Received: 25 March 2022 | Revised: 5 August 2022 | Accepted: 16 August 2022
DOI: 10.1111/jac.12623

DROUGHT STRESS

Journal of Agronomy and Crop Science WILEY

Grain yield, harvest index, water-use efficiency and nitrogen partitioning to grain can be improved by application of the plant growth regulator paclobutrazol to maize plants with reduced N supply

Birgit W. Hütsch | **Sven Schubert**

Institute of Plant Nutrition (iFZ), Justus Liebig University, Giessen, Germany

Correspondence
Birgit W. Hütsch, Institute of Plant Nutrition (iFZ), Justus Liebig University, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany.
Email: birgit.w.huetsch@ernaehrung.uni-giessen.de

Funding information
Deutsche Forschungsgemeinschaft (DFG, German Research Foundation), Grant/Award Number: HU554/5-1; Justus Liebig University Giessen

Abstract

Applications of plant growth regulators such as paclobutrazol (PAC) to grain maize frequently caused depressions in grain yield. This negative impact probably originated from treatments at an early growth stage during plant ontogenesis when the determination of potential kernel number coincided with time of regulator application. However, stability of grain yield itself is of high relevance, and it is also the key determinant for harvest index (HI) and the use efficiencies of water (WUE) and nutrients (NUE). Therefore, in a container experiment, the effect of delayed PAC application at growth stage V8 was tested with the maize (*Zea mays* L.) cultivars Galactus and Fabregas. Immediately after PAC treatment, differential N fertilization was introduced in order to meet the demand of the control plants (100% N), and with a supply of 75% N. With late PAC application (V8), grain yield depressions could not only successfully be avoided; moreover, in Galactus-75%N, a significant increase in grain yield was achieved combined with an extended duration of pollen shed by 28%. Straw yield decreased less strongly after late compared with early PAC application, leading to small, but significant increases in HI for the maize plants with 75% N supply. An increase in PAC dosage combined with later application will certainly lead to stronger decreases in straw yield, but it will also enhance the risk for grain yield depressions, and thus, an overall stronger improvement of HI is uncertain. For the time around silking, remarkable improvements of $\mathrm{WUE}_{\mathrm{grain}}$ by 18% were achieved after delayed PAC application to Galactus-75%N. After PAC treatment, significant increases in nitrogen-harvest index (NHI) and thus N partitioning to grain were achieved for Fabregas and for Galactus-75%N. Although luxurious N consumption did not occur, late PAC application showed neither an effect on N-utilization efficiency ($\mathrm{NUtE}_{\mathrm{grain}}$) nor on N-uptake efficiency (NUpE). It can be concluded that it is a very complex task to achieve the right balance between PAC dosage, stability of grain yield and optimal N supply in order to avoid both, luxurious N consumption and N deficiency, and to achieve an improvement of $\mathrm{NUtE}_{\mathrm{grain}}$ of maize plants.

KEYWORDS
gibberellin-biosynthesis inhibitor, kernel number, nitrogen-use efficiency, pollination, single kernel weight, *Zea mays*

Key points

- Applications of plant growth regulators to grain maize frequently caused depressions in grain yield.
- However, stability of grain yield itself is of high relevance, and it is also the key determinant for harvest index (HI) and the use efficiencies of water (WUE) and nutrients.
- With late paclobutrazol (PAC) application, grain yield depressions could be avoided, and combined with reduced N supply, a significant grain yield increase was achieved.
- Late PAC application and reduced N supply led to significant increases in HI and N partitioning to grain, as well as remarkable improvements in WUE_{grain} around silking.
- Late PAC application showed neither an effect on N-utilization efficiency nor on N-uptake efficiency.

1 | INTRODUCTION

In contrast to barley and wheat, the harvest index (ratio of grain yield to total above-ground biomass at physiological maturity) of grain maize was almost unchanged during the last decades and increases in maize grain yield were mainly achieved by higher plant stand densities (Duvick, 2005; Hütsch & Schubert, 2017; Russell, 1991). Concomitantly, vegetative biomass production was strongly enhanced. In contrast to maize used as whole plant silage or as bioenergy source, for grain maize production, a huge vegetative biomass is not needed but requires large amounts of nutrients and water (Hammer et al., 2009). Thus, an increase in harvest index by reduction in vegetative shoot growth may improve resource-use efficiencies. In crop production, less input of resources is beneficial for several reasons: (1) restricted fertilizer resources, for example, of phosphorus (P) are saved; (2) less fertilizer application reduces the risk of losses, for example, due to nitrate leaching, denitrification or P runoff; and (3) saving of water. Climate-change models generally predict decreased precipitations in many of the world's cropping regions, making water an increasingly limiting factor for plant growth and development (Davies et al., 2011; Ort & Long, 2014). Excessive vegetative growth can aggravate water limitations by using too much water before flowering (Passioura & Angus, 2010), as sufficient water availability during the reproductive period is particularly important for kernel setting and thus yield performance of maize (Hütsch et al., 2015; Otegui et al., 1995; Setter & Parra, 2010; Yang & Grassini, 2014; Zinselmeier et al., 1999). According to Duvick (2005), more emphasis on improving WUE with the aim of achieving better drought resistance is needed.

For maize crops, reduction in vegetative shoot growth can be achieved by the application of gibberellin-biosynthesis inhibitors such as paclobutrazol (PAC) or uniconazole (UCZ). PAC- and UCZ-treated maize plants showed decreased plant height and thicker culms due to reduced elongation growth of the internodes (e.g. Hütsch & Schubert, 2018, 2021a, 2021b; Iremiren et al., 1997; Kamran et al., 2018; Schluttenhofer et al., 2011). These growth regulators allow the induction of reduced GA concentrations during selective phases of maize ontogenesis and not during the entire growth period as in the dwarf mutants, which show abnormalities in flower sexuality and are therefore not suitable for commercial maize production (Bortiri & Hake, 2007; Irish, 1996; Rood et al., 1980; Xu et al., 2004).

Most studies on nutrient-use efficiencies focused on nitrogen (N), as it plays versatile key roles in plant metabolism. Nitrogen is strongly associated with the source component via Rubisco in leaf tissue enabling photosynthesis, and via the stay-green capability of leaves (Ciampitti & Vyn, 2012). Nitrogen deficiency reduces leaf area index, leaf area duration and photosynthetic rate (Lemcoff & Loomis, 1986; Sinclair & Horie, 1989; Wolfe et al., 1988). Consequently, grain yield was reduced by suboptimal N supply resulting from decreases in kernel number and single kernel weight (Jacobs & Pearson, 1991; Lemcoff & Loomis, 1994; Paponov et al., 2020; Paponov & Engels, 2005; Uhart & Andrade, 1995b). This negative impact of N deficiency could be attributed either to effects on assimilate partitioning to the maize cob during the critical period around silking (Uhart & Andrade, 1995a) or to direct effects on enzymes involved in kernel development (Below et al., 2000). In addition, N-induced alterations in hormone metabolism are closely linked to the regulation of vegetative and generative plant growth. These reports clearly demonstrate the major effect that N nutritional status exerts over maize grain yield formation during the entire growing season (Ciampitti & Vyn, 2012).

Nitrogen-use efficiency is determined by two components: N-uptake efficiency (NUpE) and N-utilization efficiency (NUtE) (Haegele et al., 2013; Moll et al., 1982). The first parameter refers to the amount of nutrient absorbed by plants relative to the available soil N. The second parameter quantifies the amount of dry matter produced per unit of absorbed nutrient (Moll et al., 1982). In our study, we focus on $NUtE_{grain}$, which is defined as grain dry matter/nutrient content of total above-ground biomass at physiological maturity (Hütsch & Schubert, 2017). With an improvement of $NUtE_{grain,}$ also the N-use efficiency of fertilizers can indirectly be enhanced, as less fertilizer needs to be applied in order to achieve maximum grain yield and the risk of losses is reduced (Hütsch & Schubert, 2017; Raun & Johnson, 1999). Another frequently used parameter is the N-harvest index (NHI), which describes the partitioning of N between the total above-ground biomass and grain (Hay, 1995; NHI is defined as grain N content/N content of total above-ground biomass at physiological maturity). Values for N-use efficiency strongly depend on the amount of plant-available N. With increasing N application, not only a decrease in N-uptake efficiency was observed, but also a successive decline in N-utilization efficiency (Barbieri et al., 2008; Gao & Chu, 2020; Haegele et al., 2013; Liao et al., 2012; O'Neill et al., 2004; Wang et al., 2020). With yield approaching the yield potential, utilization efficiencies of N as well as of P and K decreased (Setiyono et al., 2010).

