×T, N×M, N×T×M). Generally, a small reduction of OA percentage was observed with increased N fertilization. This effect was modified by different harvest times and harvest methods.

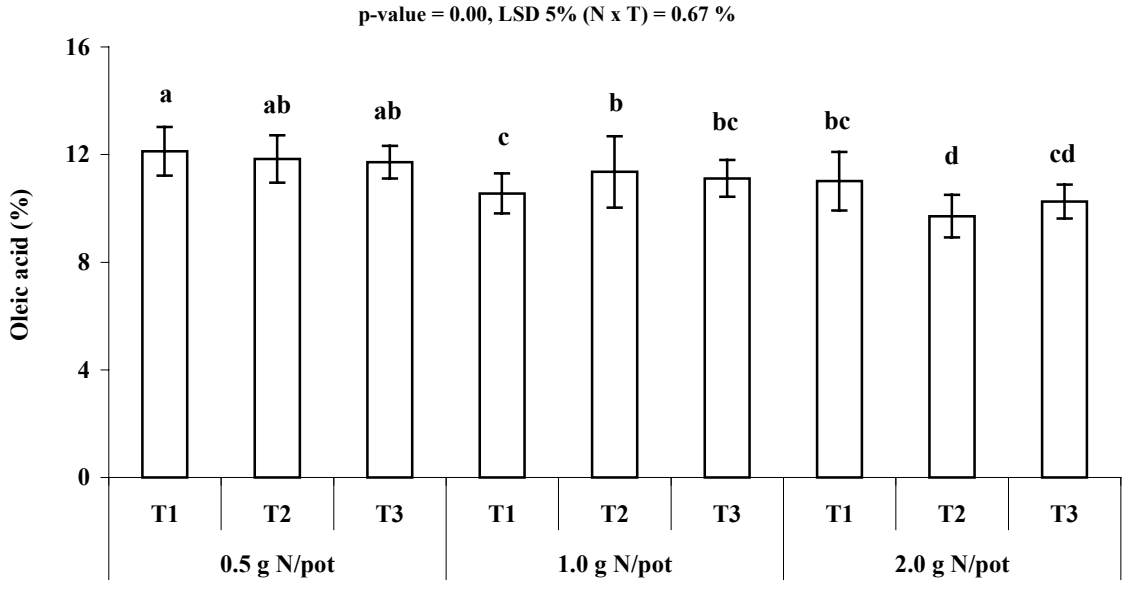
The lowest OA percentage was obtained from the pots that were defoliated before the harvest (Table 4-11). Interaction of nitrogen and harvest time shows that, under 0.5 g of N, harvest time did not have significant effect on OA percentage. Moderate level of nitrogen produced a significant reduction in the percentage of OA of the plants which were harvested at the first harvest time. As the level of nitrogen was increased to 2.0 g/pot, a reduction in the percentage of OA was observed. At the second harvest time, this reduction was stronger than that of the first and the third harvest times (Fig 4-13).

The interaction of nitrogen and harvest method on oleic acid showed that, under low and moderate level of nitrogen, the highest percentage of OA was recorded in the pots from which

the plants were hanged after harvest (M3). In contrast to that, the same method (M3) produced the lowest percentage of OA when high level of nitrogen was applied (Fig 4-14).

Linoleic acid

The percentage of linoleic acid (LA) varied from 70.3 % in $N_1T_1M_1$ to 73.8 % in $N_3T_2M_2$ (Table 4-11). Nitrogen had significant influence on LA accumulation. As nitrogen was increased from 0.5 to 1.0 g/pot, the LA percentage increased to 73.0 %. Additional nitrogen application reduced the LA to 72.5 %. In this experiment the interaction between nitrogen, harvest time and harvest method on the percentage of LA indicated that, with low nitrogen application (0.5 g of N/pot) and when the harvested capsules were dried at 40 °C, the LA percentage gradually increased with delayed harvest. In contrast, under moderate and high levels of nitrogen and delayed harvest a reduction in the percentage of LA was observed.



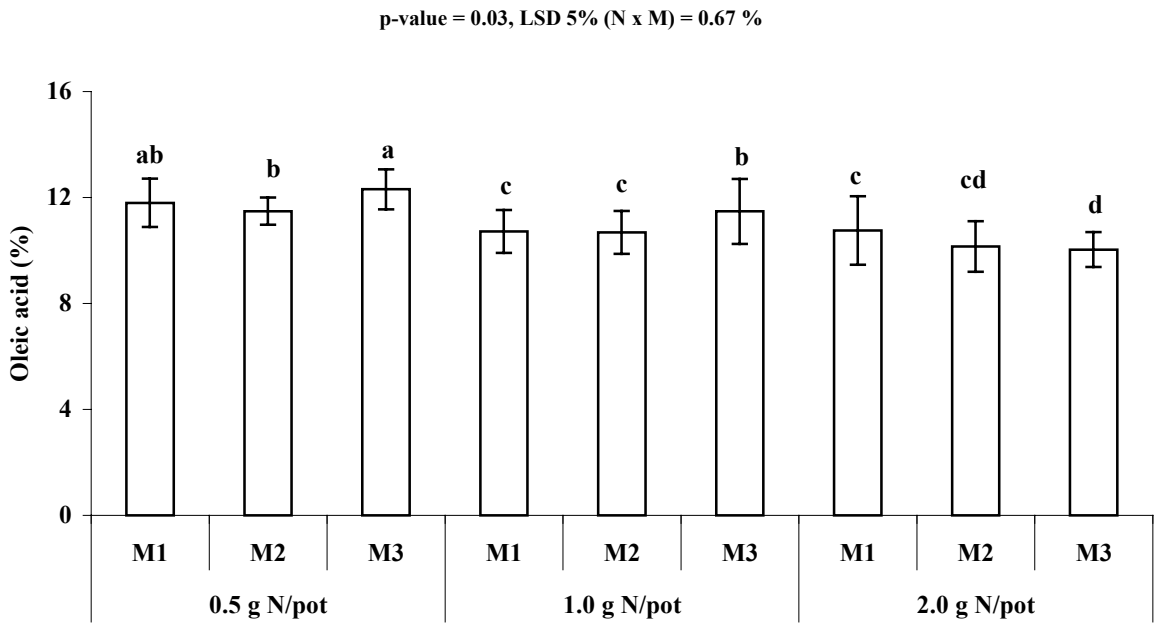
N1: 0.5 g of N/pot, N2: 1.0 g of N/pot, N3: 2.0 g of N/pot, T1: first harvest time, T2: second harvest time, T3: third harvest time, M1: drying capsules under 40 °, M2: defoliation by herbicide, M3: conventional harvest with accelerating the dryness by hanging , bars at the top of the columns mean standard deviation

Fig 4-13. Interaction between nitrogen fertilization and harvest time on OA percentage of evening primrose seed oil, pot experiment (S2) spring 2005

Table 4-11. Effect of nitrogen application, harvest times and harvest methods on the percentage of oil, raw protein and fatty acid composition of *Oenothera biennis* seeds, Pot experiment, spring 2005 (S2)

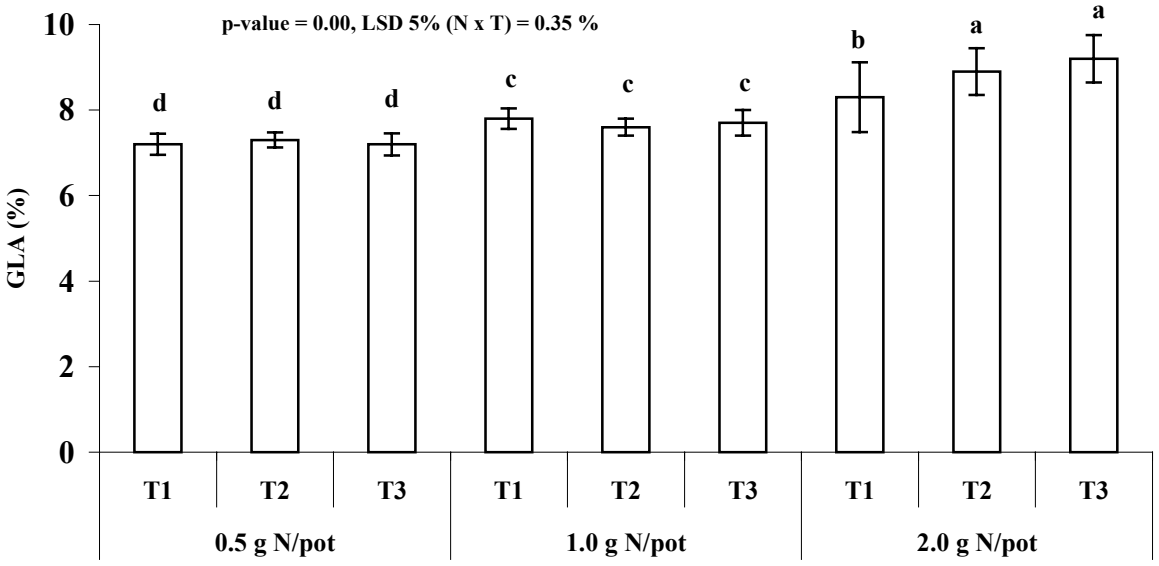
N	T	M	SO (%)	SP (%)	PA (%)	SA (%)	OA (%)	LA (%)	GLA (%)
0.5 g N	T1	M1	27.6	12.3	5.4	2.5	12.0	70.3	7.0
		M2	27.2	12.7	5.5	2.4	11.2	72.8	7.3
		M3	29.4	12.4	5.6	2.5	13.1	71.6	7.2
	T2	M1	29.7	12.1	5.6	2.4	12.0	71.8	7.3
		M2	30.1	12.4	5.5	2.3	11.7	72.5	7.2
		M3	30.5	13.8	5.5	2.3	11.8	72.6	7.2
	T3	M1	28.1	12.9	5.5	2.3	11.4	73.2	7.0
		M2	-	-	-	-	-	-	-*
		M3	27.7	12.6	5.6	2.4	12.0	71.8	7.4
1.0 g N	T1	M1	29.3	13.9	5.6	2.3	10.7	73.5	7.7
		M2	27.8	14.5	5.6	2.4	10.1	73.7	7.8
		M3	26.5	13.3	5.6	2.3	10.8	72.9	8.0
	T2	M1	27.7	13.9	5.7	2.5	10.1	73.6	7.8
		M2	26.7	13.8	5.7	2.4	11.2	72.7	7.6
		M3	27.0	13.8	5.7	2.4	12.8	71.5	7.4
	T3	M1	27.0	13.3	5.6	2.4	11.4	72.6	7.8
		M2	-	-	-	-	-	-	-*
		M3	27.2	13.7	5.5	2.4	10.8	73.2	7.6
2.0 g N	T1	M1	22.3	15.1	5.7	2.4	12.2	70.9	8.2
		M2	22.5	15.6	6.1	2.3	11.0	72.2	8.4
		M3	25.8	15.4	6.0	2.2	9.9	73.2	8.3
	T2	M1	26.2	15.7	5.9	2.2	10.2	72.7	8.6
		M2	26.5	15.4	5.6	2.1	9.3	73.8	9.1
		M3	26.5	14.9	5.7	2.3	9.6	72.8	8.2
	T3	M1	25.8	16.0	5.7	2.2	9.9	72.5	9.6
		M2	-	-	-	-	-	-	-*
		M3	25.4	16.3	5.6	2.2	10.6	72.5	8.7
0.5 g N			28.8	12.6	5.5	2.4	11.2	72.1	7.2
1.0 g N			27.4	13.8	5.6	2.4	11.0	73.0	7.7
2.0 g N			25.2	15.6	5.8	2.2	10.3	72.5	8.7
	1		26.5	13.9	5.7	2.4	11.2	72.1	7.8
	2		28.1	13.9	5.6	2.3	11.1	72.7	7.9
	3		26.8	13.9	5.6	2.3	11.0	72.5	8.0
		1	27.1	13.8	5.6	2.4	11.1	72.4	7.9
		2	26.8	14.1	5.7	2.3	10.8	72.8	7.9
		3	27.4	13.9	5.6	2.3	11.3	72.5	7.9
LSD $_{5\%}$	N		0.67	0.38	0.10	0.10	0.39	0.53	0.20
	T		0.67	ns	ns	ns	ns	ns	ns
	M		ns	ns	ns	ns	0.39	ns	ns
	N×T		1.2	ns	0.16	ns	0.67	ns	0.35
	N×M		ns	ns	ns	ns	0.67	ns	ns
	T× M		ns	ns	0.16	ns	ns	ns	ns
	N×T×M		2.17	ns	ns	ns	1.20	1.60	ns

N: Nitrogen, T: harvest time; T1: first harvest, T2: second harvest, T3: third harvest, M: harvest method; M1: drying the capsules under 40°C, M2: defoliation by herbicide, M3: conventional harvest with accelerating the dryness by hanging, SO: seed oil, SP: seed protein, PA; palmitic acid, SA; stearic acid, OA: oleic acid, LA; linolenic acid, GLA; γ- linolenic acid, *Siccation at the third harvest time was not applied



N1: 0.5 g of N/pot, N2: 1.0 g of N/pot, N3: 2.0 g of N/pot, T1: first harvest time, T2: second harvest time, T3: third harvest time, M1: drying capsules under 40 °C, M2: defoliation by herbicide, M3: conventional harvest with accelerating the dryness by hanging, bars at the top of the column mean standard deviation

Fig 4-14. Interaction between nitrogen and harvest method on OA percentage of evening primrose seed oil, pot experiment (S2) spring 2005



N1: 0.5 g of N/pot, N2: 1.0 g of N/pot, N3: 2.0 g of N/pot, T1: first harvest time, T2: second harvest time, T3: third harvest time, bars at the top of the columns mean standard deviation

Fig 4-15. Interaction between nitrogen fertilization and harvest time on GLA percentage of evening primrose, pot experiment (S2) spring 2005

Gamma linolenic acid

In the spring 2005 experiment the gamma linolenic acid (GLA) percentage of evening primrose varied from a minimum of 7.0 % in $N_1T_1M_1$ to a maximum of 9.6 % in $N_3T_3M_1$ (Table 4-11). In this experiment, different levels of nitrogen significantly influenced the percentage of GLA. An interaction between nitrogen fertilization and harvest time on GLA percentage indicated that with 0.5 g of N and 1.0 g of N no significant effect of harvest time on GLA percentage was observed. In contrast, a delay in harvest date in pots containing 2.0 g of nitrogen produced a significant increase in the GLA percentage (9.2 % at the third harvest).

