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Effects of increased autumn temperatures and sub-acute levels of ammonia on post-winter development of four cultivars of winter oilseed rape (*Brassica napus* L.)

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Summary

Early planting dates, warm autumns and increased N mineralisation rates may advance the growth of winter crops and also a high supply of nitrogen poses risks of excess biomass accumulation before the winter leading to reduced frost hardiness and excessive leaf loss. Although the number of frost days is currently declining in temperate Europe, events of frost may still create damage in the future in winter crops that have accumulated too much biomass in the preceding autumn.

Here we report on a study in which the responses of four cultivars of winter oilseed rape (OSR) to elevated autumn temperatures and sub-acute levels of ammonia (NH_3) were compared. It has been suggested that high concentrations of the gas, e.g. in livestock intensive regions may both act as an airborne fertiliser and reduce the frost hardiness of plants. Advancing treatments were imposed for 88 days in the autumn and consisted of pot grown plants kept in greenhouses without (ELVT) and with elevated concentrations of ammonia (ELVT+A; 195 µg m^{-3} NH_3). Reference plants (AMB) were raised outdoors, where temperatures were 1.4°C lower than in the greenhouses.

After the treatments plants were all overwintered outside to study whether the pre-winter growth advancement was still discernible in the following spring. Shed leaves were collected weekly to follow how much shoot biomass was lost during and after the winter. Cultivars responded differently to warming and exposure to ammonia. Up to the winter shoot biomass was strongly increased by the advancing treatments. However, final shoot mass in the following summer did not differ between cultivars and was unaffected by the higher temperatures in the preceding autumn. Nevertheless, significantly more biomass was observed in ammonia fumigated plants. Higher autumn temperatures increased leaf shedding and advanced flowering and senescence in the next spring so that plants showed a significantly reduced seed mass, harvest index and oil yield at the final harvest. Obviously, the growth advancement in the preceding autumn by elevated temperatures negatively affected the availability of resources in the following spring. In contrast, plants that were grown at both elevated ammonia and temperature in the autumn showed a delayed flowering, higher shoot and seed mass, increased harvest index and oil yield. We conclude that growth advancement by elevated autumn temperatures without the re-supply of nutrients increases leaf shedding during winter. Nevertheless, the loss of resources in winter for re-growth in spring will certainly be of minor importance for yield formation as compared to the frost damage resulting from late spring frosts.

Introduction

Oilseed rape (OSR) is currently the most important bioenergy crop in the temperate regions and much effort has been made to improve yields and the oil quality of this species in recent decades. Climatic changes may shift the current OSR crop belt in the near future and in its current cultivation range increasing temperatures may affect the phenology, speed up the crop development and eventually reduce

the seed output due to the shorter crop growth cycle. At the same time, seed maturation, yield and oil quality may directly be affected by planting dates and high temperatures (MORRISON and STEWART 2002; AKSOUH-HARRADJ et al., 2006; PELLET et al., 2008; BAUX et al., 2008; FARAJI et al., 2009). It may also be hypothesised that with the predicted climatic changes, new pests and diseases will occur in the future, while the rising winter temperatures may not be suited to the vernalization requirements of some winter crop cultivars any more. Fig. 1 shows time series for OSR yield increments since 1961 and changes in the average duration of the culture period between the years 1951 and 2008 for Germany. Because sowing and harvest dates have been moved to earlier and later dates, respectively, average winter OSR culture periods increased from the 1950s to the 1980s but then decreased since the 1990s. At the same time OSR flowering occurred 15 days earlier in the decade 1999 to 2008 as compared to the climate normal period (Fig. 2). It is unclear, however, whether and how strong the introduction of new cultivars has had an effect on the observed phenological changes and the development of yields. There is also no information available whether yield increments over time and changes in crop duration are interrelated.
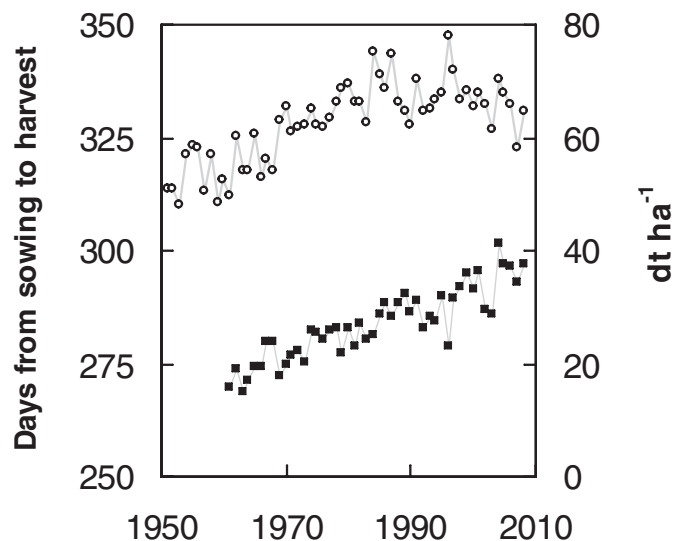

Fig. 1: Changes in the average duration of the winter OSR cultivation period (○, y-axis to the left) and yields (■, y-axis to the right) in Germany. Phenological records (1951-2008) stem from the database of Deutscher Wetterdienst (DWD) and data on oilseed rape yields in Germany (1961-2008) from FAOSTAT.