In our previous study on the effect of PAC application to six maize cultivars, strong reductions in straw yield were obtained resulting in significant improvements in the harvest indices in the range of 8%–36% (Hütsch & Schubert, 2021a). The PAC-treated plants had a strongly decreased water consumption during vegetation and, as a consequence, four cultivars showed a significantly enhanced water-use efficiency (WUE_{grain}) during the critical period of kernel setting. However, concomitantly significant depressions in grain yield occurred (between 9% and 20%) due to PAC treatment, which were solely based on decreases in kernel number per cob, as single kernel weights were either slightly increased or unchanged (Hütsch & Schubert, 2021a, 2021b). In addition, after PAC application, significant reductions in cob length were observed (Hütsch & Schubert, 2021b). As PAC did not affect flowering of the maize plants (e.g. start and duration of pollen production, start of silking, anthesis-silking interval), the negative impact probably originated from an earlier growth stage during plant ontogenesis when the number of kernel primordia and cob length is determined. Floret initiations start at the 9-leaf stage (approximately V5), during early vegetative growth, and last for about 5–10 days (Zhao et al., 2021). The number of spikelets with florets formed on an ear is the primary determinant of potential yield as it sets the upper limit for the number of kernels per plant (Jacobs & Pearson, 1992). Thus, in our previous study, the time of PAC application coincided with the start of floret initiation and the determination of potential yield (Hütsch & Schubert, 2021b).

However, the stability (or even increase) of grain yield after application of plant growth regulators is of high relevance, as it is also a key determinant for the harvest index and the use efficiencies of water and nutrients. Improvements in these parameters are scarce when grain yield depressions occur. Consequently, increases in nutrient-use efficiencies could not be achieved so far, even after reduced application of the macronutrients N, P and K according to the lower demand of the smaller, PAC-treated maize plants (Hütsch & Schubert, 2021b). Luxurious nutrient consumption after PAC application could not be avoided with less NPK supply (reduced to 85% and 78% of the optimal dosage for untreated control plants), and decreases in grain yield due to less kernel numbers and shorter cobs were strengthened with reduced fertilizer supply (Hütsch & Schubert, 2021b).

With delayed PAC application time, floret initiation can probably be protected and grain yield depressions avoided or even increased due to a higher kernel number per cob, as shown by Zhao et al. (2021) for treatment of maize plants with ethephon. Therefore, in the present study, a container experiment was conducted with the maize cultivars Galactus and Fabregas, which showed the best performance among six tested cultivars with respect to improvement of harvest index and water-use efficiency after PAC treatment (Hütsch & Schubert, 2021a). The plant growth regulator was applied once at a later growth stage (V8), and thereafter, differential N fertilization was introduced in order to meet the demand of untreated control plants (100% N), and with a supply of 75% N, slightly less than the approximate requirement of PAC-treated plants. Our investigations focused on the following hypotheses: with application of PAC at growth stage V8, (a) grain yield depressions can be avoided, (b) further improvements of water-use efficiency are achieved, and (c) N-harvest index and N-use efficiencies are increased with 75% N supply.

2 | MATERIALS AND METHODS

2.1 | Plant cultivation, PAC application and measurements during growth

The experiment was conducted at the experimental station of the Institute of Plant Nutrition in Giessen (50°35′53.30″N, 8°40′1.56″E) during the vegetation period of 2021. Maize plants (*Zea mays* L., *cv.* Galactus and *cv.* Fabregas) were cultivated according to Hütsch and Schubert (2018, 2021a, 2021b) using the container technique. 120 L plastic containers were filled with 140 kg of a Luvisol subsoil (strong sandy loam: 18.9% clay, 28.2% silt, 52.9% sand; N_{min}: 0.8 mg N kg^{-1}, CAL-P: 10.3 mg P kg^{-1}, CAL-K: 88.5 mg K kg^{-1}; pH [$CaCl_2$] 5.6 prior to liming). The air-dry soil was mixed with $CaCO_3$ (2.5 g kg^{-1} soil; pH [$CaCl_2$] 7.4 after liming) and filled into the containers in four layers: three layers with 30 kg soil moistened with 3 L deionized water each and a topsoil layer (approx. 0–30 cm), which was fertilized with 40 g compound fertilizer ('Blaukorn') per container, consisting of 4.8 g N, 2.1 g P, 5.6 g K, 0.5 g Mg, 2.4 g S, 0.004 g Zn and 0.008 g B. Additionally, 0.32 g Zn, 0.16 g Cu and 0.08 g Mn were applied per container. The topsoil layer was moistened with 5 L deionized water.

On 18 May 2021, the maize cultivars Galactus and Fabregas were sown. Seventeen days after sowing (DAS), the number of plants was reduced from nine to four per container, and water content was adjusted to 60% maximum water-holding capacity (WHC). For the determination of 100% WHC, a container filled with 140 kg air-dry soil was put into a water basin enabling water to infiltrate through holes at the container bottom until reaching the soil surface. The container was then removed from the water basin and let drain freely until weight constancy, which resembles the amount of water held in the soil against the gravity. This amount equals 100% WHC and accordingly the value for 60% max. WHC was calculated. In addition to the weight of container, soil and water, during vegetation, increasing amounts of plant material were also accounted for. During the whole vegetation period, water content was adjusted to 60% max. WHC by weighing the containers and applying water at least twice daily. Water supply was always recorded for each container. With this experimental setup, plant roots could exploit a rather large soil volume (120 L per container, soil depth 80 cm). The plants grew in a vegetation hall under natural light conditions. The average daily temperature during the vegetation period ranged from 13 to 31°C with a mean of 21.8 ± 0.3°C. The containers were set up in a completely randomized design, and their position was changed at least once a week.

For each genotype, two treatments were set up (control and PAC application), which were subdivided into two fertilization regimes: 100% N supply with additional fertilizer applications (10 g 'Blaukorn' per container) four times during the vegetation period, on 16 June, 28 June, 8 July and 19 July. This application of in total 80 g 'Blaukorn' per container is according to the requirement of maize plants grown under control conditions. In the second fertilization regime, additional compound fertilizer (10 g 'Blaukorn' per container) was added only once during vegetation, on 16 June, and the N amount was reduced to 75% with the three last applications. The other macronutrients, which are also contained in the compound fertilizer, were supplemented in order to achieve a supply similar to the 100% N treatment. The reduction in N supply to 75% was slightly below the optimal dosage for the smaller PAC-treated plants, according to our previous studies (Hütsch & Schubert, 2021a, 2021b). The different N fertilization regimes were started only 2 days before PAC application in order to avoid changes in synchronization in plant ontogenesis due to variation in N supply. As PAC was applied at a defined growth stage, the same application date for all maize plants was advantageous. Otherwise, varying weather conditions (temperature, solar radiation) at different dates can affect uptake and metabolism of PAC within the plants and impair results. With two maize cultivars, control and PAC treatment, two fertilization regimes and four replicates, the experiment consisted of 32 containers in total.

On 30 June (43 days after sowing, DAS) between 12 AM and 1 PM, the growth regulator PAC was applied at stage V8, when the collar of the 8th leaf was visible in 96% of all plants, and 2% had already reached V9. This rather late growth stage was chosen in order to avoid negative effects on kernel primordia initiation and thus potential kernel number. Both cultivars received the same dosage of 3 mg a.i. (active ingredient) PAC per plant. For this dosage, 52.42 mg PAC (22.9% w/w) was dissolved in 1 L of deionized water and poured onto the soil surface of each container, which resulted in the desired application of 3.0 mg a.i. PAC per plant. All solutions were prepared fresh in the morning of the application day. In order to ensure fast uptake of the chemicals by the plant roots, no water was applied to the containers on this day. The soil of the control treatment received 1 L deionized water only.

Growth stages were determined on the following dates: 28 and 30 June, 7 and 14 July (41, 43, 50 and 57 DAS, respectively). Plant height (measured from the shoot base to the tip of the longest leaf) was monitored on 30 June and 7, 14 and 21 July (43, 50, 57 and 64 DAS, respectively). On 43 DAS (0 DAA, days after application), leaf number was counted, and on 77 DAS (34 DAA), culm diameter was determined above the second node.

From 17 July until 30 July, production of fresh pollen and start of silking were recorded for each plant daily at peak pollen shed (10 to 11 AM). From these data, the duration of pollen production and the anthesis-silking interval ASI (start silking minus start pollen production) were determined. For better comparisons of grain yield per plant and of yield components, such as kernel weight and kernel number per cob, tillers were removed immediately after appearance. Axillary branches were not produced. Additional cobs on the main culm were not removed, as the effect of PAC application on this trait was evaluated. Start of senescence (yellowing of older leaves, appearance of red-coloured culms) was also recorded. Insecticides against *Oscinella frit* L. and European corn borer were applied when required.