4.4 Effects of storage time and storage temperature on the seed quality

As it can be inferred from Table 4-12 the most of the measured parameters involved in the quality of evening primrose seed (i.e. oil and gamma linolenic acid percentage, acid value and peroxide value) were significantly affected by storage time or by interaction of storage time and storage temperature.

4.4.1 Oil and protein percentage

Oil percentage

From the results of four months of storage in which the oil analysis of the seed samples were carried out, it can be inferred that there is a significant effect of storage time on seed oil percentage of evening primrose.

The oil percentage of seeds varied from a maximum of 26.1 % after one month of storage to a minimum of 24.8 % after four months of storage. During the first two months of storage, no significant difference in the percentage of seed oil was observed. As the storage time increased, a rapid decrease of the percentage of oil compared to the pervious months was recorded (24.3 % in contrast to control 26.0%). In this experiment, different storage temperatures did not significantly influence the percentage of oil of the seeds and no interaction between storage time and storage temperature on the percentage of oil was observed.

Protein percentage

The percentage of raw protein varied from a minimum of 13.4 % in control to a maximum of 15.3 % in samples stored under low temperature over a period of four months. A significant influence of storage time on the percentage of raw protein of seed was found. As storage progressed, an increase in the percentage of raw protein of stored seed from 13.4 % to 15.4 %

was detected. Different temperatures did not significantly affect the percentage of raw protein. No interaction between treatments on the percentage of raw protein was observed in this experiment.

Table 4-12. Effect of storage conditions (times and temperature) on quality changes in evening primrose seed

ST	TEM	SO (%)	PA (%)	SA (%)	OA (%)	LA (%)	GLA (%)	AV (%)	PV*	SP (%)
Control		26.0	6.1	2.1	10.3	73.4	8.8	2.1	1.2	13.4
ST1	TEM1	25.7	6.2	2.1	10.5	72.7	8.8	2.2	2.8	13.4
	TEM2	26.8	6.2	2.1	10.5	73.2	9.2	2.1	2.3	13.6
	TEM3	25.8	6.3	2.1	10.7	72.4	8.9	2.3	2.6	14.9
ST2	TEM1	25.8	6.1	1.9	10.6	72.5	8.7	2.4	3.3	14.4
	TEM2	26.3	6.1	1.8	10.2	72.6	8.9	2.3	2.7	14.5
	TEM3	25.4	6.0	1.9	10.7	72.2	8.6	2.7	3.8	13.8
ST3	TEM1	25.0	6.1	1.9	10.7	71.6	8.7	2.6	15.5	15.0
	TEM2	25.4	6.1	1.8	10.4	72.3	8.8	2.5	16.0	15.1
	TEM3	24.9	6.0	1.9	10.8	71.7	8.6	2.9	16.9	15.2
ST4	TEM1	24.5	6.0	1.8	10.7	71.5	7.7	3.1	22.9	15.1
	TEM2	24.9	6.2	1.9	10.6	72.0	7.8	2.7	18.6	15.3
	TEM3	24.3	6.3	1.8	10.8	71.2	7.3	3.4	22.5	15.1
ST1		26.1	6.3	2.1	10.5	72.8	9.0	2.2	2.6	14.0
ST2		25.8	6.1	1.8	10.5	72.4	8.7	2.5	3.3	14.3
ST3		25.1	6.1	1.9	10.6	71.9	8.7	2.7	16.1	15.4
ST4		24.5	6.2	2.2	10.7	71.6	7.9	3.1	22.5	15.4
	TEM1	25.4	6.1	2.0	10.6	72.9	8.5	2.5	9.2	14.3
	TEM2	25.8	6.2	2.1	10.4	71.7	8.9	2.3	8.2	14.8
	TEM3	25.3	6.2	1.9	10.7	71.6	8.4	2.7	10.1	14.4
LSD 5%										
ST		0.73	ns	0.10	0.14	0.16	0.26	0.25	0.65	0.55
TEM		ns	ns	ns	0.11	0.13	0.21	0.20	0.50	ns
ST×TEM		ns	ns	ns	ns	0.28	ns	0.44	1.12	ns

ST: seed storage time (month after storage), TEM: storage temperature; TEM1: room temperature conditions, TEM2: low temperature (4 °C), TEM3: high temperature (35°C), SO: seed oil, PA: palmitic acid, SA: stearic acid, OA: oleic acid, LA: linoleic acid, GLA: γ-linolenic acid, AV: acid value, PV: peroxide value, SP: seed protein, * meq O_2/kg

4.4.2 Fatty acid composition

Palmetic and stearic acids

The fatty acid composition of evening primrose seed samples stored under different temperature conditions are shown in Table 4-12. As the data indicate, different storage temperatures and times did not significantly influenced the percentage of palmitic acid (PA). The percentage of stearic acid (SA) of seed samples was significantly influenced by storage time. The percentage of stearic acid was very low and the maximum was 2 %.

Oleic acid

The percentage of oleic acid (OA) was significantly influenced by both storage time and temperature (Table 4-12). During the storage period, there was a regular increase in the percentage of OA of the seed samples from 10.3 % to 10.7 %. In comparison with room temperature (21 °C) and high temperature (35 °C), low temperature of 4 °C led to a significant decrease of OA percentage (10.4 %).

Linoleic and gamma linoleic acid

With increasing storage time, a reduction in the amount of linoleic acid was observed. Under all temperature conditions, the lowest percentage of linoleic acid (LA) was observed after four months of storage. The interaction between storage temperature and storage time on LA percentage indicated that, during the whole storage periods except the second month of storage the reduction in the LA percentage of evening primrose seed oil stored under low temperature was lower than the samples stored under room and higher temperatures (Table 4-12). As storage progressed a reduction in the percentage of GLA was observed. The lowest GLA percentage was recorded four months after storage. During the storage, the percentage of GLA was significantly influenced by temperature. The lowest percentage of GLA (8.4 %) was recorded with the samples stored under high temperature. However, the reduction rate was not apparent in evening primrose seeds stored under low temperature conditions.

4.4.3 Free fatty acid percentage

The results of this experiment show that both storage time and storage temperature strongly influenced the free fatty percentage of evening primrose seed. During the storage period, the samples that were stored under the lower temperature conditions produced the lowest percentage of FFA (Table 4-12). After the first month of storage, no significant differences between different temperatures were observed. At the second month of storage, the FFA of samples stored under low temperature was significantly lower than that of samples stored under high temperature conditions. The observed interaction between storage time and storage temperature on free fatty acid percentage indicates that, during first three months of storage the FFA of seed samples which were stored under high temperature (35 °C) conditions was significantly higher than those of samples which were stored under low temperature and room conditions. On the other hand, four months after storage the FFA of seed sample stored under low temperature was significantly lower than other temperature conditions.

4.4.4 Peroxide value

In this experiment, the peroxide value of evening primrose seed varied from a minimum of 1.2 meq O_2/kg in control to a maximum 22.9 meq O_2/kg in the seed samples which were stored at the room temperature conditions for a period of four months. The results indicate that storage time of four months and storage temperature had a significant effect on lipid peroxide value (PV) of evening primrose seed samples. As storage progressed, PV gradually increased. During the last two months of storage, the level of PV strongly increased from 3.3 % after the second month to 16.1 % and 22.5 % after the third and fourth months of storage, respectively. During the first month of storage, no significant differences between different storage temperatures were observed. After four months of storage the PV of seed samples subjected to low temperature conditions was significantly lower than those of samples which were stored under room and higher temperature conditions (Table 4-12).

4.5 Study on seed germination

4.5.1 Effect of day length and temperature on seed germination

The present work was dedicated to clarify the effect of day length and temperature on evening primrose seed germination. The results of the experiment presented in Table 4-13 indicate a strong influence of temperature and day length on seed germination of evening primrose. Under all photoperiodic conditions, no seed germination occurred at temperatures ranging between 5°C and 10°C.

Under dark conditions, 12 d after seed imbibition and increase in temperature from 10 °C to 15 °C, the germination percentage was 0.5 %. At 15 °C two weeks after seed sowing, only a low percentage of germination was recorded (1.5, 6 and 5% under dark conditions, 8 and 24 h/d length photoperiod, respectively). With 15 °C, no germination was observed under the 16 h/d photoperiod. Under 20 °C temperature conditions after 4 d of imbibition, the seeds which were subjected to 8 hours of day length started to germinate (8.5 %). At 16 h day length after eight days imbibing water by the seeds, a small fraction of the seeds (7.5 %) germinated. The seeds that were subjected to a 24 hours day length started to germinate 6 d after imbibition (18.5 %). At the end of the period (14 d) the highest number of germinated seeds (73.5 %) was observed both under 8 and 24 h/day lengths.

Table 4-13. Effects of different temperatures and photoperiodic conditions on the germination of *Oenothera biennis* seeds (germination rate in %)

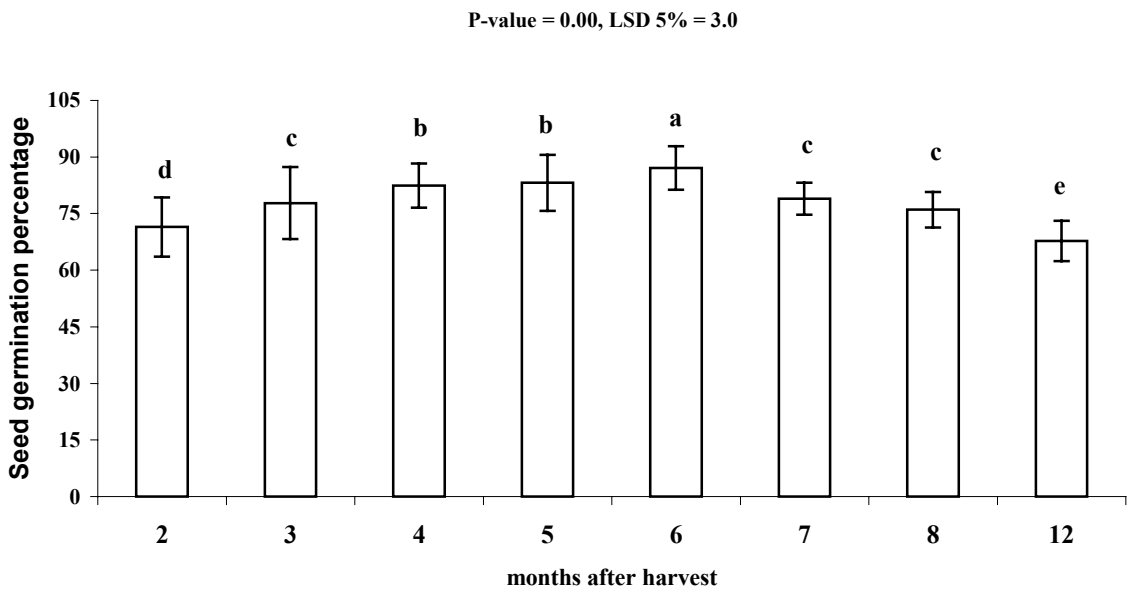
TEM	DL	2nd	4th	6th	8th	10th	12th	14th (DAI)
5°C		0	0	0	0	0	0	0*
10°C		0	0	0	0	0	0	0*
15°C	DL1	0	0	0	0	0	0.5	1.5
	DL2	0	0	0	0	0	0	6.0
	DL3	0	0	0	0	0	0	0
	DL4	0	0	0	0	0	2.0	5.0
20°C	DL1	0	0	0	3.0	6.0	18.0	24.0
	DL2	0	8.5	14.0	29.5	37.0	65.0	73.5
	DL3	0	0	0	7.5	23.0	34.0	39.0
	DL4	0	0	18.5	54.0	63.0	66.5	73.5
5°C		0	0	0	0	0	0	0
10°C		0	0	0	0	0	0	0
15°C		0	0	0	0	0	0.6	3.1
20°C		0	2.1	8.1	23.5	32.3	45.9	52.5
	DL1	0	0	0	0.8	1.5	4.6	6.4
	DL2	0	2.1	3.5	7.4	9.3	16.3	19.9
	DL3	0	0	0	1.9	5.8	9.0	11.0
	DL4	0	0	4.6	13.5	15.8	16.6	18.4
$LSD_{5\%}$	TEM	-	0.9	1.1	2.9	4.5	5.0	5.2
	DL	-	0.9	1.1	2.9	4.5	5.0	5.2
	TEM×DL	-	1.8	2.2	5.8	8.9	10.0	10.4

TEM: temperature, DL: day length, DL1: dark, DL2: 8h/day, DL3: 16 h/day, DL4: 24 h/day, DAI: days after seed water imbibing, * with 5 and 10 °C under all day length conditions germination was zero

4.5.2 Effect of harvest time and harvest method of the plants on seed viablity

The results show that during one year of storage under room conditions, the seed germination percentage of evening primrose gradually increased up to six months and then there was a reduction from there on (Fig 4-16). One month after storage, the stored seed samples had a germination rate of 71 %. This rate increased during the first 6 months of storage up to 87 %. At the end of the investigation, nearly the same level of germination (68 %) was observed as at the beginning of storage (71 %).

The germination of evening primrose was significantly influenced by different harvest times and methods. In the first month of the experiment, the early harvested seeds had the lowest germination percentage. Between the middle and late harvested seeds, no significant difference was observed after the first month of storage. A significant difference was observed between the early harvested seeds and the seed samples harvested at the middle and late times.