Effects arising from changes in the flowering window due to deviations from the normal climatic program can be manifold. Earlier onset of flowering may be especially critical if pollinators are still absent and if late frosts occur. The flowering window of OSR is about one month in total and adverse climatic conditions may well occur during this rather long time period affecting at least part of the

flowers. Because the climate in central Europe may become more variable in the future, the uniform phenological development may be impaired if the heat accumulation over time and the photoperiodic response of crops and cultivars do not match any more. Besides changes in the beginning of flowering, the alteration of the plant phenological program by climatic changes may also affect the timing of root and leaf development, which drive the resource acquisition and nutrient use efficiency (NUE) of plants (NORD and LYNCH, 2009). While the timing of fertilizer application may be adapted to these new conditions, the availability of water in the future will depend on the timing and intensity of rainfall. However, variations in precipitation patterns under climatic change are currently much more difficult to forecast than changes in temperatures.

Responses of oilseed rape to higher spring temperatures
PELTONEN-SAINIO et al. (2009 a, b) addressed the current and future potential of OSR farming in Northern Europe suggesting that cropping of winter instead of spring varieties may be more favourable in a warmer climate. Information is lacking, however, on problems with OSR cropping in the southern European regions that might arise from climatic changes. The suitability of irrigated oilseed rape cultivation as a winter rotational crop in semiarid regions of the South-Western US was addressed by ADAMSEN and COFFELT (2005). Planting the same spring varieties as used in the Great Plains in November at Maricopa Agricultural Center (Arizona) lead to acceptable yields but plants produced too much vegetative biomass (straw) at the expense of seed mass. A reason for the lower harvest index may be the high temperatures in the study region that affected flowering and seed filling. MORRISON and STEWART (2002) found that floral fertility and seed filling were significantly reduced at high air temperatures

above 29.5°C. It may thus be suggested that the increasing frequency of hot spells even in the cool and temperate regions may reduce the seed set of OSR. This could be the case in both winter and spring cultivars and, indeed, PELTONEN-SAINIO et al. (2007) have attributed the yield losses observed in Finnish OSR over the past 15 years to the increased occurrence of summer heat. In contrast to this study, the halt in OSR farm yields since 1985 in the UK has been attributed to crop management factors (shorter rotations, direct drilling, lower application rates of nutrients) rather than to changing climatic factors and failures in germplasm improvement (BERRY and SPINK, 2006). Still, the authors suggest that an increase in temperatures by 3°C will reduce the seed filling period by 13 days. Using historical crop yield and climate data from Canada, KUTCHER et al. (2010) found that the beginning of July, i.e. the beginning of the flowering period, was the critical period in the determination of OSR yields. Low yields were strongly associated with high July temperatures whereas high yields were associated with greater-than-average precipitation, and to a lesser extent, cooler-than-average nocturnal temperatures.