2.2 | Harvest and analyses of plant material

For each maize cultivar, treatment and N fertilization rate, four containers with four plants each were harvested at physiological maturity 139 and 140 DAS (4 and 5 October). Plant height, straw dry mass per container, cob dry mass per plant, cob length, maximal cob diameter (measured at cob base), kernel dry mass (80°C drying), kernel number per cob and individual kernel weight were determined after harvest. The dried straw and grain material were milled to fine powder and dry-ashed. Phosphorus (P) concentrations were determined colorimetrically. Total N concentrations were measured using an elementary analyser (Unicube® trace, Elementar Analysensysteme GmbH, Langenselbold, Germany). Sample digestion was carried out via catalytic combustion at a temperature of 950°C, with the nitrogen-containing components in the sample forming N_2, which was measured with thermal conductivity detection.

The nutrient concentrations are given as mg nutrient g^{-1} dry matter. For the determination of the nutrient content per plant, which reflects net nutrient uptake of the above-ground biomass, the concentrations were multiplied with the corresponding plant dry weights.

2.3 | Calculation of efficiency parameters and statistical analysis

Harvest index (HI), water-use efficiency of grain (WUE_{grain}), nitrogen-uptake efficiency (NUpE), nitrogen-utilization efficiency of grain ($NUtE_{grain}$) and nitrogen-harvest index (NHI) were calculated according to the following equations:

$$\text{HI} = \text{Grain yield} / \text{Total above} - \text{ground biomass at physiological maturity} \quad (1)$$

$$\text{WUE}_{\text{grain}} = \text{Grain dry matter} / \text{Total water consumption} \quad (2)$$

$$\text{WUE}_{\text{grain}}\ \text{after PAC application} = \text{Grain dry matter} / \text{Water consumption from start PAC application until physiological maturity} \quad (3)$$

$$\text{NUpE} = \text{Total N content of plant shoot}/\text{Fertilizer N supply} \tag{4}$$

Fertilizer N supply was 2.40 and 1.80 g N per plant in the 100% N and 75% N treatment, respectively; available soil N (0.03 g N per plant) was not accounted for.

$$\text{NUtE}_{\text{grain}} = \text{Grain dry matter}/\text{N content of total above} - \text{ground biomass at physiological maturity} \tag{5}$$

$$\text{NHI} = \text{Grain N content}/\text{N content of total above} - \text{ground biomass at physiological maturity}. \tag{6}$$

Means ± standard errors (SE) were calculated from four replicates per growth regulator treatment, N-fertilizer supply and maize cultivar. After two-way ANOVA (factors: growth regulator treatment and N supply) using *Rstudio*, multiple comparisons of means were conducted following adjustment with the *fdr*-test. Statistically significant differences are indicated with different letters ($p \le 5\%$). Differences between the two cultivars were not statistically evaluated.

3 | RESULTS

3.1 | Vegetative plant growth and development, time of flowering

Already 7 days after application (DAA) of the plant growth regulator paclobutrazol (PAC), distinct decreases in plant height were observed, and at 14 DAA, the PAC effects were significant for both N treatments and cultivars causing growth reductions of 11%–15% (Table 1). Low N supply did not affect plant height during the entire growth period including physiological maturity (results not shown). Culm diameter of Galactus was significantly increased in the PAC treatment, and N supply showed no effect for both cultivars (Table 1).

In most cases, start of pollen production was unaffected by PAC treatment and N supply, apart from Galactus-75%N with a significant earlier start due to PAC (Table 1). With PAC application, pollen shed of Galactus was significantly extended by 21% and 28% with 100% N and 75% N supply, respectively, and pollen production lasted significantly longer with PAC-75%N than PAC-100%N (Table 1). For Fabregas, duration of pollen production showed no significant effects of PAC treatment or N fertilization (Table 1). For Galactus, silking started significantly earlier in the PAC treatment with 75% N supply, which was also earlier than PAC treatment with 100% N (Table 1). Fabregas showed no effects of PAC treatment or N supply on silk appearance. For both cultivars, the anthesis-silking interval (ASI) was not significantly affected by PAC application or N supply (Table 1).

3.2 | Senescence, yield determinants and harvest index

At the end of July, 10 days after the last N fertilization, first N-deficiency symptoms were observed on plants of Galactus-75%N with chloroses on the oldest leaves and red colour of the culm base. At this time, N-deficiency symptoms on Fabregas plants were not yet visible. Overall, senescence of plants with 75% N supply started 2 weeks earlier than with 100% N, and PAC treatment delayed onset of senescence by approximately 4 to 5 days.

In this container experiment, losses of whole cobs or of kernels due to infection with cob smut did not occur. At maturity, all plants had produced one kernel-carrying cob (Figure 1). Galactus and Fabregas control plants with 100% N supply generally showed good kernel development over the entire cob, and after PAC application, kernel abortion occurred in the apical part of some cobs. With 75% N, the cobs looked shorter and thinner than with optimal N supply, and particularly for Fabregas, more barren cob tips were observed after PAC treatment (Figure 1).

The grain yield was either unaffected by PAC application, or for Galactus-75%N, it was significantly increased by 9% in comparison with the untreated control (Figure 2a). Suboptimal N supply caused

TABLE 1 Plant height at 14 DAA (days after application of the plant growth regulator paclobutrazol, PAC), culm diameter at 34 DAA, start and duration of pollen production, start of silking and anthesis-silking interval (ASI; start silking minus start pollen production) of two maize cultivars under control conditions and after application of paclobutrazol (PAC), with 100% and 75% N-fertilizer supply

Maize cultivar	N-fertilizer supply	Treatment	Plant height	Culm diameter	Pollen production		Silking	Anthesis-Silking Interval
			(cm)	(mm)	Start (DAS)	Duration (d)	Start (DAS)	ASI (d)
Galactus	100% N	Control	270.0 ± 3.6 a	23.3 ± 0.1 b	63.6 ± 0.2 a	3.9 ± 0.3 c	64.0 ± 0.3 ab	0.4 ± 0.2 a
		PAC	231.5 ± 3.4 b	24.5 ± 0.0 a	63.6 ± 0.2 a	4.7 ± 0.1 b	63.4 ± 0.3 b	−0.2 ± 0.1 a
	75% N	Control	266.7 ± 2.5 a	22.8 ± 0.3 b	63.8 ± 0.2 a	4.4 ± 0.2 bc	64.5 ± 0.2 a	0.7 ± 0.1 a
		PAC	237.2 ± 0.9 b	24.3 ± 0.2 a	62.2 ± 0.3 b	5.6 ± 0.3 a	62.5 ± 0.1 c	0.4 ± 0.4 a
Fabregas	100% N	Control	268.4 ± 1.5 A	23.2 ± 0.4 A	63.1 ± 0.2 A	4.6 ± 0.4 A	64.4 ± 0.2 A	1.3 ± 0.1 A
		PAC	238.6 ± 6.8 B	23.6 ± 0.6 A	62.8 ± 0.3 A	4.2 ± 0.2 A	63.5 ± 0.3 A	0.7 ± 0.1 A
	75% N	Control	268.3 ± 2.1 A	22.2 ± 0.3 A	63.2 ± 0.6 A	4.3 ± 0.3 A	64.5 ± 0.9 A	1.3 ± 0.3 A
		PAC	227.6 ± 4.4 B	23.6 ± 0.2 A	63.6 ± 0.3 A	3.7 ± 0.5 A	64.5 ± 0.2 A	0.9 ± 0.2 A

Note: Data show means of four replicates ±SE; significant differences ($p \le 5\%$) within each cultivar are indicated by small (Galactus) or capital letters (Fabregas). PAC dosage per plant: 3 mg a.i. for both cultivars.

Abbreviation: DAS, days after sowing.

significant decreases in grain yield by 17% to 25% compared with 100% N. After PAC treatment, the straw yield was significantly reduced (between 9% and 12%) with exception of Fabregas-100%N, where the reduction was not significant (Figure 2b). N fertilization showed no significant effect on straw yield. Significant improvements of the harvest index by up to 10% were obtained with PAC application to 75% N supply of both cultivars; with 100% N, the increases were not significant (Figure 2c). Suboptimal N fertilization led to a decrease in harvest indices in all cases (Figure 2c). Similar grain and less straw yields resulted in higher harvest indices of Fabregas compared with Galactus (Figure 2a–c).

For both cultivars, kernel number per cob was not significantly affected by PAC treatment (Figure 3a). N supply had only an effect on Fabregas with significantly less kernels in PAC-75%N than in PAC-100%N (Figure 3a). Single kernel weight of Fabregas-75%N was significantly increased due to PAC treatment (Figure 3b). Suboptimal N supply caused significant decreases in kernel weight in all cases, whereby Galactus showed stronger effects than Fabregas (on average −20% vs. −11%, respectively; Figure 3b). Cob length of Galactus-75%N was significantly increased by 10% due to PAC, and cobs were shorter with 75% N than with 100% N fertilization (Figure 3c). For Fabregas-100%N, cob length was decreased by PAC application, and a similar reduction in the untreated control was caused by 75% N compared with 100% N (Figure 3c). The cob diameter was unaffected by PAC treatment, but 75%N supply resulted in strong and significant decreases between 29% and 33% for both cultivars (Figure 3d).