* Germination did not measured during 9th, 10th and 11th months of storage, bars at the top of the columns mean standard deviation

Fig 4-16. *Oenothera biennis* seed germination rate under room temperature during 12 months of storage

Table 4-14. Effect of harvest times and harvest methods on seed germination of evening primrose during 12 months after harvest

T	M		MO1	MO2	MO3	MO4	MO5	MO6	MO7	MO12
T1	M1		64.7	72.0	82.0	84.7	87.3	75.3	72.6	68.8
	M2		60.7	81.3	87.0	86.0	88.7	76.7	78.7	70.4
	M3		72.0	87.3	84.0	79.3	86.7	76.0	71.3	67.5
T2	M1		65.3	70.0	82.7	80.0	82.0	78.0	74.3	66.4
	M2		74.7	86.0	80.7	75.3	92.0	80.7	76.0	67.7
	M3		75.3	75.3	86.0	77.3	90.7	86.3	82.0	69.0
T3	M1		77.7	74.0	75.3	80.7	80.7	79.3	80.0	61.7
	M2		73.3	70.7	78.4	91.3	88.7	75.3	72.7	68.2
	M3		79.3	83.3	85.3	94.0	86.7	82.7	76.7	69.9
T1			65.8	80.2	84.3	83.0	87.6	76.0	74.0	68.8
T2			71.8	77.1	83.1	77.6	88.4	81.7	77.4	67.7
T3			76.8	76.0	79.8	83.3	85.2	79.1	76.0	66.6
	M1		69.2	72.0	80.0	81.7	83.6	77.6	75.7	65.6
	M2		69.6	79.3	82.1	84.2	89.8	77.6	75.8	68.8
	M3		75.6	82.0	85.1	83.6	87.9	81.7	76.7	68.8
LSD 5%		T	5.8	ns	ns	5.1	ns	2.7	ns	ns
		M	ns	ns	ns	ns	ns	2.7	ns	ns
		T×M	ns	ns	ns	8.8	ns	4.7	ns	ns

T: harvest time, T1: early, T2: middle, T3: late harvest time, M: harvest method; M1: conventional harvest, M2: defoliation by herbicide, M3: defoliation by flame, MO: month of storage after harvest

This indicates that delayed harvest time led to increase germination rate of the seeds. During the second and third months of the experiment, no significant difference in the seed germination was observed. Four months after storage, significant differences were observed between different seed samples. At this time, seed samples that were harvested at the late time, showed the highest germination rate. Six months after storage a significant influence of harvest method on seed germination was observed. After both four and six months of storage, an interaction between both treatments on seed germination was observed.

After four months of storage, the seeds of plants that were defoliated by flame and were harvested at the early time showed the lowest germination percentage. On the other hand, the highest seed germination rate was recorded in the seed samples of the plants that were defoliated with flame and harvested at the late harvest time.

Table 4-15. Effect of different storage times (month) and storage temperatures on the germination percentage of *Oenothera biennis* seeds

ST	TEM	2nd	4th	6th	8th	10th	12th	14^{th} (DAI)
ST0	TEM0	10.0	61.3	67.3	67.3	67.3	67.3	67.3
ST1	TEM1	34.0	66.0	74.7	76.7	76.7	76.7	76.7
	TEM2	45.3	63.3	75.3	76.0	75.3	79.3	84.0
	TEM3	14.0	84.0	84.0	84.0	84.0	84.0	84.0
ST2	TEM1	23.3	65.3	80.7	82.0	82.0	82.0	82.0
	TEM2	22.0	80.0	82.7	92.7	92.7	92.7	92.7
	TEM3	15.3	60.0	72.7	72.7	72.7	72.7	72.7
ST3	TEM1	8.7	76.7	78.0	78.0	78.0	78.0	78.0
	TEM2	6.0	76.7	80.7	80.7	83.3	83.3	83.3
	TEM3	2.7	70.0	71.3	75.3	75.3	75.3	75.3
ST4	TEM1	41.3	76.0	76.0	76.0	76.0	76.0	76.0
	TEM2	26.0	60.7	64.7	64.7	68.0	68.0	68.0
	TEM3	19.3	75.3	78.0	78.0	78.0	78.0	78.0
ST1		31.1	71.1	77.3	78.2	78.2	78.2	78.2
ST2		20.2	68.4	78.7	82.4	82.4	82.4	82.4
ST3		5.8	74.4	76.7	78.9	78.9	78.9	78.9
ST4		28.9	70.7	72.9	74.0	74.0	74.0	74.0
	TEM1	26.8	71.0	77.3	78.2	78.2	78.2	78.2
	TEM2	24.8	70.2	75.8	80.0	79.8	80.8	82.0
	TEM3	12.8	72.3	76.5	77.5	77.5	77.5	77.5
$LSD_{5\%}$	ST	7.2	ns	ns	ns	ns	ns	ns
	TEM	5.6	ns	ns	ns	ns	ns	ns
	ST×TEM	12.4	ns	ns	ns	ns	ns	ns

ST: storage time (month from 0 to 4), TEM: storage temperature (TEM0 = control, TEM1 = room temperature (21 °C), TEM2 = low temperature (4°C), TEM3 = warm temperature (35 °C), DAI = Days after water imbibing

Six months after storage, the seeds of chemically defoliated plants that were harvested at the early and middle times showed a higher germination percentage than those of conventionally

harvested seeds. In contrast, seeds that were conventionally harvested at the late harvest time showed the highest germination percentage.

4.5.3 Effect of different storage times and temperatures on seed germination

The results of the effect of storage time and temperature on seed germination rate show that, after two days imbibition of water, the seed germination was significantly influenced by different storage times and temperatures (Table 4-15). During the first two months after storage the total germination percentage of *Oenothera biennis* seeds was more than that of the control. Under all temperature conditions a strong reduction in the percentage of total germination was observed after three months of storage. At the first three months of storage, the highest seed germination percentage was recorded in the seed samples stored under low temperature. On the other hand, after four months of storage the same seed samples had the lowest germination rate. In most cases, there was no big difference in the germination percentage under different temperature regimes.

5. Discussion

5.1 Seed yield performance and seed quality of evening primrose in field experiments

The results of the field experiments showed a significant effect of harvest date on seed yield in the autumn 2004 experiment (Table 4-2). The influence of harvest date on seed yield in this experiment could be due to the longer growing period of plants from 398 d in early harvest and 427 d in late harvest. This was caused by the longer growing stages of FB-DT (from flower beginning to desiccation) and FB-T (flower beginning to harvest). It can be supposed that an elongated growing stage from flower beginning to desiccation leads to formation of more branches and capsules per plant. That could be cause production of more seeds per plant and higher seed yield per area.

In the autumn experiment, the highest seed yield was obtained from plants that were harvested at the late harvest time, in which more than 75 % of capsules were brown. The finding of the present study is in agreement with the findings of Simpson and Fieldsend (1993). In a field experiment, they demonstrated that in *Oenothera biennis* L. cv. "Merlin" the highest seed yield was achieved when 75 % of capsules were brown.

In contrast to the autumn experiment, both spring experiments resulted in a reduction of seed yield during the early and late harvest dates. The low percentage of matured seeds at the early harvest time could be the reason why the seed yield was lower than at the middle time-point. On the other hand high seed shattering may have led to lower seed yield from plants which were harvested at the late harvest time-point than from plants which were harvested at the middle time-point.

The large difference in seed yield from the spring experiments could have been caused by the delayed sowing time in spring 2005 (Table 3-7). This led to a shorter total growing period of 161 d (early harvest) to 167 d (late harvest). In contrast, in spring experiment in 2004 the total growing period varied from 155 (early) to 176 (middle) and 187 (late), respectively. It can be supposed that the short vegetation period during the spring experiment in 2005 is the main reason why the seed yield was low in this experiment. This result is in accordance with that of Fieldsend and Morison (2000 b) who demonstrated that a delay in sowing time led to a decrease the seed yield of evening primrose.

During the first spring experiment, the seed yield of defoliated plants-especially plants that were defoliated with herbicide- was higher than those of non-defoliated plants (Table 4-2). It can be supposed that the desiccation process improved the ripeness, thereby increasing the

number of matured seeds. The results observed in the spring experiment from 2004 appear to confirm the hypothesis for an effect of defoliation on seed yield of evening primrose, however this hypothesis was not confirmed in the autumn experiment 2004 and spring experiment 2005. Since the results of the different experiments did not correspond, additional investigations with more cultivars of evening primrose seem to be necessary.

As is presented in Table 4-2, no interaction between harvest method and harvest time on seed yield was observed in the field experiments. These observations do not agree with the hypothesis that the effects of defoliation vary under different harvest times.

The variation in TSW (Table 4-2) could be related to the duration of the seed development (82 d for late harvest time in contrast to 50 and 71 d growing stage for early and middle harvest times, respectively). It seems that there is a negative relationship between seed yield and TSW in evening primrose. Higher seed yields means are caused by higher capsules and/or higher number of seeds per capsules. Higher number of capsules per plant as well as higher number of seeds per capsules increases the nutrient competition (dry matter accumulation) and results in reduced seed size. Due to direct relationship between seed size and seed yield, this could be the reason why TSW decreases with higher seed yield.

In the spring experiment of 2005, the lowest percentage of oil was recorded at the early harvest date and there was a big difference between oil percentage at different harvest dates, especially between early harvest and the fallowing harvests (Table 4-3). This is probably because of an early harvest reducing the growing period and hence limits the time for oil accumulation.

Several studies on evening primrose (e.g. Reiner and Marquard 1988; Court et al. 1993, Fieldsend and Morison 2000 a) suggest that a positive correlation exists between seed oil content and temperature during seed filling. From the climatic data (Table 3-4), it can be inferred that, plants, which were harvested at the early harvest date, received a total temperature sum of 500°C less than plants, which were harvested at the middle and late date. It is therefore possible that the temperature requirements for plant oil synthesis were not achieved in this harvest time. As the oil accumulation increases gradually during seed development, it could be assumed that a low percentage of matured seed at the first harvest date in the spring experiment 2005 is the main reason for the low seed oil percentage.

In the different treatments tested, an early defoliation was found to reduce the percentage of oil of the seeds. In plants that were chemically defoliated, the observed reduction was more

than that of plants that were defoliated by flaming. The basis of lipid synthesis is the supply of sufficient carbon atoms from the carbohydrate metabolic pathways (Stumpf, 1989). For that reason the shortening of the photosynthesis by defoliation leads to reduced lipid synthesis and seed oil percentage, as observed during the early harvest time.
In comparison with the conventional harvest, defoliation by both flaming and herbicide treatment improved oil accumulation at the middle and late harvest times (Table 4-3). After certain stages of seed development defoliation usually accelerates the maturity of seeds (Growley and Fröhlich 1998). Since the seed oil content is directly related to the seed maturity, a higher number of matured seeds results in a higher percentage of oil and vice versa. This could be a reason why defoliation during the middle and late harvest times increased the percentage of seed oil in the autumn experiment in 2004 (Table 4-3). The data obtained on seed oil percentage lead to the conclusion that results observed in the field experiments confirm the hypothesis in which defoliation affects the seed quality of evening primrose.

The effect of flaming on plant defoliation appeared to be slower than that of herbicide. The flame-defoliated plants might have a longer photosynthesis activity than those of chemically defoliated plants. This could be the reason why flame-defoliated plants in the spring experiments with delayed harvest produced a higher oil percentage plants that were defoliated with herbicide. The observed results confirm the hypothesis that the effect of defoliation can be affected by parameters like harvest time.

The reduction of raw protein percentage during the late harvest in the spring experiment of 2005 could be explained by the negative correlation between the percentage of oil and raw protein in oilseeds (Yin and Vyn 2005).
Results of the present study also show that in some cases the percentage of oleic acid (OA) of evening primrose seed oil is decreased by delaying in harvest (Table 4-4). This confirms the previous observations by Yaniv et al. (1989), who showed that the percentage of OA decreased during seed development. Due to the fact that the percentage of polyunsaturated fatty acids (e.g. linoleic and γ-linolenic acids) increases during seed development (Yaniv and Perl 1987), the inverse relationship between mono and polyunsaturated fatty acids in seed oil could be the reason why oleic acid was reduced with delayed harvest.
In the spring experiment in 2005, the plants that were conventionally harvested produced a lower percentage of oleic acid than those of the plants that were defoliated before harvest

(Table 4-4). As described above, the percentage of saturated and monounsaturated fatty acids decrease during seed development. The percentage of polyunsaturated fatty acids reaches a maximum at maturity. Based on the results obtained here it could be supposed that in the spring experiment 2005 the defoliation was applied early. Compared to defoliated plants the non-defoliated plants had at least two weeks longer for photosynthetic activity. This higher assimilation presumably increased the number of full-ripened capsules and seeds, resulting in higher polyunsaturated fatty acids than in defoliated plants.
The lowest percentage of linoleic acid (LA) was observed in the plants that were harvested at the early date. This confirms the results of Fieldsend and Morison (2000 a), who showed that the percentage of LA increases by delaying the harvest and is expected since the linoleic acid accumulation gradually increases during the seed development (Yaniv et al. 1987).

In spring 2005 experiment, plants which were defoliated using flame produced a higher percentage of LA than those defoliated using herbicide. As mentioned previously it seems that the effect of flaming on plant defoliation is slower than that of herbicide treatment. It seems that plants defoliated by flaming continue to photosynthesis for a relatively longer period than plants defoliated by herbicide. The higher percentage of LA observed in flame-defoliated plants could have come from a possible increase in the dry matter accumulation due to the extended photosynthetic activity (Table 4-2).

During the both spring experiments the highest GLA percentage was recorded in the early harvested seeds (when all capsules were green) and GLA gradually decreased by delaying in harvest (Table 4-4). The fact that the highest GLA percentage was recorded in early harvested plants is not in agreement with the results of Yaniv et al. (1987) who found that the GLA percentage of spring evening primrose increased when the harvest time was delayed. These observations bear no direct relationship with the duration of seed development, seed dry matter accumulation or with the weather conditions during these times. It could be that the GLA percentage is correlated with other fatty acids like oleic acid and linoleic acid, which are synthesized prior to GLA formation.
It has long been known that in oilseed crops (e.g. oilseed rape, sunflower and flax) the extent of desaturation in the fatty acid composition of the seed oil is inversely related to the prevailing temperatures during seed maturation (Canvin, 1965). This also seems to be the case with evening primrose (e.g. Levy et al., 1993), and crops grown at warmer latitudes tend to produce oil with lower γ-linolenic acid content (Simpson and Fieldsend, 1993). On the other

hand, in some studies it has been described that low temperature increases the percentage of GLA (Yaniv 1989; Brandle et al.1993; Levy et al. 1993; Fieldsend and Morison, 2000a; Fieldsend and Morison, 2000b; El-Hafid et al. 2002). In present study, early harvested plants were exposed to a lower temperature sum during their growth period, especially from flowering to harvest, in comparison the plants that were harvested at the middle and late dates (Table 3-4). As described previously the extent of fatty acid unsaturation is inversely related to temperature, this is considered to be an adaptive phenomenon that can regulate the fluidity of particular cell membranes and allow proper physiological function at the lower temperature. The higher solubility of oxygen in aqueous solution at lower temperatures may increase desaturase activity by increasing the availability of the oxygen substrate (Stymne and Stobart 1987). Higher activity of desaturase under low temperature could be one of the reasons why the GLA percentage of evening primrose was reduced by delayed harvest in the spring experiments.