In contrast to the negative effects of high temperatures during flowering and the seed determination period 19-25 days after mid-flowering the earlier flowering caused by warmer springs may also be regarded as a positive response. This is due to the fact that light reflection by the flowers will be reduced earlier so that leaf area and the assimilation of carbohydrates may overall profit. One would then expect higher yields provided the absence of thermal stress later on. BERRY and SPINK (2006) state that bringing forward flowering by one week could improve yields but it is unclear whether the earlier flowering and increased yields in Germany (Fig. 1) are interrelated. Still, the strong yield depressions by 20% in the hot summer 2003 and the warm spring in 2007 indicate adverse climatic influences on OSR and give some idea on the effects of thermal stress in the future.
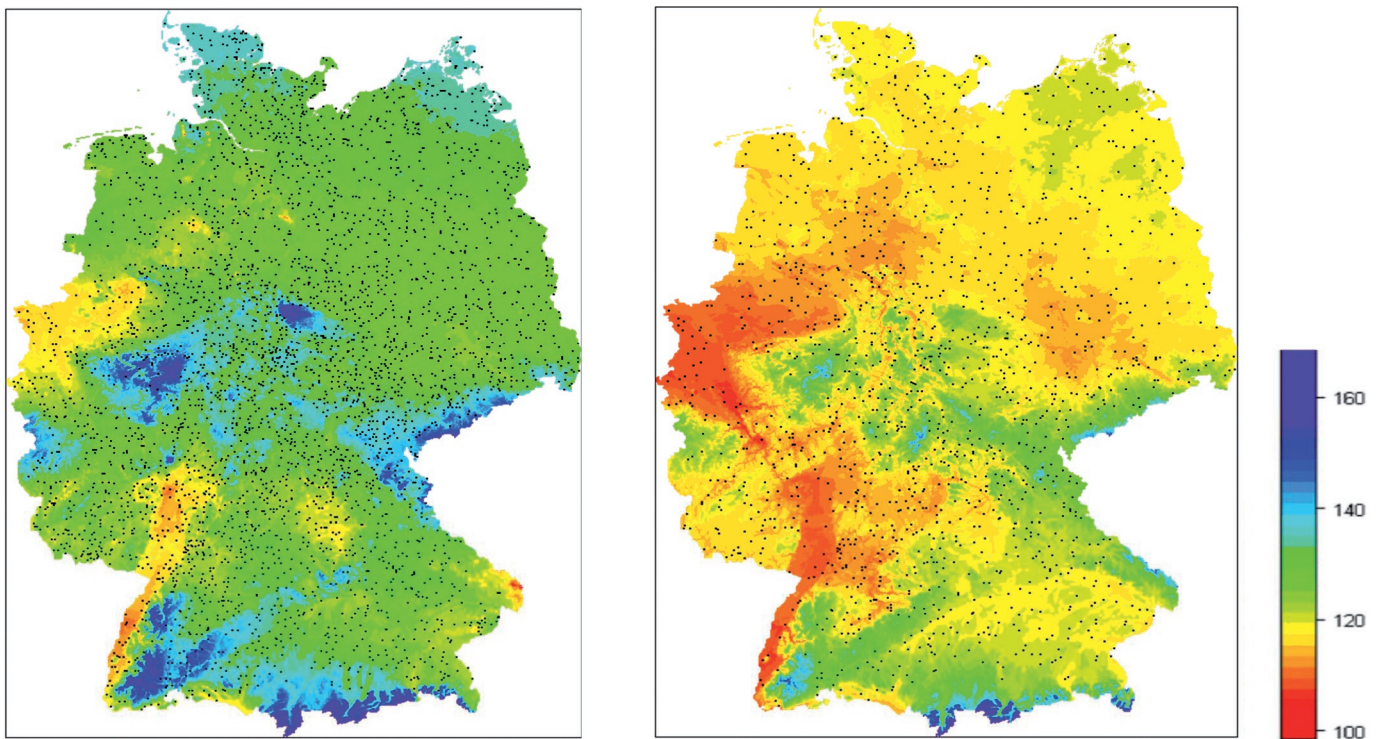
Fig. 2:   Beginning of flowering of winter OSR in Germany during the WMO climate normal period 1961-1990 (left) as compared to the warmer decade 1999-2008 (right). Phenological data for over 3500 stations (black dots) from Deutscher Wetterdienst (DWD). Maps were produced on the basis of a digital elevation model (1 km x 1 km) with local trend surface prediction (BIVAND et al., 2008) using the statistical programming environment R. Numbers in scale represent days of the year (DOY, with DOY 1 = 1 January). In most regions flowering occurs 10 to 15 days earlier nowadays than during the climate normal period.

Responses of oilseed rape to elevated autumn and winter temperatures

Despite heat stress and heat spells probably occurring more frequently in the future, frost will still play a significant or even increasing role in a warmer world (GU et al., 2008). This is because more severe damage or post-freeze setback may turn up in plants that experienced a strong pre-freeze phenological advancement due to warmer conditions.

In winter crops, sufficient biomass and cryoprotective compounds must be accumulated between the sowing and the onset of the winter to increase their frost hardiness. On the other hand, too much shoot biomass and premature shooting will increase the danger of frost damage due to the exposure of aerial plant parts and the occurrence of frost drought. In order to prevent premature shooting under climate change, farmers may apply plant growth regulators to keep plants in the rosette stage but this investment may narrow the benefits. To respond to the increased likelihood of shooting before the onset of winter, sowing of winter OSR cultivars could eventually be moved to later dates in the near future. In spring cultivars, however, sowing dates should be as early as possible because late planting will result in lower yields, a reduced harvest index and lower oil contents (GROSS, 1964; KONDRA, 1977; JOHNSON et al., 1995).

While the concept of accumulated thermal time (growing degree days, GDD) is successfully applied to predict the development of spring and winter OSR (MORRISON et al., 1989) in spring, not much information is available on the relationship between heat accumulation and pre winter growth of winter OSR. According to DIEPENBROCK (2006a) winter OSR should be sown in Germany between 15 August and 15 September at a density of 60 to 90 seeds per square metre. Until winter plantlets should be in the 6- to 8-leaf stage and have an optimum shoot dry weight of approximately 1 g per plant. This will give plants a high chance to survive the winter. Growth completely ceases below 2°C and low temperatures will induce the accumulation of phospholipids in plant leaves, which renders them more resistant to frost dehydration (RAPACZ, 1998, 1999, 2002; CHRISTEN and FRIEDT, 2007). Another prerequisite to withstand frost is the existence of a thick root collar containing large amounts of soluble carbohydrates, which will also benefit a rapid re-growth in spring (LÄÄNISTE et al., 2007). During the winter a mean of 50% of the dry shoot mass will normally be lost, but higher losses may occur due to frost damage (DIEPENBROCK, 2006b). LÄÄNISTE et al. (2007) have addressed the importance of sowing dates on winter hardiness of OSR in Estonia, which is currently the northern border for the winter cultivars of this crop. Based on three years trials they found that winter survival was strongest in plants that had received 416 growing degree days (GDD above a base temperature of 5°C). While plants from late sowing treatments did not survive the winter also the plants from the early sowing variants were severely affected by frost damage. The authors found that at 500 GDD the percentage of overwintering plants was below 50%.