3.3 | Water consumption and water-use efficiency (WUE)

Water consumption, determined for the entire growth period, was significantly decreased by PAC in Galactus-100%N (Table 2). In addition, this PAC effect was also observed in Galactus-75%N, when water consumption from time of PAC application until physiological maturity was considered (Table 2). With 75% N supply, significantly less water was consumed by plants of both cultivars. For Galactus-75%N, water-use efficiency (WUE_{grain}) was significantly improved in the PAC treatment by 15%. A rather small, but significant increase was also observed for Fabregas-100%N with PAC, when WUE_{grain} was calculated with water consumed after PAC application (Table 2). WUE_{grain} was decreased with 75% N supply with one exception: Galactus, with water consumption starting from PAC application, where PAC offset the negative impact of 75% N reaching the level of 100% N (Table 2). Comparable effects of PAC and N supply were obtained when WUE_{grain} was calculated for the time around silking (start silking ±2 weeks; Table 2).

3.4 | Nitrogen concentrations, contents and utilization efficiencies

In the present growth regulator experiment, the maize plants were supplied with two rates of N fertilization: optimal (100% N) and suboptimal (75% N) supply. In Figure 4, the N concentrations in grain and straw dry matter are combined for cultivars Galactus and Fabregas, grown under control conditions or after PAC treatment. For both cultivars, N concentrations in grain and straw were unaffected by PAC application, whereas with 75% N supply, significant decreases were observed (exception: straw, Fabregas-75%N, with PAC; Figure 4a,b). The N contents of grain, straw and total above-ground biomass per one plant are shown in Figure 5 for both maize cultivars. In Galactus-75%N, the N content of grain was significantly increased by 13% due to PAC treatment, whereas it was decreased in straw (Figure 5a,b). In addition, N content of straw was also significantly decreased after PAC application to Fabregas-100%N (Figure 5b). For both cultivars, reduction in N supply to 75% caused significant decreases in N content in control and PAC treatments, which lay in the range of 22%–26% in the total above-ground biomass and thus mirrored the 25% less N fertilization very well (Figure 5c). For both cultivars, it can be asserted that decreased shoot N uptake due to low fertilizer supply relied on both, decreased N concentrations in grain and straw (Figure 4a,b) and decreased grain yields (Figure 2a).

Parameters, which characterize the internal and external efficiency of N use, are combined in Figure 6. According to the increase in N-harvest index (NHI) after PAC application, the allocation of N in the total above-ground biomass to the maize kernels was significantly improved by up to 6% (one exception: Galactus-100%N; Figure 6a). In addition, similar improvements due to PAC were observed for the P-harvest index (increases by up to 5%; results not shown). With less N fertilization, either significant decreases in NHI or no changes were observed for Galactus and Fabregas, respectively (Figure 6a). PAC treatment showed neither an effect on N-utilization efficiency ($NUtE_{grain}$) nor on N-uptake efficiency (NUpE; Figure 6b,c). Only for Fabregas-75%N, a significant increase in $NUtE_{grain}$ was found in comparison with 100% N, whereas suboptimal N supply did not affect NUpE (Figure 6b,c).

4 | DISCUSSION

4.1 | With delayed PAC application grain yield depressions can be avoided

With late PAC application at growth stage V8, grain yield depressions were not only successfully avoided; moreover, for Galactus-75%N, a significant increase in grain yield was achieved (Figure 2a). Accordingly, the yield determinants kernel number and single kernel weight were also unaffected by PAC in most cases (exception: increase in kernel weight of Fabregas-75%N; Figure 3a,b). The improvement in grain yield of Galactus-75%N was most likely due to a combination of an increase in cob length and of kernel number, although the latter was not significant (Figure 3a,c). Delayed PAC treatment had obviously no negative impact on floral initiation and formation of spikelet primordia and thus on the potential kernel number per cob. This supports the study of Zhao et al. (2021), who found similar results when comparing an early ethephon application to maize plants at the 9-leaf stage with a late application at the 13-leaf stage. This matches exactly our study at the V5 growth stage when

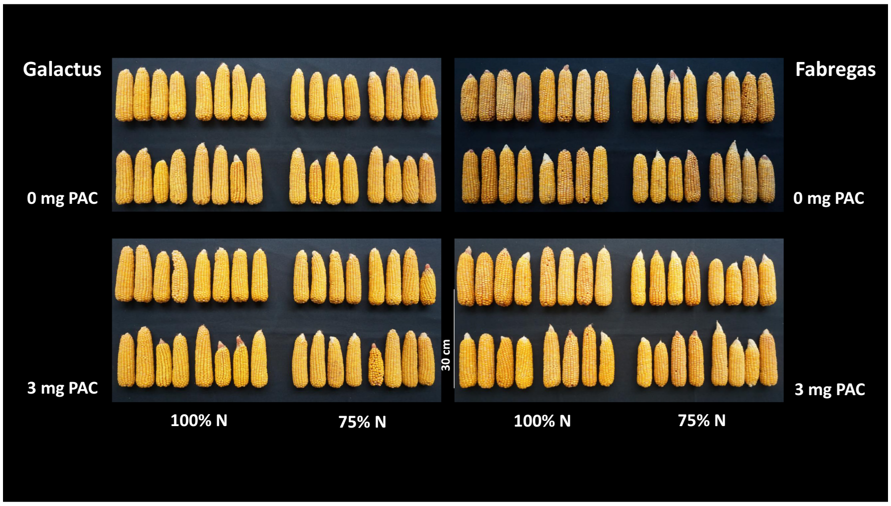

FIGURE 1 Effect of the plant growth regulator paclobutrazol (PAC) and differential nitrogen supply (100% and 75% N) on cob development of maize cultivars Galactus and Fabregas at maturity (at 139/140 days after sowing (DAS) and 96/97 days after PAC application (DAA); PAC dosage per plant: 3 mg a.i. for both cultivars.

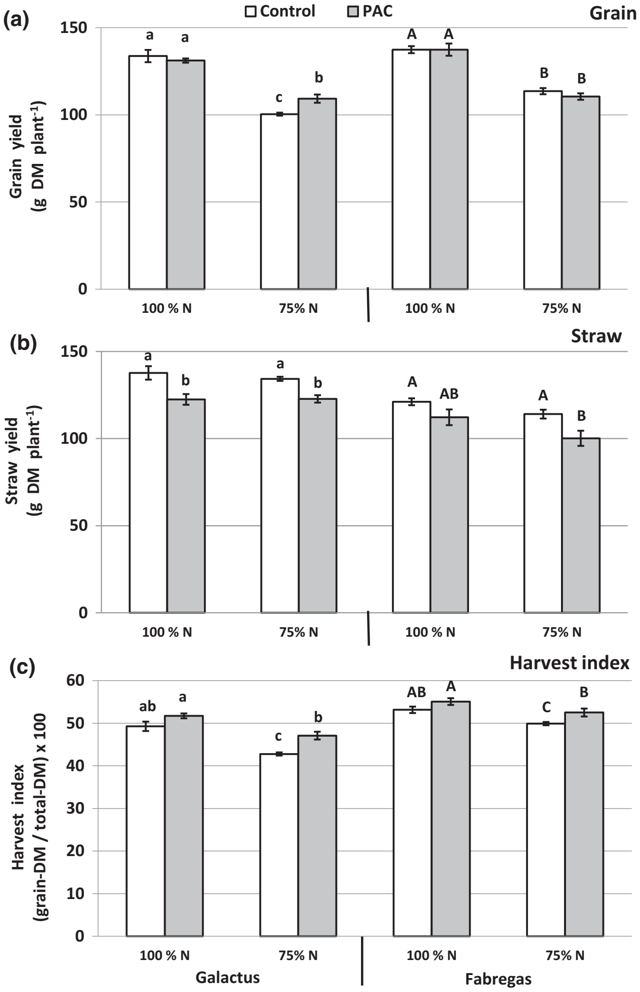

FIGURE 2 Grain dry matter yield (a), straw dry matter yield (b) and harvest index (c) of two maize cultivars under control conditions and after application of the plant growth regulator paclobutrazol (PAC), with 100% and 75% N-fertilizer supply; data show means of four replicates ±SE; significant differences ($p \le 5\%$) within each cultivar are indicated by small (Galactus) or capital letters (Fabregas). PAC dosage per plant: 3 mg a.i. for both cultivars.

the plants had developed 9 leaves (Hütsch & Schubert, 2021b), and at the V8 growth stage the plants had produced 12 leaves.