Comparing with the spring experiment in 2004, the lower GLA percentage observed in the spring experiment in 2005 is not in agreement with the observation of Honermeier et al. (2005), who showed that the percentage of GLA in spring evening primrose was gradually increased by delaying sowing time. Although with delaying sowing time a high GLA percentage was expected in the spring experiment in 2005, the higher total temperature received by the plants (2314 °C and 2577 °C temperature sum in S1 and S2 experiments, respectively) might have caused the GLA percentage in the spring experiment of 2005 (S2) to be lower than in the spring experiment of 2004 (S1).

Based on the experimental results and the hypothesis that defoliation reduces the heterogenity of seed ripeness with increasing seed yield, oil percentage and γ-linolenic acid percentage, it can be concluded that no clear and constant effect of defoliation could be observed on those parameters. This was the case although in some cases the above mentioned parameters were significantly influenced by different methods of defoliation. To clarify the hypothesis more field experiments with another cultivars of *Oenothera biennis* are recommended.
From the all measured seed quality parameters, the interaction between harvest time and harvest method had significant and clear effects only on the percentage of oil of spring evening primrose seeds. These observations confirm the hypothesis that the effect of defoliation on seed quality of evening primrose varies depending on different harvest times.

5.2 Seed yield performance and seed quality of evening primrose in pot experiments

In the pot experiments, it was found that the seed yield of evening primrose was positively influenced by different amount of nitrogen in all cases (Tables 4-6, 4-8 and 4-10). A similar result was reported by Sekeroglu et al. (2006) in evening primrose, El Hafid, et al. (2002) in borage, Hocking et al. (1997) and Cheema et al., 2001 in canola and Indian mustard. These studies showed that at certain levels of nitrogen the seed yield of the experimental plants was higher than those of control plants. The positive effect of nitrogen on seed yield is probably a result of new branches (new capsules) being formed due to an increased rate of plant growth and growing period. An adequate application of nitrogen leads to rapid leaf growth in crop plants, enabling them to intercept more solar radiation and thus resulting in increaser photosynthesis. This is manifested in the plant producing more pods, which could be the reason behind the increased seed yield (see Tables 4-6, 4-8 and 4-10).

In the spring 2005 experiment, the observed reduction in the seed yield of plants harvested at the late harvest time and supplied with 2.0 g of Nitrogen per pot (Fig 4-8) could be due to the formation of extra branches (without ripe capsules) and leaves. It could be concluded that the vegetative growth of plant during this time induced a strong competition between capsules and foliage, which presumably delayed or reduced the seed maturation.

In both the autumn experiment of 2004 (A) and the spring experiment of 2005 (S2) plants which were harvested at the early harvest time produced the lowest TSW, while those with a delayed harvest showed an increase in TSW (Tables 4-8 and 4-10). The relatively higher amount of non-ripened capsules and immature seeds in early harvested plants is one of the reasons why thousand seed weight of the early harvested plants is low.

In most cases the number of capsules per main stem, number of capsules per side shoots and number of capsules per pot were strongly increased by increasing the nitrogen (Tables 4-8 and 4-10). The observed results confirmed numerous investigations with other oil crops. For instance, Cheema et al. (2001) showed that additional nitrogen application increased the number of pods in canola. Nitrogen improves the vegetative growth of the plant, increasing the number of side shoots, which in turn supports production of more capsules and increases total seed yield. This could be the main reason why additional nitrogen improves the number of harvested capsules per pot.

The long growth period could be the reason why the highest number of capsules per pot was achieved from plants that were harvested at the late time-point.

Different harvest methods could indirectly influence the number of capsules in different parts of the plant. For instance, defoliated plants could be dryer than those of non-defoliated plants could during threshing. Thus, the possibility of capsules detaching could increase during harvesting process. Delaying threshing after swathing desiccated crops will increase capsule shattering and seed loss in evening primrose. On the contrary, the possibility of capsules shattering from green plants (non-defoliated) is lower than plants that are dried before harvest (defoliated plants). For most crops, harvest can normally commence within 4-10 d after desiccation (Palmer and Sanderson 1976). However, adverse weather conditions such as rainfall, cool temperatures and high humidity will slow plant desiccation and keep seed moisture levels high; this can delay commencement of harvest beyond 10 d after desiccation also influence the total number of harvested capsules.

In autumn-sown plants with nitrogen levels, the number of capsules per side-branch the pots which were harvested at the early harvest date was higher than at the middle and late harvest times (Table 4-8). The reason for this difference cannot be explained in terms of nitrogen application or harvest time, but might rather to be due to an interaction between treatments and environmental conditions.

As expected the highest number of capsules per pot was recorded in plants that were fertilized with higher levels of nitrogen and harvested at the late harvest time. It can be concluded that with a long growing period nitrogen stimulates the production of extra branches and leaves to produce more capsules. Delayed harvest gives sufficient time to produce a higher number of capsules and seeds per plant.

In the autumn experiment the number of capsules was lower on the plants which were defoliated by herbicide or conventionally harvested than on plants whose capsules were directly harvested and dried (method M1). This is probably because the degree of capsule loss from plants which were conventionally harvested (M3) or defoliated before harvest (M2) were higher than those of plants whose capsules were directly harvested and dried at 40 °C (M1).

The results indicate that seed yield of evening primrose increased as N fertilization rate increases, while the oil content of the seed declined. This inverse relationship might be due to a reduced availability of carbohydrates for oil synthesis at high N supply. The negative influence of N fertilization on the oil content of the oil seeds is consistent with other reports

Reiner (1989) in evening primrose, Zhao et al. (1993); Hocking (1995); Hocking et al. (1997), Cheema et al. (2001) and Rathke et al. (2005) in canola. The physiological reason for the negative correlation could be related to the competition for carbon skeletons during carbohydrate metabolism. In a study, Lambers and Poorter (1992) indicated that the synthesis of both fatty acids and amino acids requires carbon compounds from the decomposition of carbohydrates. Since the carbohydrate content of proteins is lower than that of oils, increased N supply intensifies the synthesis of proteins at the expense of fatty acid synthesis and thus, reducing the oil content of the seed.

The observed reduction in the seed oil percentage at the late harvest date in the spring experiment of 2005 (Table 4-11) concurs with the results of Simpson and Fieldsend (1993), who showed that the oil content of evening primrose seed tended to reduce with delayed harvest. Similar result have been reported in other oil seed crops, for example by Stymne and Stobart (1987) in linseed (*Linum usitatissium*), Chung et al. (1995) in sesame (*Sesamum indicum* L.) and Growley and Fröhlich (1998) in camelina (C*amelina sativa*). Although there are some reports that the seed oil content of oil seed plants gradually reduces after maturity, the reason for this phenomenon is not still clear (Chung et al. 1995).

In accordance with the chronology of fatty acid synthesis, there is a negative correlation between the percentage of total monounsaturated and polyunsaturated fatty acids in vegetable oils, based on the observed positive influence of nitrogen on the percentage of polyunsaturated fatty acids like oleic and γ-linolenic acids (Tables 4-7, 4-9, 4-11). This might explain why the percentage of oleic acid decreased with additional nitrogen fertilizer. Thanapornpoonpong (2004) demonstrated that additional nitrogen reduced the percentage of oleic acid of quinoa (*Chenopodium quinoa*) seeds. In his experiment, the percentage of oleic acid was decreased with increasing rates of nitrogen, while the percentage of linoleic acid was increased. He concluded that the effect of nitrogen on percentage of different fatty acids of quinoa seed oil can be explained by an indirect influence of the nitrogen supply on the availability of other nutrients, or direct increase in the activity of involved enzymes in polyunsaturated fatty acid production. Based on the results of the present study and the findings of Thanapornpoonpong (2004), it can be supposed that the increases of total polyunsaturated fatty acids in evening primrose might be related to a reduction of monounsaturated OA. In other words, N application in evening primrose indirectly reduces the OA percentage while increasing the seed dry matter that is result to higher percentage of polyunsaturated fatty acids like LA and GLA.

The observed positive influence of nitrogen on linoleic acid percentage does not conform with Reiner et al. (1989) who demonstrated a negative effect of nitrogen on LA accumulation. Recently Sekeroglu and Özgüven (2006) reported that although N application did not have a clear influence on the LA percentage of *Oenothera biennis* cultivated under dryland conditions, the LA percentage still increased with certain levels of nitrogen. This observation could be explained by the fact that an adequate nitrogen supply not only encourages leaf development, but it can also materially assist in retaining leaves in active photosynthesis (Cechin and Fumis 2004). High photosynthetic activity can accelerate capsule and seed maturity. As the highest polyunsaturated fatty acid accumulation is expected in fully-ripened seed, this might be a reason why nitrogen increases the percentage of linoleic acid in evening primrose seeds.

The percentage of GLA was positively affected by nitrogen application in both the A and S2 experiments (Tables 4-9 and 4-11). In these experiments, the percentage of GLA significantly increased, when the level of nitrogen was increased from 1.0 to 2.0 g/pot. GLA significantly increased. These observations not confer with Sekeroglu and Özgüven (2006), who reported that under different field conditions the GLA percentage was reduced by nitrogen application. On the other hand, Hafid et al. (2002) reported that, although the amount of GLA of the seed of borage (*Borago officinalis* L.) was not significantly influenced by N fertilizer, compared to controls the plants that were supplied with N fertilizer still produced more GLA. Both of the experiments mentioned above were carried out in field conditions, where the interaction between nitrogen and other nutrients as well as climatic conditions could lead the observed conflicts. Hence, more investigations in both field and pot experiments seems to be necessary to obtain reliable information about the effect of different nutrients especially nitrogen, on the fatty acid composition of evening primrose seed. In general, the results obtained in pot experiments showed no clear effect of defoliation on the different measured parameters like seed yield, the percentage of oil and γ-linolenic acid. The observations in pot experiments do not confirm the hypothesis that defoliation reduces the heterogenity of seed ripeness and affects seed yield and seed quality. Since in the pot experiments in most cases no interaction was observed between the harvest method, the harvest date and the nitrogen fertilization, the hypothesis that the effect of defoliation depends on harvest time and nitrogen application could not be confirmed.

Despite the above interpretation of results of both field and pot experiments, it is not easy to say how many days after flowering can be recommended as harvest time. The parameters such as plant variety, climate conditions, soil fertility, sowing time, etc. strongly influence the time of harvest. Generally, according to this study the average of the period after flowering to harvest in autumn and spring sown plants is different. In addition, nitrogen can influence this period. In total, 70-75 days after flowering is the recommended harvest time of the spring sown *Oenothera biennis L*. cv. "Anothera" in Germany. In autumn sown plants, the duration between flowering and harvest is longer than spring-sown plants (90-95 days after flowering).

5.3 Comparison of the performance of autumn and spring evening primrose

For evaluating the hypothesis that autumn and spring sown evening primrose have equal performance, three field experiments as well as three pot experiments were carried out. All experiments were executed separately. Because of the different sowing times and cultivation requirements a randomization of spring and autumn sown evening primrose within the same experiment was not possible. This means that only an indirect comparison of the experiments and their measured parameters was possible. Nevertheless, conclusions about the performance of autumn and spring evening primrose are possible based on the results of experiments in field and pot conditions.

Field Experiments

In field experiments, a large variation in seed yield of the evening primrose was observed (Table 4-2). There was no clear difference in the seed yield of autumn and spring evening primrose. In many instances, variation in the seed yield in different experiment years can be described by agronomic factors such as poor crop establishment or weed and disease pressure. However, it can be suggested that a significant proportion of the potential of seed production even of well-grown crops may be lost during or after harvest. Another aspect could be the climatic conditions during experimental years. Climate data show that plants in the spring 2004 experiment were exposed to lower temperatures than those of the autumn 2004 and spring 2005 experiments (Table 3-4). It can be concluded that the lower temperature, especially during capsule formation might improve seed filling.

The effects of temperature on seed formation of evening primrose were investigated by Yaniv et al. (1989) who observed an inverse relationship between seed yield and air temperature. Indeed, seed yield gradually reduced with increasing daily temperature. The reason why seed yield of evening primrose is negatively influenced by high temperature is not clear. It can be

supposed that high temperature might accelerate the plant development and shorten the period of seed filling. This effect may reduce the seed size (TSW) of evening primrose.

A negative relationship between TSW and seed yield was observed in the autumn 2004 and spring 2005 experiments (A and S2). This observation was confirmed by Fieldsend and Morison (2000), who showed that the TSW of autumn and spring evening primrose is related to seed yield.

Fieldsend and Morison (2000a) showed that the time needed for oil accumulation in evening primrose ranged from only 28-32 days. In recent trials both in spring and in autumn experiments, this duration was a minimum of 50 days in spring-sown plants and a maximum of 106 days in autumn sown plants (Table 4-1). In all experiments, the duration after flowering to harvest was longer than that the range of oil accumulation recommended by Fieldsend and Morison (2000 a).

As shown in Table 4-3, in comparison with the spring 2004 experiment the percentage of oil was higher in autumn-sown plants. A similar observation was reported by Fieldsend and Morrison (2000 a) who demonstrated that the seeds from autumn crops tend to contain more oil, but with a lower γ-linolenic acid content, than seeds from spring-sown crops. Obtained results in field experiments show that the oil percentage of evening primrose seeds in the spring 2005 experiment was higher than those of plants in the spring 2004 experiment. This finding is in agreement with Fieldsend and Morrison (2000a), who concluded that although using improved cultivars such as cv. Merlin can reduce the risk of producing low seed yield in different years, large differences in oil and γ-linolenic acid percentage can occur between years, even for a given cultivar and time of sowing.