Effects of N-availability on the frost hardiness of plants

Another important non-climatic factor, which may strongly advance the development of winter crops, is of course the availability of fertilisers. While P and K fertilisers may be applied to the seedbed to increase yields, nitrogen availability in autumn does not have pronounced effects on the final yields as long as the fertilisation is adequate in the following spring. According to COLNENNE et al. (2002) even severe autumn N deficiencies will allow enough shoot mass accumulation to ensure sufficient re-growth in spring. In fact, excess fertilisation in autumn may increase the danger of frost damage in crops due to the stronger built up of shoot biomass and the exposure of aerial plant parts to frost desiccation.

Apart from soil-N, elevated concentrations of ammonia near livestock farms may significantly contribute to the N-supply of plants (SOMMER, 1988, SOMMER et al., 1991, 2009). The uptake rates of gaseous ammonia have been shown to be elevated at higher soil fertilisation rates, which may be explained by the higher leaf area index at higher availability of soil N that will lead to higher N deposition to the crop. Also BÖHME et al. (2003) found that crop biomass and canopy area determine how much nitrogen will be scavenged from the atmosphere and made available for additional plant growth. While N utilisation from airborne ammonia will be largest in growing stands, plants will switch from sinks of NH_3 to active sources during the senescence due to shifts in compensation points. Bi-directional fluxes of ammonia between the atmosphere and OSR leaves have been studied by HUSTED et al. (1995, 1996) and MASSAD et al. (2009) under controlled conditions and by NEMITZ et al. (2000) and SCHJOERRING et al. (2000) in the field.

Whereas it has been suggested that even low environmental concentrations of ammonia may reduce the frost hardiness of plants from acidic heathlands (e.g. *Calluna*, SHEPPARD and LEITH, 2002; SHEPPARD et al., 2008), exposure to extremely high concentrations (1000 nl L^{-1}) of ammonia did not affect low-temperature hardening of conifers and wheat (CLEMENT et al., 1995, 1999). Physiological mechanisms potentially involved in the direct effects of ammonia on the frost hardiness of plants relate to the reduced accumulation of osmotic compounds (polyamines and carbohydrates) and phospholipids allowing plant tissues to adapt to and withstand low temperatures. While these relationships have not been well established, indirect effects of ammonia on the frost sensitivity of plants may occur due to the increase in shoot biomass.

Scope of the study

We assumed that higher autumn temperatures and sub-acute concentrations of ammonia as an additional N-source would first stimulate pre-winter plant growth and later increase the risk of leaf loss in winter oilseed rape. We further postulated different responses in different cultivars and that carry-over effects from the autumn treatments would be recognisable in the formation of yields and the quality of seeds in the following spring. To test these hypotheses, we grew different cultivars of OSR in two consecutive experiments with an early and a late sowing date under ambient and elevated temperatures to quantify the additional biomass production of selected cultivars under warmer autumn conditions. We used a third treatment, in which plants were exposed to both elevated temperatures and sub-chronic concentrations of ammonia to study the additional stimulation of shoot growth. Plants from the late sowing were over-wintered to observe how the treatments affected the shedding of leaves during winter and how much biomass and seeds were produced in the following spring. Performing such pot-based studies under controlled climatic conditions and collecting the shed leaves at fixed intervals may serve to address the resource use efficiency as well as the progress of senescence in higher temporal resolution as compared to field trials, in which shed biomass cannot be accounted for.

Materials and methods

Plant cultivation

Two individual experiments with different sowing dates were performed to investigate the effects of elevated autumn temperatures and sub-acute levels of ammonia on the growth of winter oilseed rape (OSR). While plants from the first experiment (early sowing date) were harvested before the winter to address the plant responses in late autumn, only the plants of the second experiment (late sowing date) were overwintered to study the effects of elevated autumn

temperatures and ammonia exposures on yield formation and seed quality in the following spring. In the first experiment, OSR was sown on 15 August 2008 (DOY 228), which is three days earlier than the sowing date in Hohenheim in recent years. In the second experiment the crop was sown five weeks later on 22 September 2008 (DOY 266). After sowing, each of the 48 pots (4 per cultivar * 4 cultivars * 3 treatments) was fixed in packs of 6 pots on polystyrene racks placed above individual plastic water containers to be able to follow water consumption over time. Water consumption was registered for each of the pots and water was re-filled on demand to the plastic containers at known amounts.