Apart from the extent of spikelet formation on an ear, maize kernel number at maturity is also a function of fertilization of the florets, which requires synchronization of pollen shed and silking (Jacobs & Pearson, 1991, 1992). In our previous study, start and duration of pollen production, start of silking and ASI were unaffected by PAC treatment (Hütsch & Schubert, 2021b). This is in contrast to the present experiment with delayed PAC application which resulted in a significant increase in duration of pollen shed of Galactus at the extent of 21% and 28% with 100% N and 75% N supply, respectively (Table 1). Therefore, an improved fertilization of developing kernels could also have contributed to the grain yield increase in Galactus-75%N with PAC application (Figure 2a). After successful fertilization, particularly at the apical part of the cob kernel abortion can occur and can contribute to the determination of the final kernel number. Only for Fabregas-75%N, PAC application obviously extended the degree of barren cob tips (Figure 1). This probably resulted in the smaller kernel number, which was counteracted by an increased kernel weight and eventually by an unchanged grain yield (Figure 3a,b; Figure 2d). For the maize cultivar Fabregas, we have occasionally observed this compensatory effect of an enhanced kernel weight after reduction in kernel number (Hütsch et al., 2015; Hütsch & Schubert, 2021a).

Grain yield decreases due to reduced N supply were almost exclusively resulting from smaller kernels with stronger effects on Galactus than on Fabregas (Figure 2a, Figure 3b). In addition, with 75% N, the cob diameter was decreased by about 30% (Figure 3d). Nitrogen plays a key role for assimilation during early cob development and during grain filling. First N-deficiency symptoms were observed on the oldest leaves of Galactus-75%N at the end of July, a few days after flowering had been terminated. Chlorosis of the older leaves point to a mobilization of N in order to feed the developing cob as a plant organ with high sink strength at this growth stage. Obviously, N limitation had not yet prevailed when N was needed for cytokinin biosynthesis, a prerequisite for cell

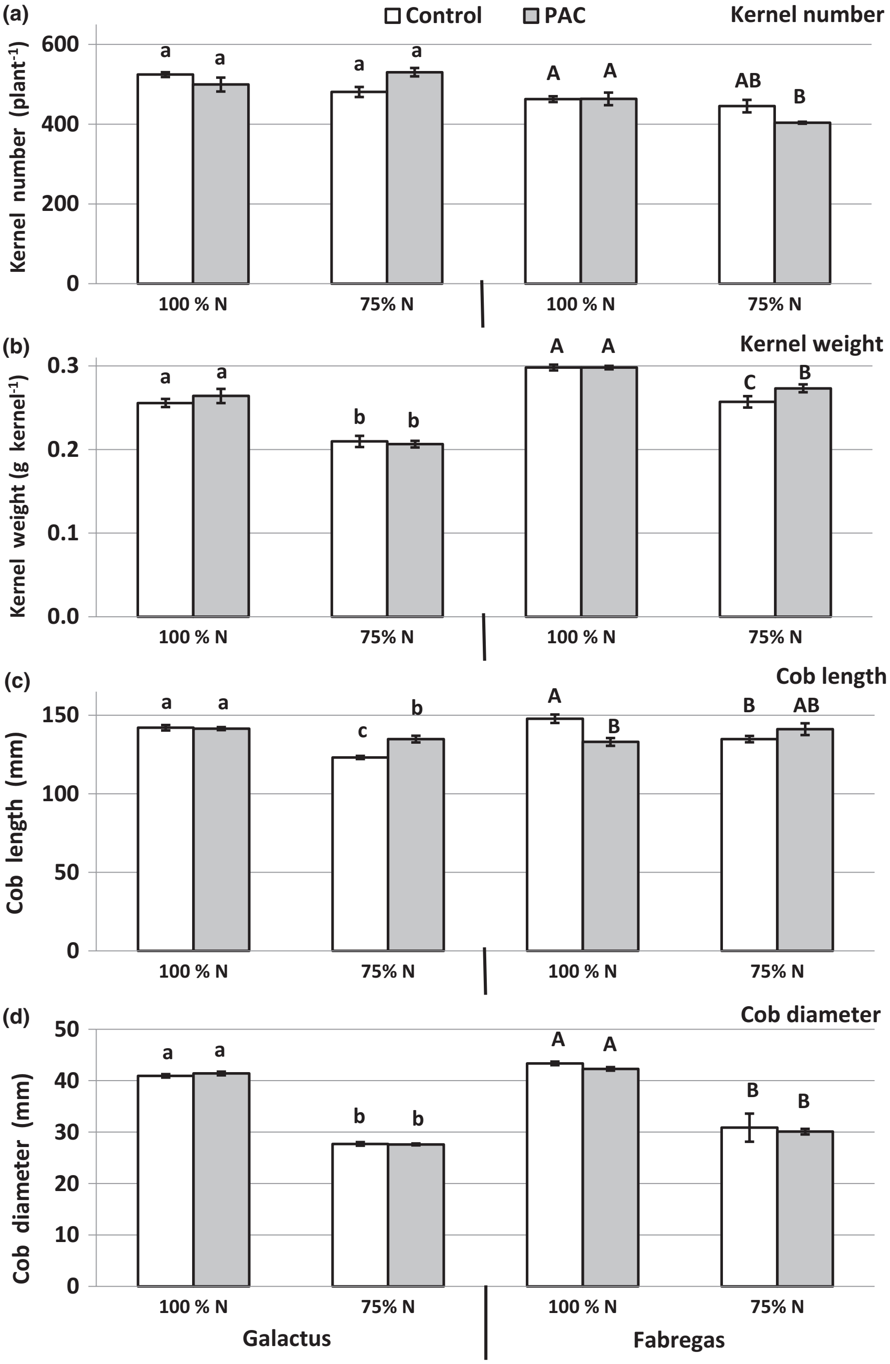

FIGURE 3 Kernel number (a), single kernel weight (b), cob length (c) and cob diameter (d) of two maize cultivars under control conditions and after application of the plant growth regulator paclobutrazol (PAC), with 100% and 75% N-fertilizer supply; data show means of four replicates ±SE; significant differences ($p \leq 5\%$) within each cultivar are indicated by small (Galactus) or capital letters (Fabregas). PAC dosage per plant: 3 mg a.i. for both cultivars.

division and kernel setting during early cob development (Dietrich et al., 1995; Hütsch & Schubert, 2022; Jameson & Song, 2016; Lur & Setter, 1993a, 1993b; Rijavec et al., 2011). A lack of N more likely limited starch deposition in the endosperm of maize kernels (during grain filling), primarily through an influence on the synthesis of metabolic enzymes needed for starch production followed by decreased activities (Seebauer et al., 2004; Singletary & Below, 1990; Singletary et al., 1990). According to Mueller et al. (2019), the greatest impact of N treatment was realized in the linear phase of kernel growth, when 90% accumulation of grain dry weight occurs, and thus, N limitations during this phase lowered final kernel weight. This explanation of the impact of reduced N supply on grain filling is supported by the result that for Fabregas chloroses on the older leaves occurred later than for Galactus, and subsequently causing less decreases in kernel weight (−11% compared with −20% on average for Fabregas and Galactus, respectively; Figure 3b).

Besides the allocation of assimilates to the maize grain, the straw biomass is the second determinant of the harvest index. After application of 3 mg a.i. PAC at a late growth stage, the straw yield was decreased between 7% and 12% (Figure 2b), which is a rather small change compared with our previous study with earlier application of a similar PAC dosage to Galactus and a smaller dosage to Fabregas (2 mg a.i.). The main difference between both application times was the distribution of the growth regulator in a larger shoot biomass, which diminished its impact on vegetative growth. It should be tested, if an increase in PAC dosage combined with the later application will lead to stronger decreases in straw yield without grain yield depressions, and thus to further improvements

TABLE 2 Total water consumption, water-use efficiency WUE_{grain} calculated with total water consumed, water consumption from date of paclobutrazol (PAC) application until physiological maturity, WUE_{grain} calculated with water consumed from PAC application till maturity of two maize cultivars under control conditions and after application of the plant growth regulator PAC, with 100% and 75% N-fertilizer supply; data show means of four replicates ±SE