The percentage of raw protein in autumn evening primrose was higher than in the spring plants. It has been long known that there is a negative correlation between oil and protein percentage in oil seeds (Pouzet 1995; Yin and Vyn 2005). The higher percentage of raw protein in the autumn experiment did not follow this relationship. In comparison with spring evening primrose, a higher percentage of raw protein in seeds of autumn evening primrose could be a disadvantage.

Between autumn and spring sown evening primrose the lowest oleic acid (OA) percentage was observed in the spring 2004 experiment. Climatic data show that in this experiment the temperature was lower than the autumn 2004 experiment. As described above, due to a positive effect of low temperature on seed maturity, a higher quantity of matured seed could be the reason for reduced OA in the spring 2004 experiment.

According to Yaniv et al. (1989) the percentage of OA in autumn sown plants is higher than those of spring plants. Although the difference between the spring 2004 and autumn 2004 experiments confirms this presumption, the higher OA percentage in the spring 2005 experiment than the autumn 2004 experiment was not in agreement with the observations of Yaniv et al. (1989).

The LA percentage of the seeds in the autumn and spring 2004 experiments was relatively similar (Table 4-3). In comparison with autumn sown plants, the LA percentage of the seed oil strongly decreased in the spring 2005 experiment. From the results of the different experiments, it can be concluded that LA percentage was not influenced by autumn or spring sowing times in this study.

A big variation in the GLA percentage was observed in autumn and spring sown plants (Table 4-3). In both spring experiments, the GLA percentage was relatively higher than that of the autumn experiment. Although this observation does not in confirm with Yaniv et al. (1989), who reported that the percentage of GLA in autumn evening primrose is higher than in spring evening primrose, similar results were reported by Fieldsend and Morison (2000 a). They demonstrated that the percentage of GLA of evening primrose sown in spring was higher than autumn sown plants.

Generally among different experimental years, a big variation in the percentage of fatty acids was observed. The variation in the percentage of different fatty acids of evening primrose seed oil during different experimental years was confirmed in a study by Fieldsend and Morison (2000 a). Their observations indicated that the changes in the percentage of fatty acids during seed development were different between years. For instance, in comparison with the first experiment the percentage of PA, SA and OA in autumn-sown *Oenothera biennis* c.v. “Merlin” strongly decreased in the second year of experiment. From the obtained results, it can be concluded that climatic conditions have a stronger influence on the fatty acid composition of evening primrose seed oil than the sowing time and harvest treatments.

It is known from other grain crops like wheat that a lower demand of vernalization improves the tolerance of the crops to late sowing (Beese 2004). The term vernalization means the promotion of flowering in response to prolonged exposure to low temperature (i.e. winter), a useful adaptation for plant species that flower in the spring. While the physiology of vernalization has been extensively studied in many species, the molecular mechanism of vernalization remains largely unknown (Michaels and Amasino 2000).

The cultivar "Anothera" used in this study was selected by the German breeding program of the Pharma Plant Institute in Arten (Thuringia). It seems that the vernalization demand of Anothera is low.

This led to a good ability for spring sowing and annual cultivation. By using this cultivar nearly the same seed yield performance can be reached in autumn as well as spring sown evening primrose. To confirm the hypothesis in which the performance of spring-sown evening primrose is equal to autumn sown plants, further field experiments that include several cultivars of evening primrose are necessary.

From the results of the field experiments, it can be concluded that there is no great difference in the seed yield and seed oil quality of spring and autumn evening primrose. Thus, the hypothesis that the performance of spring-sown evening primrose is equal to autumn sown plants was confirmed with the obtained results.

Pot experiments

Since the experimental design in spring 2004 was different, only the results of the autumn 2004 experiment and the spring 2005 experiment are discussed in this respect. Under same treatments the seed yield of spring evening primrose (S2 experiment) was higher than autumn sown plants (Tables 4-8 and 4-10). The response of plants to nitrogen in spring evening primrose seems to be more efficient than autumn plants.

Fieldsend and Morison (2000 b) showed that in most cases the seed yield of spring evening primrose (*Oenothera biennis* cv. Merlin) was higher than autumn-sown plants. In their experiment, spring evening primrose had higher number of capsules, higher harvest index and lower capsule length than autumn evening primrose.

In this study the seed formation period of spring and autumn sown plants were similar, therefore this is possibly not the reason for the big difference in seed yield between the autumn and spring experiments. According to the observed results of the field and pot experiments, it can be concluded that when the seeds of evening primrose were planted early in spring as compared to sowing any time in autumn, the plants got enough low temperature for vernalization but without causing any low stress. These conditions and the environmental situations during spring and summer seems to provide favorable growing conditions for spring evening primrose to grow faster and produce more seed than autumn sown evening primrose, which were under low stress.

The TSW was higher in spring evening primrose than in autumn sown plants (Tables 4-8 and 4-10). These observations agree with Fieldsend and Morison (2000 b) and Honermeier et al.

(2005). In the observation of Honermeier et al. (2005), the TSW of early spring sown evening primrose was higher than that of autumn sown plants. Fieldsend and Morison (2000 b) reported that during the first year of their experiments the TSW of spring sown *Oenothera biennis* cv. Merlin was much higher than that of autumn sown plants. From these results and the findings of other scientists with *O. biennis* cv. "Anothera" it can be concluded that the seed yield and TSW of evening primrose are not influenced by spring or autumn sowing dates.

In both autumn and spring, experiments the highest seed oil percentage was observed in spring-sown evening primrose supplied with 0.5 g of N/pot conventionally harvested at the second harvest date (Tables 4-9 and 4-11). Although under the same treatment conditions the highest seed oil percentage was achieved in autumn sown plants, the maximum oil percentage in this experiment was lower than that of spring experiment (30.5 % and 28.9 % in the spring and autumn experiments, respectively). This observation confirms the results of Honermeier et al. (2005) who reported that in most cases the oil content of early spring sown *O. biennis* cv. "Anothera" was higher than that of autumn sown plants.

As well as field experiments the percentage of raw protein in autumn evening primrose seeds was higher than spring evening primrose in these experiments (Tables 4-9 and 4-11). The higher percentage of raw protein in the seed of evening primrose in autumn cannot be explained by the known negative correlation of seed oil and protein percentage. Although the difference in the percentage of raw protein in autumn and spring evening primrose was on the whole not high, a lower percentage of oil and a higher percentage of raw protein in seeds of autumn evening primrose is a weak point for autumn sown plants.

In both experiments, the maximum GLA percentage was recorded in plants supplied with 2.0 g of N/pot and harvested at the late harvest date under different harvest methods (Tables 4-9 and 4-11). No difference was observed in the GLA accumulation between autumn and spring experiments. These results agree with the results reported by Honermeier et al. (2005), who did not observed any significant difference on GLA percentage of autumn and spring evening primrose.

Overall, it can be concluded that the fatty acid composition of evening primrose seed oil has the main effect on the fluctuation in the proportions of individual fatty acids, while external factors such as sowing time, environmental and husbandry factors have a smaller effect on the ratio of these fatty acids. Furthermore, it was found both in field and pot experiments that there was no large difference in the seed yield and seed quality of autumn and spring evening

primrose. These results confirm the hypothesis that the performance of spring and autumn evening primrose can be equal.

5.4 Seed quality of evening primrose during storage

The susceptibility of evening primrose oil to oxidation has been proven to be due to the presence of high levels of polyunsaturated fatty acids especially GLA. Results of pervious studies by other researchers showed that there is an inverse relationship between the concentrations of polyunsaturated fatty acids and the oil stability (Ahmadkhan and Shahidi 2000, Morello et al. 2004). Although there are some investigations about the effect of storage time and temperature on oil seed plants (Growley and Fröhlich 1998, Ahmadkhan and Shahidi 2000, Morello et al. 2004, Martini et al. 2005), no information about the effect of storage conditions (time and temperature) on the quality of evening primrose seed oil is presented.

From the results obtained in the present experiment, it can be seen that under different storage periods the oil percentage of evening primrose seeds was strongly different (Table 4-12). Compared to the results of control samples, the oil percentage of the seeds gradually decreased during storage and the lowest percentage of oil was recorded in seed samples that were stored for a period of four months. These results are in conformity with studies of sunflower seeds presented by Sisman and Delibas (2004). These researchers showed that, during a period of three months of storage, independently of storage conditions the percentage of oil gradually decreased with increasing storage time.

In vegetables oils oxygen dependent deterioration of lipids is known as rancidity. Oxidative rancidity development has been recognised as the predominant cause of oil deterioration and reduction during storage (Ahmadkhan and Shahidi 2000, Morello et al. 2004). Due to the presence of double bonds in unsaturated fatty acids, these fatty acids are susceptible to oxidation, which is a reaction between unsaturated fatty acids (regardless of whether they are in their free state or esterified as triacylglycerol molecule) and the triple oxygen ($3O_2$) molecule (Choe and Min 2006). It is well known from chemistry that the possibility of oxidation increases with additional oxygen concentration and the duration of exposure (the length of storage).

The main enzymes involved in seed oil degradation are acyl-CoA oxidase, malat synthase, citrate synthase, catalase and lipases (Kindl 1987). Most of these enzymes are aerobic and their activities either partially or wholly require the presence of oxygen. The longer the storage period the higher the oxygen availability and vice versa. This could explain why the percentage of oil of stored seeds reduces during storage.

In this study the oil percentage of evening primrose seed was not significantly influenced by different temperature conditions (Table 4-12). These observations are in confirmation with Martini et al. (2005), who reported that in a period of four months of storage they did not find a clear variation tendency in the oil percentage of stored sunflower seeds at different storage temperatures (10 °C, 21 °C and 37 °C).
In present study the decline in oil percentage during storage was accompanied by a strong increase in the percentage of raw protein was observed. It is not clear why this occurred, but it may be due to the negative correlation between seed oil and protein.

Fatty acid composition and the proportions of different fatty acids of the seed oil during storage depend on the degradation rate of different fatty acids (Ahmadkhan and Shahidi 2000). As presented in Table 4-12, in contrast to the percentage of linoleic and γ-linolenic acids the percentage of oleic acid and free fatty acid gradually increased during storage. It can be supposed that the high proportion of linoleic acid and γ-linolenic acid could be converted to monounsaturated fatty acid (OA).
Morello et al. (2004) showed that, the proportions of linoleic and linolenic acids (polyunsaturated fatty acids) strongly decreased in olive oil over a period of 12 months of storage at room temperature. Opposite to that, the proportions of oleic acid, stearic acid, palmetic acid and free fatty acid in stored samples increased markedly during this period of storage.
In the present study, the lowest reduction in the linoleic and γ-linolenic acids of the stored seed samples was observed in samples that were stored under low temperatures. This suggests that the fatty acid composition is more stable under low temperature than at higher temperatures. The reason for this is not related to a single parameter. It could be supposed that the activity of enzymes that are involved in fatty acid oxidation is strongly related to temperature (Linder 2000).
The observed similarity in the percentage of seed linoleic acid during the second month of storage under both low and warm temperature conditions did not fit the expectation of the effects of temperature or storage time on fatty acid composition. Parameters such as seed age, physiological reactions in the seed and the proportions of other fatty acids might have influence on the percentage of linoleic acid.

As discussed previously in other oilseed crops, the conditions and the duration of storage have a strong influence on free fatty acid (FFA) accumulation in the oil (Villiers et al. 1986; Ulha et al. 2003; Gomenz-Alenson et al. 2004; Abramovic and Abram 2005; Neg et al. 2005;

Sisman and Delibas 2005). The free fatty acid percentage in the oil is one of the main parameters affecting the quality of stored oilseeds. The initial hydrolysis of oil is by lipases, enzymes that catalyze the three stage hydrolytic cleavage of the fatty acids esters bonds in triacylglycerols (TAGs), ultimately to yield glycerol and free fatty acids (Bewley and Black, 1994). The results presented here show that the free fatty acid percentage of evening primrose seed oil is strongly influenced by both storage time and storage temperature. During storage, the samples that were stored at the low temperature produced the lowest FFA. As it has been discussed with Linder (2000), the activity of lipases is strongly related to temperature. The lower activity of lipases under low temperature conditions may account for the low the percentage of free fatty acid in the seed samples stored under low temperatures. In comparison with seed samples that were stored under low temperature conditions, the samples that were kept in room temperature conditions produced a higher proportion of FFA. The seed samples that were stored at 35 °C showed a more rapid increase in FFA accumulation compared to samples stored at 21 °C and 4 °C.

In a study with quinoa (*Chenopodium quinoa*) seeds, which were stored at the different storage temperatures (25, 35, 45 and 55 °C) over a period of 30 days, Neg et al. (2005) demonstrated that there is a strong interaction between storage time and storage temperature on free fatty acid accumulation of quinoa seed oil. They suggested that this interaction between storage time and temperature is probably due to the significant effect of these parameters on the activity of lipases. It can be suggested that high FFA accumulation of evening primrose seed oil in a long storage period and high temperature could be due to higher lipase hydrolysis activity coupled with higher activity of other enzymes involved in fatty acids degradation.

In evening primrose seed samples stored at the high temperature conditions, after one month of storage the peroxide value rose sharply to 2.6 meq O_2 /kg, and after four months it reached 22.5 meq O_2/kg (Table 4-12). A similar result in the peroxide value of vegetable oil has been observed by other scientists, e.g. Gomenz-Alonso et al. (2004) in olive, Abramovic and Abram (2005) in camelina.

In an experiment with sunflower and soybean oil Ullah et al. (2003) demonstrated that, at room temperature with fluorescent light and dark conditions, the peroxide value of both oil samples sharply increased during a five weeks of storage.

The higher stability of seed oil of plants under low temperature conditions was also confirmed by Abramovic and Abram (2005) in the seed oil of *Camelina sativa*. They showed that the

peroxide value of the seeds under 8 °C and dark conditions was strongly lower than seeds which were stored at room temperature under dark conditions.