The four cultivars used in the experiments 'Asgard' (A), 'Express' (E), 'Lorenz' (L) and 'Viking' (V) were provided by Norddeutsche Pflanzenzucht Hans-Georg Lembke KG (NPZ Holtsee). Seed lots of cultivars A, E, L and V had thousand seed weights (TSW) of 4.9, 4.5, 4.7 and 4.3, respectively. Five seeds per pot were sown at a depth of 1.5 cm in plastic containers (truncated cone, L=13 cm, B=13 cm, H=13 cm, A=169 cm^2, V=1.7 L) and reduced to one plant per pot representing a final density of 60 plants m^{-2}. Pots were filled with 0.65 kg standard soil (Fruhstorfer LD 80, Archut-Hawita Gruppe, Vechta) and equipped with glass fibre wicks (Type 6000, Hecker Werke GmbH, Weil im Schönbuch) for automatic watering. At water saturation the substrate has a maximum water content of 73.6 wt. % and 27.7 vol. %. The soil substrate is approved after EN 12580 and contains bark humus, volcanic clay and peat (35% organic matter in dry mass and pH 6.1). It is enriched with Radigen® slow release micronutrients and Aquaflow® slow release NPK fertilisers and has a macronutrient content of 150 mg L^{-1} N and P$_2$O$_5$ each as well as 250 mg L^{-1} K$_2$O. Accounting for the pot volume and its area the initial nitrogen availability represented a value of 150.9 kg ha^{-1}. Until winter no further nutrients were supplied, because plants were harvested in the rosette stadium. In the late sowing experiment, which lasted until seed maturation, plants received 100 mL of a nutrient solution (VDI, 2008) on 23rd of March 2009, representing a second gift of 0.5 g N per pot. The VDI standard solution is used for curly kale (*Brassica oleracea* L.) cultures and for its sufficient sulphur concentrations was believed to be well suited for OSR pot cultures. The total N availability (slow release fertiliser plus the nutrient solution) amounted to 0.755 g pot^{-1} and this quantity was taken into account to derive the N economy parameters nitrogen use efficiency (NUE) and nitrogen recovery in the seeds.

Treatments and monitoring of ammonia and climate
Three treatments were applied in the experiments. One set of plants was kept under ambient conditions (AMB) in a fenced premise which was covered by a transparent roof to exclude rainfall. The two other treatments were grown in greenhouses (Floratherm® Alltop KA 200 Krieger GmbH Herdecke, L 200, B: 185 cm, H: 210 cm, V: 7 m^3) to raise the air temperatures by ca. 2°C above the ambient conditions (ELVT). In addition to the elevated temperatures one of these warm treatments was subjected to elevated ammonia concentrations (ELVT+A). Both greenhouses, i.e. treatments ELVT and ELVT+A, were equipped with a charcoal filter unit, a ventilator and flexible tubes (Ø15 cm), through which air was lead into the greenhouse. Air was exchanged 54 times per hour in both of the greenhouses. To enrich the ELVT+A treatment with ammonia, pure NH$_3$ (quality 3.8, 40 L flask, SWF Friedrichshafen) was lead via a Teflon tube into the air stream. Outlet pressure of the flask was reduced from 8 to 1 bar resulting in an average flow of 1.5 mL min^{-1} of the gas. This supply was calculated to yield a set ammonia concentration of about 200 µg m^{-3} (285 ppb) in the ELVT+A treatment. In order to avoid placement effects the treatments were switched between the two greenhouses bi-weekly. At these dates also the pots were randomly re-arranged.

Ammonia concentrations were monitored daily in the ELVT+A and weekly in the other treatments using the Radiello® passive sampler method (Sigma Aldrich, FSM, 2006). The adsorbing cart-ridge is coated with phosphoric acid, which adsorbs ammonium at a sampling rate of 235 mL min^{-1}. The quantity of ammonium was determined against standards at 635 nm by visible spectrometry (Beckman Coulter DU 640B, Fullerton) in the form of indophenol which develops from the reaction between phenol and sodium hypo-chlorite in the presence of cyanoferrate. In order to calculate ammo-nia concentrations, the NH$_4^+$ mass per sampler was divided by the product of the duration (minutes) of the exposure and the sampling rate. In order to obtain information on the temperatures and relative air humidity in the three treatments calibrated Tinytag temperature loggers (Gemini Data Loggers, UK) were operated on a 10 min rou-tine inside and outside the greenhouses.

Measurements on oilseed rape
In the early sowing experiment, germination and numbers of germi-nated seedlings were recorded. On day 7 after germination (7 DAG) one seedling per pot was harvested at random and the number of seedlings per pot was reduced to 3 individuals to reduce plant com-petition. Two more harvests took place 14 and 21 days after germina-tion (14 DAG and 21 DAG) leaving just one plant per pot. Leaf area and fresh weight of plant fractions leaf and stem were determined. Harvested plants were put into labelled paper bags and were dried at 80°C for 24h in a drying cabinet. After drying, dry matter (DM) of the seedlings was determined and absolute (AGR) and relative growth rates (RGR) were calculated accordingly. Calculations of growth rates were based on average plant weights (n=4 per treatment and cultivar). Leaf greenness was determined weekly with a SPAD-meter (Minolta SPAD 502) to follow differences in chlorophyll contents in the different treatments. Five measurements were taken on the top of the youngest completely unfolded leaf and averages of these measurements were recorded.