Maize cultivar	N-fertilizer supply	Treatment	Total water consumption ($L\ plant^{-1}$)	WUE_{grain} ($g\ L^{-1}$) (grain-DM/Total water consumption)	Water consumption from PAC application till maturity ($L\ plant^{-1}$)	WUE_{grain} ($g\ L^{-1}$) (grain-DM/water consumption from PAC application till maturity)	WUE_{grain} ($g\ L^{-1}$) (grain-DM/water consumption start silking ±2 weeks)
Galactus	100% N	Control	38.2 ±0.4 a	3.51 ±0.12 ab	30.9 ±0.4 a	4.33 ±0.14 a	9.34 ±0.54 ab
		PAC	36.9 ±0.2 b	3.55 ±0.03 a	29.6 ±0.1 b	4.43 ±0.03 a	10.02 ±0.26 a
	75% N	Control	34.7 ±0.3 c	2.89 ±0.02 c	27.4 ±0.4 c	3.67 ±0.05 b	7.16 ±0.11 c
		PAC	33.8 ±0.5 c	3.24 ±0.12 b	26.0 ±0.3 d	4.21 ±0.13 a	8.46 ±0.36 b
Fabregas	100% N	Control	39.2 ±0.4 A	3.51 ±0.04 A	32.1 ±0.5 A	4.28 ±0.05 B	9.63 ±0.31 AB
		PAC	38.1 ±0.6 A	3.61 ±0.04 A	31.0 ±0.6 A	4.43 ±0.04 A	10.51 ±0.25 A
	75% N	Control	35.0 ±0.6 B	3.25 ±0.02 B	28.2 ±0.7 B	4.03 ±0.05 C	8.53 ±0.20 C
		PAC	33.7 ±0.5 B	3.28 ±0.06 B	26.8 ±0.4 B	4.13 ±0.05 C	9.36 ±0.42 BC

Note: Significant differences ($p \leq 5\%$) within each cultivar are indicated by small (Galactus) or capital letters (Fabregas). PAC dosage per plant: 3 mg a.i. for both cultivars.

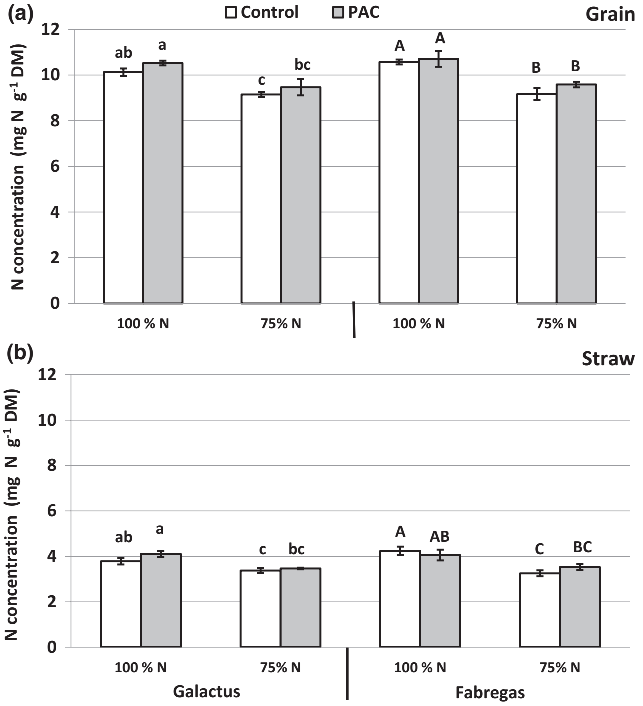

FIGURE 4 Nitrogen (N) concentrations in grain (a) and straw (b) of two maize cultivars under control conditions and after application of the plant growth regulator paclobutrazol (PAC), with 100% and 75% N-fertilizer supply; data show means of four replicates ±SE; significant differences ($p \leq 5\%$) within each cultivar are indicated by small (Galactus) or capital letters (Fabregas). PAC dosage per plant: 3 mg a.i. for both cultivars.

of HI. Although in the present study, rather small but significant increases in HI were obtained after PAC application to maize plants with 75% N supply, they were achieved in combination with enhanced or unchanged grain yields (Figure 2a,c).

4.2 | Remarkable increases in water-use efficiency due to PAC were achieved with 75% N supply to cultivar Galactus

After PAC treatment, decreases in water consumption during the entire growth period and for the timespan from PAC application until maturity were obtained, although only for Galactus the effects were significant (Table 2). On average, the total water consumption decreased by 3% to 4% due to PAC, whereas in our previous study, the reduction lay at 14% for both cultivars (Hütsch & Schubert, 2021a). With late PAC application, plant height depressions were less strong than with an early treatment (13% vs. 35% on average of Galactus and Fabregas for late vs. early application, respectively), resulting in only a slight compaction of the plant canopy making reduced transpirational water losses due to a higher humidity less probable (Hütsch & Schubert, 2021a). In addition, as total biomass production was less diminished after late PAC treatment, less water could be saved due to the reduced need of stomatal opening for CO_2 assimilation. Considering the small

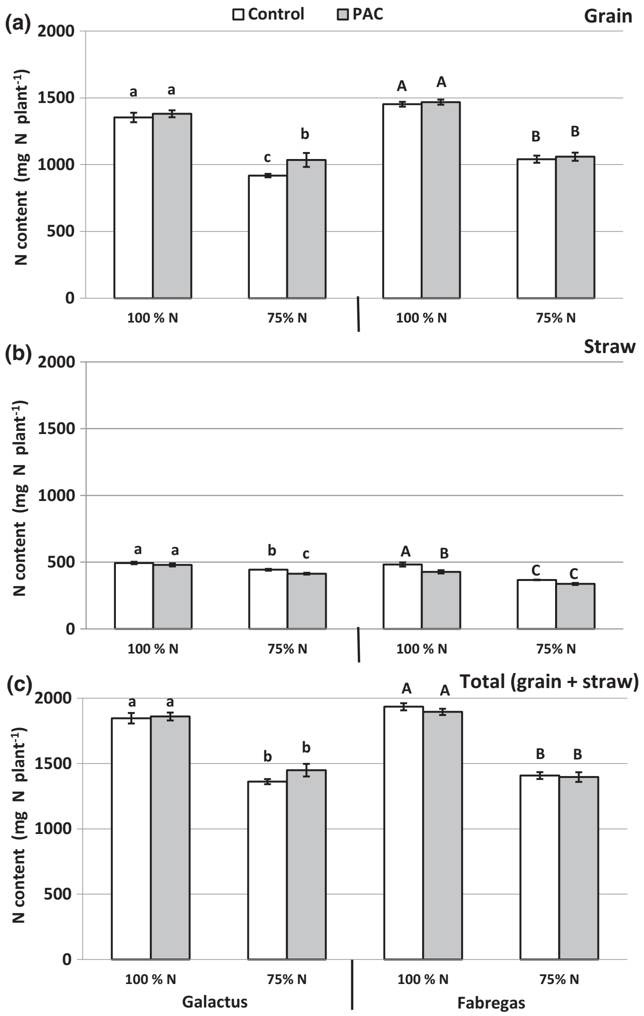

FIGURE 5 Nitrogen (N) contents in grain (a), straw (b) and grain plus straw (c) of two maize cultivars under control conditions and after application of the plant growth regulator paclobutrazol (PAC), with 100% and 75% N-fertilizer supply; data show means of four replicates ±SE; significant differences ($p \le 5\%$) within each cultivar are indicated by small (Galactus) or capital letters (Fabregas). PAC dosage per plant: 3 mg a.i. for both cultivars.

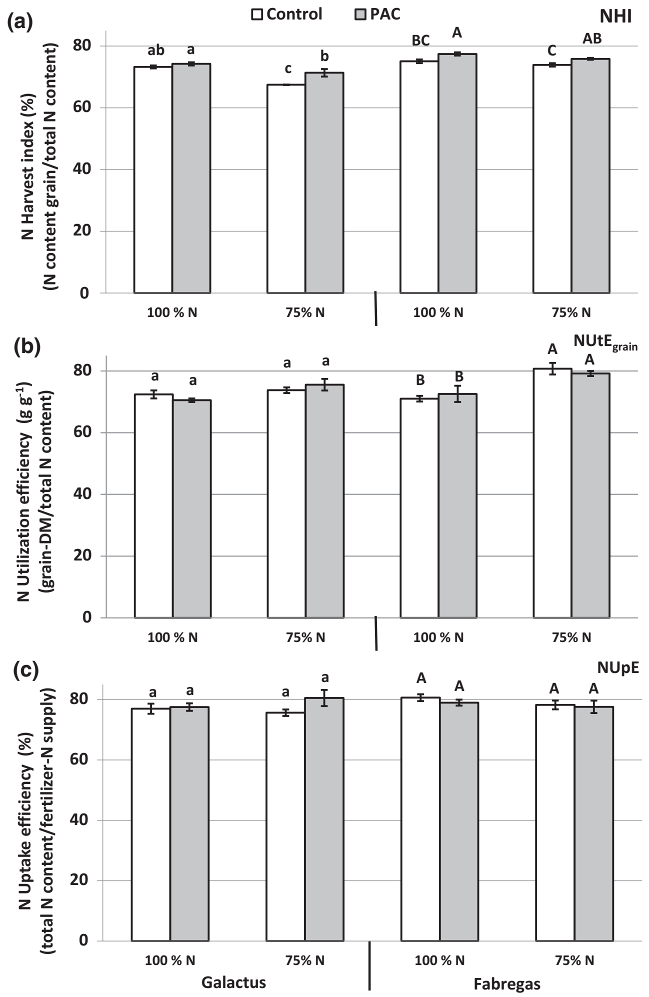

FIGURE 6 Nitrogen-harvest index NHI (a), nitrogen-utilization efficiency NUtE$_{grain}$ (b) and nitrogen-uptake efficiency NUpE (c) of two maize cultivars under control conditions and after application of the plant growth regulator paclobutrazol (PAC), with 100% and 75% N-fertilizer supply; data show means of four replicates ±SE; significant differences ($p \le 5\%$) within each cultivar are indicated by small (Galactus) or capital letters (Fabregas). PAC dosage per plant: 3 mg a.i. for both cultivars.