The peroxide concentration gives a measure of degree of oxidative rancidity and serves as an indicator of quality change in oil (Setiowaty et al. 2000). The influence of storage condition on peroxide value of vegetable oils was reported in sunflower seed (Abramovic and Abram 2005, Kucuk and Caner 2005). In the present study with evening primrose as storage time progressed, regardless the temperature, an increase in the peroxide value was observed. The variation in peroxide value of samples stored under low temperature conditions was obviously lower than in samples stored at higher temperatures. According to Abramovic and Abram (2005), a maximum of 20 meq O_2/kg is the limited peroxide value of unrefined oil. In present study, the seed samples that were stored at 35 °C and 21 °C showed peroxide values above the maximum limit of 20 meq O_2/kg that was recommended by Abramovic and Abram. Contrary to that, after four months of storage the seed samples that were stored at low temperature conditions (4 °C) produced a peroxide value of 18.6 meq O_2/kg, which is lower than maximum limited value of peroxide in unrefined oil.

It can be concluded that in storage under low temperature conditions four months might be the maximum period of storage of evening primrose seed in which the lowest oil quality losses will occur. In contrast to the low temperature conditions, after three months of storage at high temperature, the peroxide value of samples exceeded the maximum recommended value of 20 meq O_2/kg.

Overall, it can be supposed that the most of parameters involved in the quality of evening primrose seed (i.e. oil and gamma linolenic acid percentage, free fatty acid percentage and peroxide value) were strongly influenced by different storage time and temperature. From this experiment, a higher quality of seed is expected under shorter storage time and lower storage temperature. Thus, the hypothesis that storage time and temperature during storage influence the seed quality of evening primrose has been confirmed by the obtained results of present study. Further investigations are necessary to obtain more information about the effects of storage conditions on seed quality, for example content of natural antioxidants like tocopherols.

5.5 Seed germination in evening primrose

Germination beings with water uptake by the seed (imbibition) and ends with the start of elongation of the embryonic axis, usually the radicle (Bewley and Black 1994). Besides

internal regulating factors like hormones, protein hydration, sub-cellular structural changes and respiration, external factors such as light density, day length, temperature and humidity also strongly influence the seed germination.

It is known that photoperiod and temperature have an effect on germination in evening primrose seed (Ensminger and Ikuma 1988). In the present study, the seed samples which were placed at 5°C and 10°C under all photoperiodic conditions did not germinate (Table 4-13). This means that despite the fact that *Oenothera biennis* is tolerant to low temperature, the minimum temperature for seed germination is higher than 10 °C.

In an earlier experiment, it was observed that the seeds of evening primrose that were placed in temperature below 12 °C showed a prolonged delay in germination. When these seeds were transfered to conditions with higher temperature the germination started. The results of the present study are in agreement with the observations of Ensminger and Ikuma (1988). During the experiment, the seeds that were treated with low temperature (5°C and 10°C) for a period of two weeks did not germinated, whereas the same seeds germinated when they were transferred to room temperature conditions. Ensminger and Ikuma (1987) showed that although evening primrose seeds are photosensitive, temperature has great influence on photo-demand of seeds for germination. They reported that with increasing temperature from 16°C to 32°C the demand of light for germination of seed strongly decreased.

In the present experiment it was observed that the number of germinated seeds strongly decreased when the seeds were germinated at 16 hours day length as compared to those germinated in 8 and 24 hours day lengths (Table 4-13). This observation is not explainable by the direct effect of temperature or photoperiod. Generally, a seed sample of an indeterminate plant (e.g. evening primrose) includes seeds with different maturity stages. Different matured seeds have different stages of dormancy that are regulated by different plant hormones, especially the ratio of ABA and GA. The diversity in seed maturity stage of experimental seed samples might be the cause of the observed results.

The hypothesis that the seed germination of evening primrose is significantly influenced by day length and temperature is confirmed by the results of this experiment. Higher temperature resulted in higher germination percentage and vice versa. Although no clear effect of day length on seed germination was observed, the results confirmed a strong interaction between temperature and light on the potential of seed germination in evening primrose.

Due to gradual maturity of embryo in seed during the ripening process, it was assumed that harvest time and harvest method has influence on seed germination in evening primrose. The results of this study show that the germination percentage of evening primrose seed depends

on harvest time. The germination rate was significantly different during the first, fourth and six months of storage after harvest (Table 4-14). These results can be explained by the physiological dormancy of the seed embryo, which is present in most newly harvested seeds of vegetables and wild flowers (Bewley and Black 1994, Hartman et al. 2001). In the early harvested seed samples, a high proportion of immature seeds could be the main reason for the low germination percentage. The plants which were harvested at later had more matured seeds than those of plants which were harvested earlier, leading to an increase in germination rate of late harvested seeds.

It has been long known that physiological dormancy of seeds will over a period of 2-3 months of storage (Hartman et al 2001). As can be seen in Table 4-14, during the second months of storage, the germination percentage of the seeds that were harvested at the early harvest time gradually increased. At this time, the germination percentage of early harvested seeds was similar to seeds harvested at the middle and late harvested dates.

In contrast to early harvested seeds, germination rate of seeds that were harvested at the middle and late harvest dates tended to decline with progresses storage. The reason for this reduction is not clear. Secondary dormancy develops in already dispersed, matured seeds in response to unfavourable conditions. It is characterized by a loss of sensitivity of dormancy–breaking factors such as light or temperature. This could be one reason why additional storage decreased the germination percentage of seeds that were harvested at the late harvest time. Based on the results obtained in this experiment it can be concluded that depending on harvest time a gradual increase in the seed germination rate of newly harvested seeds could be overcome the physiological dormancy of embryo (Leadem 1997). The reduction of germination with increasing storage time could further be explained by secondary dormancy of the embryo induced by a long storage period (Hartman et al. 2001).

From the results that are presented in Figure 4-16 (germination during one year), it can be concluded that evening primrose reached a good level of germination with a range from 68 % to 87 %. Seeds can be used for sowing several months after harvest. Since under field conditions different parameters like soil structure, temperature, water content and soil microorganisms can influence the germination percentage of seeds, the results are not necessarily comparable to the germination rate under field conditions.

Generally, from the results obtained it can be concluded that the seed germination rate of evening primrose was affected by the different harvest times and harvest methods of mother

plants only over the first six months of storage. These results confirmed the hypothesis that harvest time and method influence the seed viablity of evening primrose.

6. Summary

Evening primrose has a good potential to become a commercial agricultural plant for GLA production, some disadvantages such as indeterminate inflorescence, high seed shattering during ripening, length of life cycle, lack of uniformity in seed germination are still problems to be overcome for this to happen. Although several studies have carried out to find ways of removing the seed shattering as well as reducing the flowering heterogeneity, seed shattering still posses the biggest problem in the production of evening primrose. This project was an agronomic procedure aimed at optimising the appropriate harvest time and harvest method that will lead to higher quality and quantity seed (in aspect of seed yield, oil and γ-linolenic acid percentage). Experience has shown that defoliation before harvest usually accelerates seed ripening and increases the homogeneity of seed maturation. The harvest method used in these experiments was defoliation by means of flame and by chemical.

To date our knowledge is still limited about the effect of fertilizers (especially nitrogen) on seed yield and the percentage of oil of evening primrose seed. In this project, the effect of nitrogen under different harvest methods was studied by using some pot experiments.

The effect of storage conditions on the seed oil percentage and fatty acid composition of evening primrose were also investigated. In addition to these seed germination ability of evening primrose was also studied.

For field experiments seed yield, thousand seed weight, oil percentage, raw protein percentage, fatty acid composition, plant dry matter, seed dry matter were measured. The parameters measured in the pot experiments were seed yield, thousand seed weight, oil and raw protein percentage, fatty acid composition, number of side shoots per pot, number of capsules per side shoot, number of capsules per main stem and total number of capsules per pot.

Both in field and pot experiments it was observed that harvest time had influence on the seed yield of evening primrose. Due to low maturity, a significant reduction in the seed yield of early harvested plants was observed. Similar pattern of seed yield was observed in plants that were harvested late. Contrary to early harvest high seed shattering is the main reason of low seed yield in the plants that were harvested at the late date. Oil and γ-linolenic acid percentage of the seed were significantly influenced by different harvest times. Low seed maturity at the early time and advance senescence of the seed during the over ripened period are the main

reasons for the low percentage of oil. Regardless of the sowing time, in both field as well as pot experiments, it was observed that in most cases the maximum seed quantity and quality was achieved during the middle harvest time.

Despite the above interpretation of results, it is not easy to say how many days after flowering can be recommended as harvest time. The parameters such as plant variety, climate conditions, soil fertility, sowing time, etc. strongly influence harvest time. Generally, according to this study the average of the period after flowering to harvest in autumn and spring sown plants is different. In addition, nitrogen can influence this period. In total, 70-75 days after flowering is the recommended harvest time of the spring sown *Oenothera biennis L*. cv. "Anothera" in Germany. In autumn sown plants, the duration between flowering and harvest is longer than spring-sown plants (90-95 d after flowering).

Study on the effect of nitrogen on the evening primrose in pot experiments showed that although nitrogen had negative influence on the percentage of seed oil, there was positive relationship between nitrogen and most measured parameters especially seed yield, linoleic and γ-linolenic acid percentage. There is a need to carry out further investigation on the effect of nitrogen as well as other plant nutrients on evening primrose in the field conditions.

In general based on obtained results it can be concluded that in both field and pot experiments no clear effect of defoliation was observed on the different measured parameters like seed yield, the percentage of oil and γ-linolenic acid. The observations of these experiments in most cases do not confirm the hypothesis in which defoliation reduces the heterogenity of seed ripeness and effects seed yield and seed quality. In most cases no interaction between method of harvest and harvest time as well as nitrogen fertilizer was observed. The hypothesis in which effect of defoliation depends on harvest time and nitrogen application is different was not agree with the results obtained.

From the results of experiments, it can be concluded that there is not a big difference in the seed yield and seed oil quality of spring and autumn evening primrose. Thus, the hypothesis that the performance of spring-sown evening primrose is equal to autumn sown plants was confirmed with the obtained results.

The results showed that the quality of newly harvested seeds of evening primrose gradually decrease during storage. The free fatty acid percentage and the peroxide value of stored seeds strongly increased with the storage time. It was observed that the increase was lower in the seed samples that were stored at low temperature than under room temperature and higher. A negative relationship between storage time and the percentage of oil was observed in this

study. Based on the results obtained from the present study, it may be said that for oil quality and quantity the fresh seeds of evening primrose can be stored for a period of 4-6 months.
Temperature is the main factor affecting the germination of evening primrose seeds. A synergetic effect between light and temperature on evening primrose seed germination was observed in this study. Depending on harvest time a gradual increase in the seed germination percentage of newly harvested seed could be achieved by removing the physiological dormancy of embryo during storage. The reduction of germination with increasing storage time could be due to some stimulation of secondary dormancy in the embryo.

Zusammenfassung

Die Nachtkerze besitzt als Nutzpflanze ein großes Potential für die kommerzielle Produktion von Gamma-Linoleinsäure. Bei der Kultivierung dieser Pflanze müssen jedoch einige Probleme wie indeterminiertes Wachstum, hoher Samenausfall während der Reife, langer Lebenszyklus und ungleichmäßige Samenabreife gelöst werden. Obwohl sich bereits einige Studien mit der Verminderung des Samenausfalls wie auch der Reduzierung der Blühheterogenität beschäftigt haben, ist der Samenverlust immer noch das größte Problem in der Nachtkerzenproduktion. Das Ziel der Arbeit bestand deshalb darin, in Feld- und Gefäßversuchen den Einfluss unterschiedlicher Erntetermine und –Methoden auf den Samenertrag und die Samenqualität von Nachtkerze zu klären. Als Erntemethoden wurden dabei die chemische Sikkation und die thermische Behandlung der Pflanzen angewandt. Parallel zu den Feld- und Gefäßversuchen mit Nachtkerze wurde der Effekt der Lagerungsbedingungen auf den Ölgehalt und die Fettsäurezusammensetzung untersucht. Zusätzlich wurden Experimente durchgeführt, in denen Einflüsse der Erntemethode und der Lagerung auf die Keimfähigkeit der Samen untersucht wurden. In den Feldversuchen wurden die Prüfmerkmale Samenertrag, Tausendkornmasse, Ölgehalt, Proteingehalt, Fettsäurezusammensetzung sowie Ganzpflanzen- und Samentrockenmasse ermittelt. In den Gefäßversuchen wurden die Prüfmerkmale Samenertrag, Tausendkornmasse, Ölgehalt, Proteingehalt und Fettsäurezusammensetzung sowie Anzahl der Seitentriebe pro Gefäß, Anzahl der Kapseln pro Seitentrieb, Anzahl Kapseln pro Haupttrieb und Anzahl Kapseln pro Gefäß bestimmt.
Die Ergebnisse der Feld- und Gefäßversuche bringen zum Ausdruck, dass der Erntezeitpunkt einen gesicherten Einfluss auf den Samenertrag der Nachtkerze hat. Auf Grund der verringerten Zeit für die Samenreife war der Samenertrag der früh geernteten Pflanzen stark reduziert. Eine Verminderung des Samenertrages wurde auch bei den Pflanzen der letzten

Erntezeit gefunden. Im Gegensatz zur frühen Ernte ist bei der späten Ernte der hohe Samenausfall der Hauptgrund für den geringen Samenertrag. Öl- und Gamma-Linoleinsäuregehalt der Samen wurden ebenfalls signifikant durch die verschiedenen Erntetermine beeinflusst. Geringe Ausreife der Samen bei früher Ernte und erhöhte Seneszenz der Pflanzen während der Überreife sind die Hautursachen für den geringen Ölgehalt. Sowohl in den Feld- als auch in den Gefäßexperimenten wurde unabhängig von Frühjahrs- oder Herbstaussaat festgestellt, dass in den meisten Fällen die höchsten Samenerträge und die beste Samenqualität bei mittlerer Erntezeit erzielt wurde.

Der Reifetermin der Pflanzen wird durch Faktoren wie Sorte, Witterung, Nährstoffversorgung und Saatzeit signifikant beeinflusst. In den durchgeführten Versuchen wurde festgestellt, dass die Zeitspanne von der Blüte bis zur Ernte bei Frühjahrs- und Herbstaussaat der Nachtkerze verschieden ist. Auch die Stickstoffversorgung hat einen Einfluss auf diese Zeitspanne. Bei einer Aussaat im Frühjahr erreichte die Nachtkerze etwa 70 –75 Tage nach der Blüte die Samen- und Erntereife. Bei der Herbstaussaat betrug dieser Zeitraum etwa 90 – 95 Tage.