In the late sowing experiment the same growth related parameters were studied. Like in the early sowing experiment root biomass was not analysed. However, much effort was put on the harvest of dead leaf material, which was present after the winter and which accumu-lated during the senescence of the plants. Shed leaves were collected every week and shoot length and flowering phenology were recorded thoroughly. At the onset of flowering, SPAD values were determined on a weekly basis, with measurements being performed on marked leaves at a height of 80 cm. We chose a leaf from a high position instead from the rapidly senescing rosette to be able to get extended information on the whole process of senescence. At the final harvest shoot biomass was separated into stem, leaves, pod walls and seeds. While the fractions stem, leaves and pod walls were dried at 80°C, seeds were air dried to constant weight. Finally, the dry weight of all fractions was determined.

Seeds of each plant were counted with a Contador seed counting machine (Pfeuffer GmbH Kitzinigen, Germany) to derive thousand seed mass. Seed oil contents were analysed using the near infrared spectroscopy (NIRS) standard procedure outlined in VDLUFA (2002). N concentrations of leaves that had developed before the winter and seeds harvested the following spring were determined by elemental analyses and seed PK- concentrations were determined by ICP-OES following acid digestion.

Data evaluation and statistics
From the original biomass data determined at several harvests, specific leaf area (SLA), relative growth rates (RGR) and absolute growth rates (AGR) were calculated after HENDRY and GRIME

(1997). All the biological data were subjected to descriptive statistical analyses and one or two-factorial ANOVA to identify significant treatment effects as well as treatment* cultivar interactions. Posthoc Tukey Honest Significant Differences (HSD) tests were used for multiple comparisons to study whether there were significant differences in the response of the chosen cultivars. All statistical analyses and most of the graphical representations were performed using the open source programme R and different libraries (R DEVELOPMENT CORE TEAM, 2009).

Results and discussion

Climate and ammonia concentrations

The daily mean temperatures recorded inside the greenhouses (treatments ELVT and ELVT+A) and outside (AMB) are presented in Fig. 3. The temperature curve from 15 August 2008 (early sowing of OSR) to 24 June 2009 (harvest of OSR from the late sowing experiment) is also compared to the long term daily temperature means at Stuttgart, Germany. As can be seen in Tab. 1, ambient temperatures in the autumn 2008 were slightly higher than the long term average. However, in December 2008 and January 2009 mean temperatures remained below the long term average for those months. The extended frost spell early in the year therefore proved to be well suited to study the effect of advanced autumn growth on the winter hardiness of OSR.

During the warm autumn treatment (ELVT) in the early sowing experiment (15.08.-13.10.2008) temperatures were 2.4°C higher than in the AMB treatment. In the late sowing experiment, in which the treatments lasted from 22.09. to 19.12.2008 (88 d) the realised temperature increase was only 1.4°C. Mean temperatures did not differ between the two greenhouses, which served to increase the temperatures (ELVT and ELVT+A).

Mean daily ammonia concentrations during the early sowing experiment were 2.5 (± 0.7) µg m⁻³ outside (AMB), 7.3 (± 6.4) µg m⁻³ inside the untreated greenhouse (ELVT) and 275 (± 99) µg m⁻³ inside the ammonia treated greenhouse (ELVT+A). In the late sowing experiment the mean daily NH₃-concentrations ranged from 6.8 (± 6.4) µg m⁻³ outside (AMB) to 6.4 (± 3.2) µg m⁻³ inside the untreated greenhouse (ELVT) and 195 (± 93) µg m⁻³ inside the ammonia

treated greenhouse (ELVT+A). The ammonia treatments resulted in sub-acute ammonia concentrations comparable to those measured after slurry applications.

Responses during the autumn treatments

Seeds germinated at a rate of almost 100% with no significant differences between the cultivars and the three treatments in both the early and late sowing experiments. However, there were slight cultivar differences in the speed of germination in the follow order A>V>L>E, but the different speed of germination was unrelated to growth differences subsequently evolving in the three treatments. Nevertheless, the availability of seed resources had slight effects in the seedling establishment phase. In the AMB treatments of the early sowing experiment, thousand seed masses (TSM) of the cultivars were positively associated with their absolute growth rates (AGR) between 7 and 21 days after germination, while the opposite relationship was observed in the plants that were exposed to higher temperatures. At the same time, the OSR cultivar 'Viking' with low availability of seed resources (low TSM) responded more strongly to the atmospheric nitrogen fertilisation in the ELVT+A treatment than the other lines (data on growth performance in intermediate harvests not shown).

OSR plants from all cultivars and from both the early and late sowing experiments were able to absorb ammonia via their shoots and achieved a significantly higher shoot mass than in the other treatments.