amounts of water saved after PAC treatment, remarkable improvements of WUE$_{grain}$ were only obtained for Galactus-75%N, which showed a significant increase in grain yield (Table 2, Figure 2a). For the time around silking, which is particularly important for kernel set and cob development, WUE$_{grain}$ of Galactus-75%N was enhanced by 18% due to PAC (Table 2). For Galactus, the plant growth regulator was able to counteract the negative impact which 75%N supply generally exerted on WUE$_{grain}$ (Table 2).

4.3 | With PAC treatment N-harvest index was enhanced, whereas N-use efficiencies were unaffected

At physiological maturity, the N nutritional status, which can be characterized by the N concentration in the respective plant part, was unaffected by PAC application. Thus, luxurious N consumption, which was observed for the smaller, PAC-treated maize plants in earlier studies (Hütsch & Schubert, 2021a, 2021b), did not occur. Yet, reduced N supply caused significant decreases in N concentrations of grain and straw (on average − 11% vs. -16%, respectively; Figure 4). With 75% N supply, the maize plants suffered N deficiency, the main reason for the observed grain yield depressions (Figure 2a). After PAC treatment, particularly plants of Galactus-75%N were able to allocate more N into grains, resulting in a significant increase in N-harvest index (Figure 6a). Small, but nevertheless significant positive effects of PAC on NHI were also found for Fabregas (Figure 6a). This is in contrast to our previous study, where PAC did not affect NHI of both maize cultivars with optimal or reduced NPK fertilization (Hütsch & Schubert, 2021b). Thus, late PAC application was also advantageous with respect to NHI. Comparable results were obtained by Zhang et al. (2022)

who found an increase in NHI after ethephon application at the V8 stage.

However, late PAC application showed neither an effect on N-utilization efficiency nor on N-uptake efficiency (Figure 6b,c). At least for Galactus, this can be considered as a progress, as after early PAC application $NUtE_{grain}$ was significantly reduced by 19% on average of three NPK fertilization rates (Hütsch & Schubert, 2021b). We hypothesize that the avoidance of grain yield decreases with late PAC application leads to the improvement of N-use efficiency. The smaller, PAC-treated plants have less demand for N; thus, at least with 75% N supply an increase in $NUtE_{grain}$ was expected. As N concentrations showed no changes due to PAC, and thus, total N content was mainly determined by grain yield, changes in both of these factors of $NUtE_{grain}$ went always in the same direction causing no variation in control and PAC treatment. For the improvement of $NUtE_{grain}$ of maize plants, it can be concluded that it is a very complex task to achieve the right balance between PAC dosage, stability of grain yield and optimal N supply in order to avoid both, luxurious N consumption and N deficiency.

AUTHOR CONTRIBUTIONS

Birgit W. Huetsch: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review and editing. **Sven Schubert:** Conceptualization; methodology; resources; supervision; writing – review and editing.

ACKNOWLEDGEMENTS

Technical assistance by Corinna Alles-Becker, Anita Langer, Alexandra Neeb, Edeltraud Rödiger, Claudia Weimer and Lutz Wilming is highly appreciated. The authors thank Christina Plachta and Dr. Katrin Keipp for their support with the container experiments, and Dr. Feng Yan, Department of Agronomy and Plant Breeding of Justus Liebig University Giessen, for conducting the N analyses. Financial support to BWH by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation, Grant number: HU554/5-1) is gratefully acknowledged. Open Access funding is enabled and organized by Project DEAL; WOA Institution: Justus Liebig University Giessen. Open Access funding enabled and organized by Projekt DEAL.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

ORCID

Birgit W. Hütsch https://orcid.org/0000-0003-2771-5359

REFERENCES

Barbieri, P. A., Echeverría, H. E., Saínz Rozas, H. R., & Andrade, F. H.(2008). Nitrogen use efficiency in maize as affected by nitrogen availability and row spacing. *Agronomy Journal*, *100*, 1094–1100.

Below, F. E., Cazetta, J. O., & Seebauer, J. R.(2000). Carbon/nitrogen interactions during ear and kernel development of maize. In Westgate, M., Boote, K.(Eds.), *Physiology and modeling kernel set in maize*(Number 29, pp. 15–24). CSSA Special Publication.

Bortiri, E., & Hake, S.(2007). Flowering and determinacy in maize. *Journal of Experimental Botany*, *28*, 909–916.

Ciampitti, I. A., & Vyn, T. J.(2012). Physiological perspectives of changes over time in maize yield dependency on nitrogen uptake and associated nitrogen efficiencies: A review. *Field Crops Research*, *133*, 48–67.

Davies, W. J., Zhang, J., Yang, J., & Dodd, I. C.(2011). Novel crop science to improve yield and resource use efficiency in water-limited agriculture. *Journal of Agricultural Science (Cambridge)*, *149*, 123–131.

Dietrich, J. T., Kaminek, M., Blevins, D. G., Reinbott, T. M., & Morris, R. O.(1995). Changes in cytokinins and cytokinin oxidase activity in developing maize kernels and the effects of exogenous cytokinin on kernel development. *Plant Physiology and Biochemistry*, *33*, 327–336.

Duvick, D. N.(2005). The contribution of breeding to yield advances in maize (*Zea mays* L.). *Advances in Agronomy*, *86*, 83–145.

Gao, S., & Chu, C.(2020). Gibberellin metabolism and signaling: Targets for improving agronomic performance of crops. *Plant Cell Physiology*, *61*, 1902–1911.

Haegele, J. W., Cook, K. A., Nichols, D. M., & Below, F. E.(2013). Changes in nitrogen use traits associated with genetic improvement for grain yield of maize hybrids released in different decades. *Crop Science*, *53*, 1256–1268.

Hammer, G. L., Dong, Z., McLean, G., Doherty, A., Messina, C., Schussler, J., Zinselmeier, C., Paszkiewicz, S., & Cooper, M.(2009). Can changes in canopy and/or root system architecture explain historical maize yield trends in the U.S. Corn Belt?*Crop Science*, *49*, 299–312.

Hay, R. K. M.(1995). Harvest index: A review of its use in plant breeding and crop physiology. *Annals of Applied Biology*, *126*, 197–216.

Hütsch, B. W., & Schubert, S.(2017). Harvest index of maize (*Zea mays* L.): Are there possibilities for improvement?*Advances in Agronomy*, *146*, 37–82.

Hütsch, B. W., & Schubert, S.(2018). Maize harvest index and water use efficiency can be improved by inhibition of gibberellin biosynthesis. *Journal of Agronomy and Crop Science*, *204*, 209–218.

Hütsch, B. W., & Schubert, S.(2021a). Water-use efficiency of maize may be increased by the plant growth regulator paclobutrazol. *Journal of Agronomy and Crop Science*, *207*, 521–534. https://doi.org/10.1111/jac.12456

Hütsch, B. W., & Schubert, S.(2021b). Can nutrient-utilization efficiency be improved by reduced fertilizer supply to maize plants treated with the plant growth regulator paclobutrazol?*Journal of Agronomy and Crop Science*, *207*, 884–900. https://doi.org/10.1111/jac.12521

Hütsch, B. W., & Schubert, S.(2022). Stimulation of plasma membrane H^+-ATPase by auxins or fusicoccin and its relation to maize kernel setting, grain yield, and harvest index. *Advances in Agronomy*, *174*, 235–267.

Hütsch, B. W., Jung, S., & Schubert, S.(2015). Comparison of salt and drought-stress effects on maize growth and yield formation with regard to acid invertase activity in kernels. *Journal of Agronomy and Crop Science*, *201*, 353–367.

Iremiren, G. O., Adewumi, P. O., Aduloju, S. O., & Ibitoye, A. A.(1997). Effects of paclobutrazol and nitrogen fertilizer on the growth and yield of maize. *The Journal of Agricultural Science*, *128*, 425–430.