Die Erntemethode hatte in den meisten Fällen keinen gesicherten Einfluss auf die gemessenen Parameter. In der Tendenz scheint die Sikkation der Pflanzen, speziell mit einem Herbizid, die Samenreifung zu stimulieren und die Anzahl reifer Samen zu erhöhen. Somit kann diese Maßnahme indirekt den Samenertrag und die Samenölqualität beeinflussen.

Die Untersuchungen zur Stickstoff-Düngung in Gefäßversuchen zeigten, dass dieser Nährstoff einen negativen Einfluss auf den Samenölgehalt, jedoch einen positiven Einfluss den Samenertrag sowie auf den Linolsäure- und Gammalinoleinsäuregehalt der Samen hatte. Zur Validierung dieser Effekte sind jedoch weitere Untersuchungen erforderlich.

Die Ergebnisse der Nachernteexperimente zeigen, dass die Qualität der frisch geernteten Samen der Nachtkerze während der Lagerung allmählich abnimmt. Die Säure- und Peroxidwerte der gelagerten Samen steigen während der Lagerung stark an. Es wurde festgestellt, dass der Anstieg dieser Werte in den Samen bei kühler Temperatur niedriger war als bei Raumtemperatur oder noch höheren Temperaturen. Zwischen Lagerungsdauer und Ölgehalt wurde ein negativer Zusammenhang gefunden. Basierend auf den ermittelten Ergebnissen und im Hinblick auf den Ölgehalt und die Ölqualität kann gesagt werden, dass frisch geerntete Samen der Nachtkerze unter kühlen Bedingungen 4-6 Monate gelagert werden können. In Anlehnung an die Ergebnisse der Versuche zur Keimfähigkeit kann festgestellt werden, dass die Temperatur den Haupteinflussfaktor auf die Keimfähigkeit der Nachtkerzensamen darstellt. Zwischen Licht und Temperatur wurde ein synergistischer Effekt auf die Samenkeimung der Nachtkerze beobachtet. In Abhängigkeit von der Erntezeit konnte

ein gradueller Anstieg im Anteil gekeimter Samen während der Lagerung festgestellt werden. Diese wird auf eine Verminderung der Dormanz des Embryos während der ersten Monate der Lagerung zurückgeführt. Bei Langzeitlagerung (Raumtemperatur) von mehr als 6 Monaten nahm die Keimfähigkeit der Samen dagegen ab.

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Appendix

Fig 1. Design of field experiments with evening primrose, Groß-Gerau 2004-2005

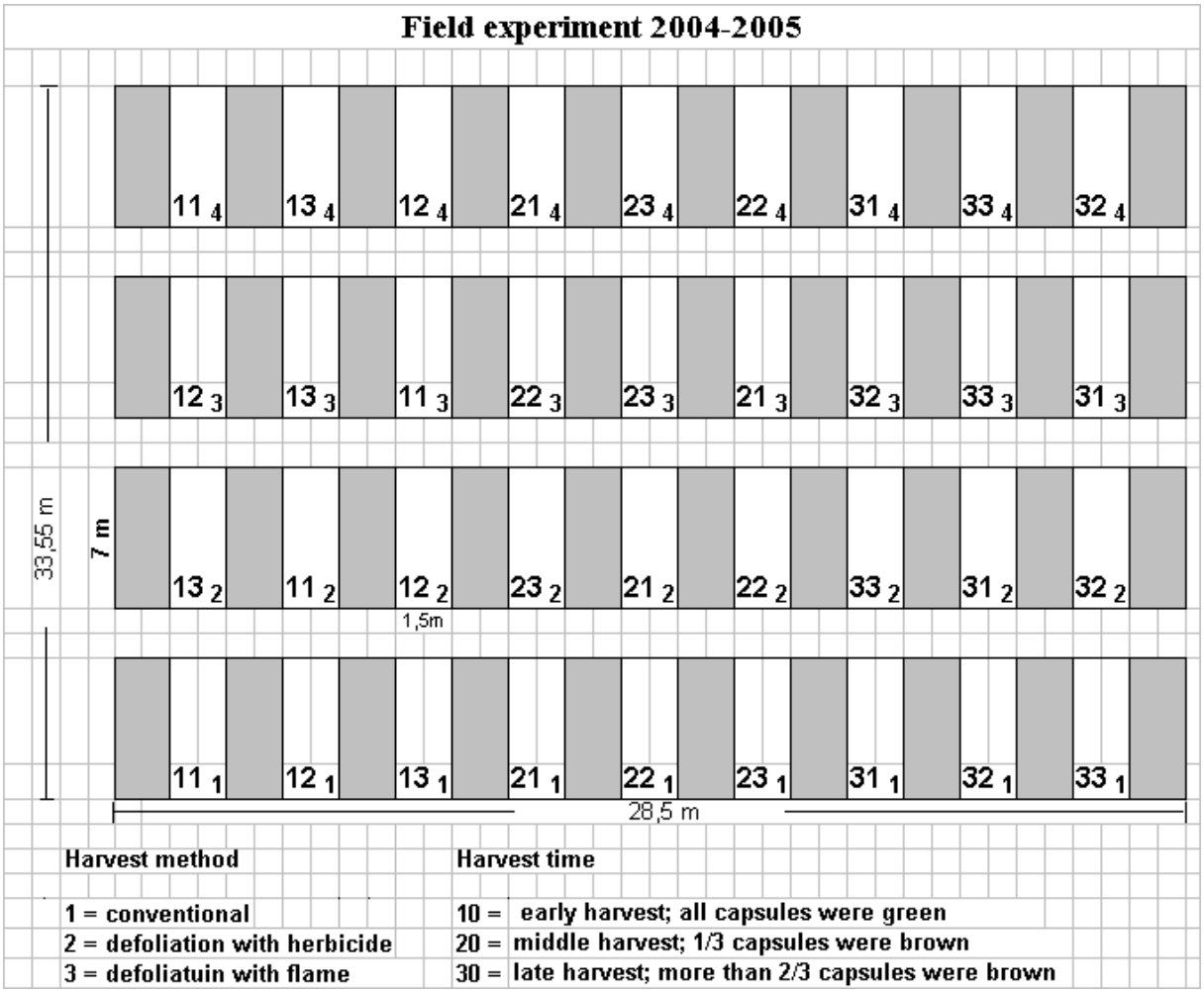

Table ap.1. Effect of different levels of nitrogen, harvest times and harvest methods on seed yield and yield components of *Oenothera biennis*, Autumn 2004 (A)

N	T	M	SY (g/pot)	SSP	CMS	CSS	CP	TSW (g)
0.5 g N	1	1	12.5	11.0	75.7	73.0	148.7	0.36
	1	2	13.2	11.3	65.7	64.0	129.7	0.42
	1	3	13.1	8.3	68.3	62.3	130.7	0.39
	2	1	12.8	9.7	77.3	66.0	143.3	0.41
	2	2	11.3	9.7	66.7	58.3	125.0	0.42
	2	3	12.7	8.7	81.0	51.0	132.0	0.39
	3	1	14.0	11.7	63.7	67.7	131.3	0.40
	3	2	12.2	8.0	82.0	64.3	146.3	0.38
	3	3	13.4	11.7	62.0	80.7	142.7	0.43
1.0 g N	1	1	22.2	13.3	106.0	182.0	288.0	0.38
	1	2	21.1	11.7	102.0	143.0	245.3	0.41
	1	3	22.1	11.0	99.0	139.0	238.0	0.44
	2	1	21.7	8.7	113.0	126.0	239.0	0.39
	2	2	22.3	10.0	100.3	153.0	253.3	0.42
	2	3	22.0	9.0	133.0	96.0	229.0	0.42
	3	1	21.6	10.0	149.0	129.7	278.7	0.40
	3	2	23.9	11.0	109.7	139.0	248.7	0.39
	3	3	23.2	10.7	108.3	129.7	238.0	0.43
2.0 g N	1	1	22.8	12.7	153.3	160.7	314.0	0.38
	1	2	24.7	11.0	134.0	174.3	308.3	0.36
	1	3	23.7	11.7	138.3	175.3	313.7	0.39
	2	1	25.0	12.3	149.3	200.7	350.0	0.41
	2	2	25.4	10.7	127.0	178.0	305.0	0.44
	2	3	24.1	9.3	110.7	166.7	277.3	0.42
	3	1	25.8	12.7	162.0	264.0	426.3	0.41
	3	2	22.1	11.3	146.0	188.0	334.0	0.42
	3	3	28.8	10.3	120.0	227.7	347.7	0.39
0.5 g N			12.8	10.0	71.4	65.3	136.6	0.40
2.0 g N			22.2	10.6	113.4	137.5	250.9	0.41
3.0 g N			24.7	11.3	137.9	192.9	330.7	0.40
	1		19.5	11.3	104.7	130.4	235.2	0.39
	2		19.7	9.8	106.5	121.7	228.2	0.41
	3		20.6	10.8	111.4	143.4	254.9	0.40
		1	19.8	11.3	116.6	141.1	257.7	0.39
		2	19.6	10.5	103.7	129.1	232.9	0.41
		3	20.4	10.1	102.3	125.4	227.7	0.41
N P-value			0.00	ns	0.00	0.00	0.00	ns
T			ns	0.02	ns	0.01	0.01	0.04
M			ns	ns	ns	ns	0.00	ns
N×T			ns	ns	ns	0.00	0.04	ns
N×M			ns	ns	ns	0.00	0.04	ns
T×M			ns	ns	ns	ns	ns	ns
N×T×M			ns	ns	ns	ns	ns	ns
N LSD 5%			2.0	1.2	13.3	14.0	17.9	ns
T			ns	1.2	ns	14.0	17.9	0.02
M			ns	ns	ns	ns	17.9	ns
N×T			ns	ns	ns	24.2	30.1	ns
N×M			ns	ns	ns	24.2	30.1	ns
T×M			ns	ns	ns	ns	ns	ns
N×T×M			ns	ns	ns	ns	ns	ns

N: Nitrogen, T: Harvest time, M: harvest method; 1: draying the capsules under 40°C, 2: defoliation by herbicide, 3: conventional harvest with accelerating the dryness by hanging, SY: seed yield, SSP: side shoots per pot, CMS: capsules of main stem, CSS: capsules of side shoot, CP: capsules per pot

Table ap. 2. Effect of different levels of nitrogen, harvest times and harvest methods on seed oil quantity and quality of *Oenothera biennis*, Autumn 2004

N	T	M	SO (%)	SP (%)	PA (%)	SA (%)	OA (%)	LA (%)	GLA (%)
0.5 g N/p	1	1	25.2	12.5	5.9	2.2	11.1	73.0	7.5
	1	2	28.2	12.8	5.4	2.2	11.3	72.4	7.3
	1	3	27.6	12.4	5.7	2.2	11.6	72.0	7.5
	2	1	28.9	12.9	5.7	2.2	11.6	72.1	7.7
	2	2	28.4	12.7	5.8	2.2	11.4	71.6	7.3
	2	3	28.7	12.3	5.7	2.1	11.2	72.9	7.3
	3	1	28.8	12.1	5.5	2.2	11.3	72.4	7.2
	3	2	28.8	13.3	5.6	2.3	12.0	70.7	7.4
	3	3	28.1	12.3	5.5	2.1	11.8	72.7	7.5
1.0 g N/p	1	1	27.0	13.6	5.7	2.2	10.3	72.5	7.9
	1	2	27.7	13.6	5.7	2.4	10.9	71.8	8.1
	1	3	28.6	14.5	5.7	2.1	10.9	71.8	7.8
	2	1	28.5	14.5	5.6	2.1	11.1	71.9	7.4
	2	2	28.3	13.7	5.6	2.2	10.5	73.0	7.8
	2	3	27.4	13.7	5.7	2.2	11.7	72.2	7.5
	3	1	27.0	13.3	5.4	2.1	11.0	71.8	7.8
	3	2	28.4	14.3	5.4	2.1	9.9	73.8	8.0
	3	3	28.3	14.1	5.0	2.2	10.8	71.9	7.3
2.0 g N/p	1	1	25.4	15.1	5.8	2.2	9.8	73.0	8.4
	1	2	26.6	14.9	5.7	2.3	9.9	72.8	8.4
	1	3	26.9	15.5	5.5	2.2	10.9	72.3	8.3
	2	1	26.7	15.8	5.5	2.0	8.9	74.0	8.9
	2	2	27.9	15.7	5.4	2.4	10.5	73.3	9.6
	2	3	26.9	15.7	5.0	2.3	9.9	73.7	9.0
	3	1	26.4	15.4	5.1	2.1	9.3	73.7	9.4
	3	2	26.6	16.4	5.3	2.2	9.3	73.6	9.1
	3	3	26.7	16.7	5.0	2.2	8.8	74.1	9.4
0.5 g N/p			28.1	12.6	5.7	2.2	11.5	72.2	7.4
1.0 g N/p			27.9	13.9	5.5	2.2	10.8	72.3	7.7
2.0 g N/p			26.7	15.7	5.4	2.2	9.7	73.4	8.9
	1		27.0	13.8	5.7	2.2	10.7	72.4	7.9
	2		28.0	14.1	5.7	2.2	10.8	72.7	8.0
	3		27.7	14.2	5.3	2.2	10.5	72.8	8.1
		1	27.7	13.9	5.6	2.1	10.5	72.7	8.0
		2	27.9	14.1	5.6	2.3	10.6	72.6	8.1
		3	27.7	14.1	5.4	2.2	10.9	72.6	7.9
N P-value			0.00	0.00	0.00	ns	0.00	0.00	0.00
T			0.02	ns	0.00	ns	ns	ns	ns
M			ns	ns	ns	0.02	ns	ns	ns
N×T			ns	ns	ns	ns	ns	ns	0.00
N×M			ns	ns	ns	ns	ns	ns	ns
T×M			ns	ns	ns	ns	ns	ns	ns
N×T×M			ns	ns	ns	ns	ns	ns	ns
N LSD 5%			0.58	0.41	0.15	ns	0.47	0.55	0.19
T			0.58	ns	0.15	ns	ns	ns	ns
M			ns	ns	ns	0.10	ns	ns	ns
N×T			ns	ns	ns	ns	ns	ns	0.32
N×M			ns	ns	ns	ns	ns	ns	ns
T×M			ns	ns	ns	ns	ns	ns	ns
N×T×M			ns	ns	ns	ns	ns	ns	ns

N: Nitrogen, T: Harvest time, M: harvest method; 1: draying the capsules under 40°C, 2: defoliation by herbicide, 3: conventional harvest with accelerating the dryness by hanging, SO: seed oil, SP: seed protein, PA; Palmitic acid, SA; Stearic acid, OA: Oleic acid, LA; Linolenic acid, GLA; γ- Linolenic acid.