Fig. 4 indicates that shoot mass was highest in the ELVT+A treatments and reached an average of 20 g DM in the early and 3.5 g DM in the late sowing experiment. The results confirm that advancing the plant development by higher temperatures and elevated ammonia concentrations may strongly increase the shoot biomass produced before the onset of the winter. In both of the experiments shoot biomass of the ELVT and ELVT+A treatments was well above a shoot dry weight of 1 g, which has been considered the minimum plant biomass for the winter survival by DIEPENBROCK (2006a). Even the plants from the late sowing experiment in the AMB treatment had approached this value indicating that OSR sowing could be moved to the beginning of September.
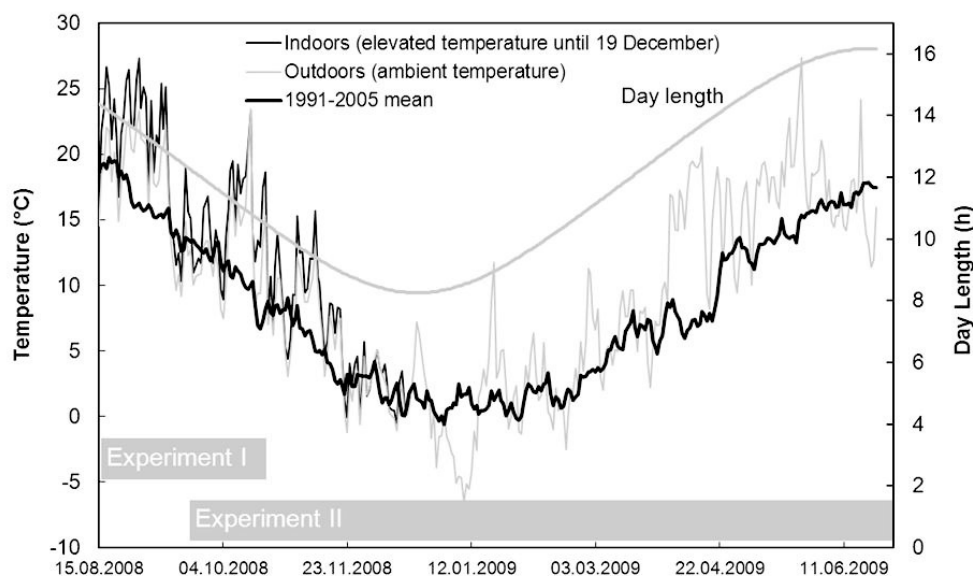
Fig. 3: Mean daily temperatures during the growth experiments as compared to the long term daily averages and changes in day length. Warm autumn treatments lasted from 15 August to 19 December 2008. Grey bars indicate the duration of the experiments I (early sowing) and II (late sowing). Long term temperatures are based on the 1991-2005 time series from Stuttgart-Airport (Data available from Deutscher Wetterdienst, DWD).

Tab. 1: Mean temperatures during the experiments, temperature sums accumulated until the individual harvests and mean shoot biomasses recorded across the four different OSR cultivars. Temperature sums (Growing Degree Days, GDD) refer to daily temperature means above a threshold of 5°C. DAG represents days after germination. Long-term data (1991-2005) are based on daily records from Stuttgart-Airport (data from DWD).

			Day	Mean temperatures (°C)				Heat units (GDD>5°C)			Shoot biomass (g DM)		
			length (hrs)	Long-term	outside AMB	inside ELVT	Diffe-rence	outside AMB	inside ELVT	Diffe-rence	outside AMB	inside ELVT	Increment (%)
Experiment 1													
Sowing date	15.08.08	DAG 0											
Harvest 1	01.09.08	DAG 7	13.9	18.1	19.8	22.9	3.1	266	330	64	0.055	0.10	96.5
Harvest 2	08.09.08	DAG 14	13.7	17.4	19.5	22.6	3.1	363	447	84	0.35	0.54	54.5
Harvest 3	15.09.08	DAG 21	13.5	16.8	18.9	21.7	2.8	442	538	96	1.46	2.44	67.4
Harvest 4	13.10.08	DAG 49	12.7	14.7	15.9	18.3	2.4	652	795	143	15.1	16.5	9.3
Experiment 2													
Sowing date	22.09.08	DAG 0											
Harvest 1	06.10.08	DAG 7	11.8	12.5	11.2	13.1	1.9	83	109	26	0.01	0.01	0.0
Harvest 2	13.10.08	DAG 14	11.6	11.9	12.6	14.6	2.0	156	193	37	0.03	0.05	111
Harvest 3	20.10.08	DAG 21	11.4	11.1	12.9	15.0	2.1	207	265	58	0.08	0.26	201
Harvest 4	13.11.08	DAG 45	10.7	9.3	11.0	12.9	1.9	261	339	78	0.89	2.49	180
Harvest 5	24.06.09	DAG 268	11.5	7.1	8.8	9.2	0.4	1263	1341	78	45.7	43.2	-5.3
Mean temperature (22.09. to 19.12.)				6.5	7.7	9.1	1.4						
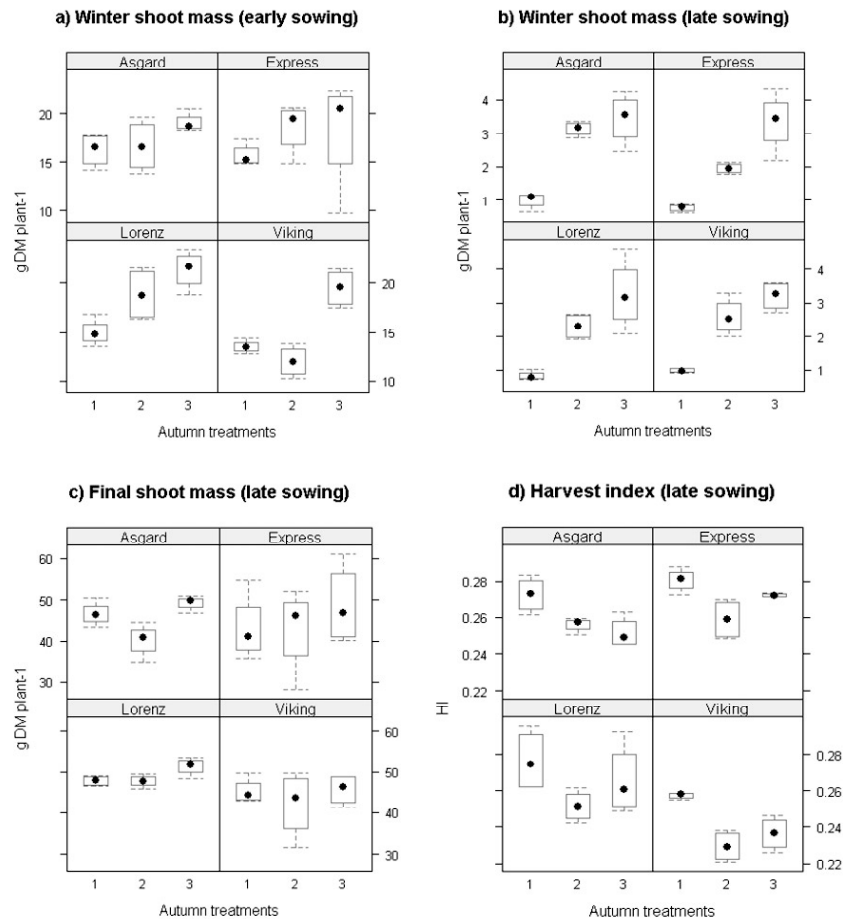
Fig. 4: Box plots showing the mean shoot biomass and harvest index of four OSR cultivars grown in early and late sowing experiments. Autumn treatments refer to ambient temperatures (AMB: 1), elevated temperatures (ELVT: 2) and elevated temperatures plus ammonia (ELVT+A: 3). Winter shoot biomass from the early sowing experiment (a) was harvested on 13 October 2008 (49 DAG) and that of the late sowing experiment (b) one month later (45 DAG). Final shoot biomass of the late sowing experiment (c) was harvested on 24 June 2009 (268 DAG) and harvest index (d) relates to the contribution of final seed to shoot mass.