Irish, E. E.(1996). Regulation of sex determination in maize. *BioEssays*, *18*, 363–369.

Jacobs, B. C., & Pearson, C. J.(1991). Potential yield of maize, determined by rates of growth and development of ears. *Field Crops Research*, *27*, 281–298.

Jacobs, B. C., & Pearson, C. J.(1992). Pre-flowering growth and development of the inflorescences of maize. I. Primordia production and apical dome volume. *Journal of Experimental Botany*, *43*, 557–563.

Jameson, P. E., & Song, J.(2016). Cytokinin: A key driver of seed yield. *Journal of Experimental Botany*, *67*, 593–606.

Kamran, M., Cui, W., Ahmad, I., Meng, X., Zhang, X., Su, W., Chen, J., Ahmad, S., Fahad, S., Han, Q., & Liu, T.(2018). Effect of paclobutrazol, a potential growth regulator on stalk mechanical strength, lignin accumulation and its relation with lodging resistance of maize. *Plant Growth Regulation*, *84*, 317–332.

Lemcoff, J. H., & Loomis, R. S.(1986). Nitrogen influences on yield determination in maize. *Crop Science*, *26*, 1017–1022.

Lemcoff, J. H., & Loomis, R. S.(1994). Nitrogen and density influences on silk emergence, endosperm development, and grain yield in maize. *Field Crops Research*, *38*, 63–72.

Liao, C., Peng, Y., Ma, W., Liu, R., Li, C., & Li, X.(2012). Proteomic analysis revealed nitrogen-mediated metabolic, developmental, and hormonal regulation of maize (*Zea mays* L.) ear growth. *Journal of Experimental Botany*, *63*, 5275–5288.

Lur, H. S., & Setter, T. L.(1993a). Role of auxin in maize endosperm development. Timing of nuclear DNA endoreduplication, zein expression, and cytokinin. *Plant Physiology*, *103*, 273–280.

Lur, H.-S., & Setter, T. L.(1993b). Endosperm development of maize *defective kernel* (*dek*) mutants. Auxin and cytokinin levels. *Annals of Botany*, *72*, 1–6.

Moll, R. H., Kamprath, E. J., & Jackson, W. A.(1982). Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. *Agronomy Journal*, *74*, 562–564.

Mueller, S. M., Messina, C. D., & Vyn, T. J.(2019). The role of the exponential and linear phases of maize (*Zea mays* L.) ear growth for determination of kernel number and kernel weight. *European Journal of Agronomy*, *111*, 125939. https://doi.org/10.1016/j.eja.2019.125939

O'Neill, P. M., Shanahan, J. F., Schepers, J. S., & Caldwell, B.(2004). Agronomic responses of corn hybrids from different eras to deficit and adequate levels of water and nitrogen. *Agronomy Journal*, *96*, 1660–1667.

Ort, D. R., & Long, S. P.(2014). Limits on yields in the Corn Belt. *Science*, *344*, 484–485.

Otegui, M. E., Andrade, F. H., & Suero, E. E.(1995). Growth, water use, and kernel abortion of maize subjected to drought at silking. *Field Crops Research*, *40*, 87–94.

Paponov, I. A., & Engels, C.(2005). Effect of nitrogen supply on carbon and nitrogen partitioning after flowering in maize. *Journal of Plant Nutrition and Soil Science*, *168*, 447–453.

Paponov, I. A., Paponov, M., Sambo, P., & Engels, C.(2020). Differential regulation of kernel set and potential kernel weight by nitrogen supply and carbohydrate availability in maize genotypes contrasting in nitrogen use efficiency. *Frontiers in Plant Science*, *11*, 586. https://doi.org/10.3389/fpls.2020.00586

Passioura, J. B., & Angus, J. F.(2010). Improving productivity of crops in water-limited environments. *Advances in Agronomy*, *106*, 37–75.

Raun, W. R., & Johnson, G. V.(1999). Improving nitrogen use efficiency for cereal production. *Agronomy Journal*, *91*, 357–363.

Rijavec, T., Jain, M., Dermastia, M., & Chourey, P. S.(2011). Spatial and temporal profiles of cytokinin biosynthesis and accumulation in developing caryopses of maize. *Annals of Botany*, *107*, 1235–1245.

Rood, S. B., Pharis, R. P., & Major, D. J.(1980). Changes in endogenous gibberellin-like substances with sex reversal in the apical inflorescence of corn (*Zea mays* L.). *Plant Physiology*, *66*, 793–796.

Russell, W. A.(1991). Genetic improvement of maize yields. *Advances in Agronomy*, *46*, 245–298.

Schluttenhofer, C. M., Massa, G. D., & Mitchell, C. A.(2011). Use of uniconazole to control plant height for an industrial/pharmaceutical maize platform. *Industrial Crops and Products*, *33*, 720–726.

Seebauer, J. R., Moose, S. P., Fabbri, B. J., Crossland, L. D., & Below, F. E.(2004). Amino acid metabolism in maize earshoots. Implications for assimilate preconditioning and nitrogen signaling. *Plant Physiology*, *136*, 4326–4334.

Setiyono, T. D., Walters, D. T., Cassman, K. G., Witt, C., & Dobermann, A.(2010). Estimating maize nutrient uptake requirements. *Field Crops Research*, *118*, 158–168.

Setter, T. L., & Parra, R.(2010). Relationship of carbohydrate and abscisic acid levels to kernel set in maize under postpollination water deficit. *Crop Science*, *50*, 980–988.

Sinclair, T. R., & Horie, T.(1989). Leaf nitrogen, photosynthesis, and crop radiation use efficiency: A review. *Crop Science*, *29*, 90–98.

Singletary, G. W., & Below, F. E.(1990). Nitrogen-induced changes in the growth and metabolism of developing maize kernels *in vitro*. *Plant Physiology*, *92*, 160–167.

Singletary, G. W., Doehlert, D. C., Wilson, C. M., Muhitch, M. J., & Below, F. E.(1990). Response of enzymes and storage proteins of maize endosperm to nitrogen supply. *Plant Physiology*, *94*, 858–864.

Uhart, S. A., & Andrade, F. H.(1995a). Nitrogen deficiency in maize: I. effects on crop growth, development, dry matter partitioning, and kernel set. *Crop Science*, *35*, 1376–1383.

Uhart, S. A., & Andrade, F. H.(1995b). Nitrogen deficiency in maize: II. Carbon-nitrogen interaction effects on kernel number and grain yield. *Crop Science*, *35*, 1284–1389.

Wang, Y., Yao, Q., Zhang, Y., Zhang, Y., Xing, J., Yang, B., Mi, G., Li, Z., & Zhang, M.(2020). The role of gibberellins in regulation of nitrogen uptake and physiological traits in maize responding to nitrogen availability. *International Journal of Molecular Sciences*, *21*, 1824. https://doi.org/10.3390/ijms21051824

Wolfe, D. W., Henderson, D. W., Hsiao, T. C., & Alvino, A.(1988). Interactive water and nitrogen effects on senescence of maize. I. Leaf area duration, nitrogen distribution, and yield. *Agronomy Journal*, *80*, 859–864.

Xu, N., York, K., Miller, P., & Cheikh, N.(2004). Co-regulation of ear growth and internode elongation in corn. *Plant Growth Regulation*, *44*, 231–241.

Yang, H., & Grassini, P.(2014). Quantifying and managing corn water use efficiencies under irrigated and rainfed conditions in Nebraska using the hybrid-maize simulation model. *Advances in Agricultural Systems Modeling*, *5*, 113–138.

Zhang, Y., Wang, Y., Liu, C., Ye, D., Ren, D., Li, Z., & Zhang, M.(2022). Ethephon reduces maize nitrogen uptake but improves nitrogen utilization in *Zea mays* L. *Frontiers in Plant Science*, *12*, 762736. https://doi.org/10.3389/fpls.2021.762736

Zhao, Y., Lv, Y., Zhang, S., Ning, F., Cao, Y., Liao, S., Wang, P., & Huang, S.(2021). Shortening internodes near ear: An alternative to raise maize yield. *Journal of Plant Growth Regulation*, *41*, 628–638. https://doi.org/10.1007/s00344-021-10326-1

Zinselmeier, C., Jeong, B.-R., & Boyer, J. S.(1999). Starch and the control of kernel number in maize at low water potentials. *Plant Physiology*, *121*, 25–35.

How to cite this article: Hütsch, B. W., & Schubert, S.(2023). Grain yield, harvest index, water-use efficiency and nitrogen partitioning to grain can be improved by application of the plant growth regulator paclobutrazol to maize plants with reduced N supply. *Journal of Agronomy and Crop Science*, *209*, 261–272. https://doi.org/10.1111/jac.12623