Results of statistical analysis for seed yield, oil, linoleic and γ-linolenic percentage

Table ap. 3. Field experiment 2004

Dependent Variable: **seed yield (Dt/ha)**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	26.755(a)		3.344	1.331	.271
Intercept	6432.040	1	6432.040	2559.733	.000
T	.682	2	.341	.136	.874
M	21.572	2	10.786	4.292	.024
T×H	4.502	4	1.125	.448	.773
Error	67.845	27	2.513		
Total	6526.640	36			
Corrected Total	94.600	35			

a R Squared = .283 (Adjusted R Squared = .070)

Table ap. 4. Field experiment 2004

Dependent Variable: **oil percentage**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	28.342(a)	8	3.543	2.570	.032
Intercept	25048.338	1	25048.338	18170.476	.000
T	1.177	2	.589	.427	.657
M	6.034	2	3.017	2.189	.132
T×H	21.131	4	5.283	3.832	.014
Error	37.220	27	1.379		
Total	25113.900	36			
Corrected Total	65.562	35			

a R Squared = .432 (Adjusted R Squared = .264)

Table ap. 5. Field experiment 2004

Dependent Variable: **linoleic acid**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.483(a)	8	.185	1.855	.110
Intercept	193598.181	1	193598.181	1937269.032	.000
T	.290	2	.145	1.453	.252
M	.040	2	.020	.198	.821
T×M	1.153	4	.288	2.885	.041
Error	2.698	27	.100		
Total	193602.363	36			
Corrected Total	4.182	35			

a R Squared = .355 (Adjusted R Squared = .164)

Table ap.6. field experiment 2004

Dependent Variable: **Gamma linolenic acid**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	12.641(a)	8	1.580	9.597	.000
Intercept	2485.001	1	2485.001	15093.540	.000
T	12.037	2	6.018	36.555	.000
M	.364	2	.182	1.106	.345
T×M	.240	4	.060	.364	.832
Error	4.445	27	.165		
Total	2502.087	36			
Corrected Total	17.086	35			

a R Squared = .740 (Adjusted R Squared = .663)

Table ap.7. Pot experiment 2004

Dependent Variable: **seed yield**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	450.015(a)	11	40.910	27.922	.000
Intercept	6682.340	1	6682.340	4560.738	.000
N	428.228	1	428.228	292.268	.000
T	5.447	2	2.723	1.859	.171
M	2.955	1	2.955	2.017	.164
N×T	9.911	2	4.955	3.382	.045
N ×M	.263	1	.263	.179	.675
T×M	.434	2	.217	.148	.863
N×T×M	2.778	2	1.389	.948	.397
Error	52.747	36	1.465		
Total	7185.102	48			
Corrected Total	502.762	47			

a R Squared = .895 (Adjusted R Squared = .863)

Table ap. 8. Pot experiment 2004

Dependent Variable: **oil percentage**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	22.338(a)	11	2.031	2.205	.037
Intercept	41875.905	1	41875.905	45471.363	.000
N	.001	1	.001	.001	.976
T	7.328	2	3.664	3.978	.027
M	.568	1	.568	.616	.438
N×T	1.129	2	.564	.613	.547
N ×M	2.774	1	2.774	3.013	.091
T× M	9.764	2	4.882	5.301	.010
N ×T ×M	.774	2	.387	.420	.660
Error	33.153	36	.921		
Total	41931.396	48			
Corrected Total	55.491	47			

a R Squared = .403 (Adjusted R Squared = .220)

Table ap.9. pot experiment 2004

Dependent Variable: **linoleic acid**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	11.296(a)	11	1.027	1.980	.061
Intercept	248127.677	1	248127.677	478457.263	.000
N	5.828	1	5.828	11.238	.002
T	.781	2	.390	.753	.478
M	2.081	1	2.081	4.013	.053
N×T	.193	2	.096	.186	.831
N×M	.061	1	.061	.117	.735
H×M	.797	2	.399	.769	.471
N ×T ×M	1.556	2	.778	1.500	.237
Error	18.670	36	.519		
Total	248157.642	48			
Corrected Total	29.965	47			

a R Squared = .377 (Adjusted R Squared = .187)

Table ap.10. pot experiment 2004

Dependent Variable: **gamma linolenic acid**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	27.868(a)	11	2.533	9.098	.000
Intercept	3099.308	1	3099.308	11129.973	.000
N	1.051	1	1.051	3.775	.060
T	4.656	2	2.328	8.359	.001
M	1.994	1	1.994	7.159	.011
N×T	.739	2	.369	1.327	.278
N×M	3.298	1	3.298	11.844	.001
H×M	10.960	2	5.480	19.679	.000
N×T×M	5.171	2	2.585	9.284	.001
Error	10.025	36	.278		
Total	3137.201	48			
Corrected Total	37.893	47			

a R Squared = .735 (Adjusted R Squared = .655)

Table ap.11. Field experiment autumn 2005

Dependent Variable: **oil percentage**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8.860(a)	8	1.108	1.412	.257
Intercept	19585.920	1	19585.920	24967.887	.000
T	.436	2	.218	.278	.761
M	6.269	2	3.134	3.996	.037
T×M	2.156	4	.539	.687	.610
Error	14.120	18	.784		
Total	19608.900	27			
Corrected Total	22.980	26			

a R Squared = .386 (Adjusted R Squared = .112)

Table ap.12. Field experiment autumn 2005

Dependent Variable: **linolenic acid percentage**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6.479(a)	8	.810	2.088	.093
Intercept	143853.801	1	143853.801	370969.689	.000
T	1.965	2	.983	2.534	.107
M	.534	2	.267	.689	.515
T×M	3.979	4	.995	2.565	.074
Error	6.980	18	.388		
Total	143867.260	27			
Corrected Total	13.459	26			

a R Squared = .481 (Adjusted R Squared = .251)

Table ap.13. Field experiment autumn 2005

Dependent Variable: **gamma linolenic acid**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.987(a)	8	.623	3.708	.010
Intercept	1853.396	1	1853.396	11022.399	.000
T	2.676	2	1.338	7.958	.003
M	.312	2	.156	.927	.414
T×M	1.999	4	.500	2.972	.048
Error	3.027	18	.168		
Total	1861.410	27			
Corrected Total	8.014	26			

a R Squared = .622 (Adjusted R Squared = .454)

Table ap.14. Field experiment spring 2005

Dependent Variable: **seed yield**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	14.207(a)	8	1.776	.564	.793
Intercept	2891.273	1	2891.273	918.837	.000
T	4.659	2	2.329	.740	.491
M	.543	2	.271	.086	.918
T×M	9.006	4	2.251	.716	.592
Error	56.640	18	3.147		
Total	2962.120	27			
Corrected Total	70.847	26			

a R Squared = .201 (Adjusted R Squared = -.155)

Table ap.15. Field experiment spring 2005

Dependent Variable: **oil percentage**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	92.300(a)	8	11.538	16.500	.000
Intercept	19845.333	1	19845.333	28380.508	.000
T	73.929	2	36.964	52.862	.000
M	2.409	2	1.204	1.722	.207
T×M	15.962	4	3.991	5.707	.004
Error	12.587	18	.699		
Total	19950.220	27			
Corrected Total	104.887	26			

a R Squared = .880 (Adjusted R Squared = .827)

Table ap.16. Field experiment spring 2005

Dependent Variable: **linolenic acid**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	16.519(a)	8	2.065	12.172	.000
Intercept	136974.578	1	136974.578	807492.055	.000
T	13.481	2	6.740	39.736	.000
M	1.916	2	.958	5.648	.012
T×M	1.121	4	.280	1.653	.205
Error	3.053	18	.170		
Total	136994.150	27			
Corrected Total	19.572	26			

a R Squared = .844 (Adjusted R Squared = .775)

Table ap.17. Field experiment spring 2005

Dependent Variable: **gamma linolenic acid**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7.576(a)	8	.947	4.255	.005
Intercept	1755.307	1	1755.307	7885.739	.000
T	5.534	2	2.767	12.431	.000
M	1.299	2	.649	2.917	.080
T×M	.744	4	.186	.835	.520
Error	4.007	18	.223		
Total	1766.890	27			
Corrected Total	11.583	26			

a R Squared = .654 (Adjusted R Squared = .500)

Table ap.18. Pot experiment autumn 2005

Dependent Variable: **seed yield**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2254.369(a)	26	86.707	19.225	.000
Intercept	32132.554	1	32132.554	7124.540	.000
N	2131.165	2	1065.582	236.265	.000
T	17.876	2	8.938	1.982	.148
M	8.407	2	4.203	.932	.400
N×T	8.962	4	2.240	.497	.738
N×M	8.309	4	2.077	.461	.764
T×M	18.775	4	4.694	1.041	.395
N×T ×M	60.876	8	7.609	1.687	.123
Error	243.547	54	4.510		
Total	34630.470	81			
Corrected Total	2497.916	80			

a R Squared = .903 (Adjusted R Squared = .856)

Table ap.19. Pot experiment autumn 2005

Dependent Variable: **oil percentage**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	83.960(a)	26	3.229	2.259	.006
Intercept	61542.584	1	61542.584	43044.204	.000
N	30.748	2	15.374	10.753	.000
T	12.861	2	6.430	4.498	.016
M	9.148	2	4.574	3.199	.049
N×T	7.344	4	1.836	1.284	.288
N×M	.192	4	.048	.034	.998
H×M	12.252	4	3.063	2.142	.088
N×T×M	11.414	8	1.427	.998	.448
Error	77.207	54	1.430		
Total	61703.750	81			
Corrected Total	161.166	80			

a R Squared = .521 (Adjusted R Squared = .290)

Table ap.20. Pot experiment autumn 2005

Dependent Variable: **linolenic acid**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	56.096(a)	26	2.158	2.076	.012
Intercept	427280.111	1	427280.111	411187.941	.000
N	23.210	2	11.605	11.168	.000
T	2.207	2	1.103	1.062	.353
M	.296	2	.148	.143	.867
N×T	6.730	4	1.683	1.619	.183
N×M	10.296	4	2.574	2.477	.055
T×M	3.153	4	.788	.758	.557
N ×T×M	10.204	8	1.275	1.227	.301
Error	56.113	54	1.039		
Total	427392.320	81			
Corrected Total	112.209	80			

a R Squared = .500 (Adjusted R Squared = .259)

Table ap.21. Pot experiment autumn 2005

Dependent Variable: **gamma linolenic acid**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	42.619(a)	26	1.639	13.903	.000
Intercept	5214.445	1	5214.445	44227.226	.000
N	34.757	2	17.378	147.397	.000
T	.605	2	.303	2.568	.086
M	.328	2	.164	1.390	.258
N×T	4.490	4	1.123	9.521	.000
N×M	.639	4	.160	1.355	.262
T×M	.235	4	.059	.497	.738
N×T×M	1.565	8	.196	1.660	.130
Error	6.367	54	.118		
Total	5263.430	81			
Corrected Total	48.985	80			

a R Squared = .870 (Adjusted R Squared = .807)

Table ap.22. Pot experiment spring 2005

Dependent Variable: **seed yield**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2771.093(a)	23	120.482	13.480	.000
Intercept	39565.533	1	39565.533	4426.775	.000
N	2095.648	2	1047.824	117.235	.000
T	197.041	2	98.521	11.023	.000
M	39.099	2	19.550	2.187	.123
N×T	253.219	4	63.305	7.083	.000
N×M	53.257	4	13.314	1.490	.220
T×M	26.224	3	8.741	.978	.411
N×T×M	103.688	6	17.281	1.934	.094
Error	429.013	48	8.938		
Total	43703.430	72			
Corrected Total	3200.107	71			

a R Squared = .866 (Adjusted R Squared = .802)

Table ap.23. Pot experiment spring 2005

Dependent Variable**: oil percentage**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	276.011(a)	23	12.000	7.911	.000
Intercept	50710.441	1	50710.441	33429.333	.000
N	146.472	2	73.236	48.279	.000
T	31.724	2	15.862	10.456	.000
M	5.788	2	2.894	1.908	.159
N×T	39.257	4	9.814	6.470	.000
N×M	14.208	4	3.552	2.342	.068
T×M	4.378	3	1.459	.962	.418
N×T×M	22.605	6	3.768	2.484	.036
Error	72.813	48	1.517		
Total	53313.100	72			
Corrected Total	348.824	71			

a R Squared = .791 (Adjusted R Squared = .691)

Table ap.24. Pot experiment spring 2005

Dependent Variable: **linoleic acid**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	48.838(a)	23	2.123	2.342	.006
Intercept	364569.351	1	364569.351	402036.963	.000
N	7.603	2	3.801	4.192	.021
T	2.555	2	1.277	1.409	.254
M	3.722	2	1.861	2.053	.140
N×T	8.745	4	2.186	2.411	.062
N ×M	6.485	4	1.621	1.788	.147
T ×M	6.732	3	2.244	2.475	.073
N×T×M	13.068	6	2.178	2.402	.041
Error	43.527	48	.907		
Total	378774.400	72			
Corrected Total	92.364	71			

a R Squared = .529 (Adjusted R Squared = .303)

Table ap.25. Pot experiment spring 2005

Dependent Variable: **gamma linolenic acid**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	34.260(a)	23	1.490	10.989	.000
Intercept	4316.440	1	4316.440	31842.587	.000
N	29.181	2	14.590	107.634	.000
T	.822	2	.411	3.033	.057
M	.090	2	.045	.332	.719
N×T	2.649	4	.662	4.885	.002
N×M	.392	4	.098	.722	.581
T×M	.360	3	.120	.886	.455
N×T×M	1.481	6	.247	1.821	.115
Error	6.507	48	.136		
Total	4513.770	72			
Corrected Total	40.767	71			

a R Squared = .840 (Adjusted R Squared = .764)