The strong increase in shoot mass due to warmer autumns and a luxurious supply of fertilisers (here airborne ammonia) may induce premature shooting and increase the frost sensitivity of plants. The maximum leaf number prior to the onset of the winter period should not exceed 8 leaves according to DIEPENBROCK (2006a), but in the early sowing experiments plants had 11.4 in the AMB and 13.3 leaves on average in both the ELVT and ELVT+A treatments. It may thus be expected that plants would have not survived the following cold winter. However, plants were harvested prior to the winter. Large differences in leaf numbers were found between the cultivars. While V had only 8.2 leaves in the AMB treatment, the other cultivars produced 11.7 (E), 12.2 (A) and 13.2 (L) leaves on average in the early sowing experiment. While A, E and V did not significantly increase leaf numbers due to warming in the ELVT treatment, cultivar L significantly increased leaf numbers to 18. It may thus be followed that L should not be sown too early, whereas V is much less temperature sensitive. The plants from the late sowing experiment produced less than 8 leaves before the winter but all plants from the late sowing experiment survived the cold winter of 2008/2009. Leaf numbers were 5.1, 7.4 and 7.7 in the AMB, ELVT and ELVT+A treatments, respectively and like in the early sowing experiment cultivar L responded strongly to elevated temperatures with 8.25 leaves on average (data not shown).

The accumulated heat sum (GDD above a threshold of 5°C) until the final harvest of the early sowing experiments was 652 and 795 in the AMB and the ELVT treatments, respectively. According to LÄÄNISTE et al. (2007) such thermal sums would be much too high for the survival of OSR under cool winter conditions. However, these findings were made in Estonia and might not be representative for milder oceanic climates. Winter hardiness of OSR in Estonia was strongest in plants that had received 416 GDD between sowing and the onset of the winter. The plants from present late sowing experiments received only 261 and 339 GDD in the AMB and ELVT treatments between sowing (21.09.2008) and 31 December but all of the plants survived the following cold winter.

Responses during the following spring

The development of plants subjected to the three autumn treatments was followed thoroughly in the following spring including the regular collection of shed leaves, the determination of shoot length and growth stages and weekly measurements of leaf greenness (SPAD). At the final harvest (24 June 2009), the biomass allocated to leaves, stems and the reproductive organs (pod walls and seeds) was determined and oil contents were analysed in the dry seeds. Total dry shoot mass averaged to 45 g plant^{-1} DM. This is comparable to the average shoot weight of 17 - 34 g DM per plant, which results from the shoot mass production of 100 to 200 dt ha^{-1} (=1 to 2 kg m^{-2}) at a density of 60 plants m^{-2}. Average seed mass averaged to 12 g plant^{-1}, which is also comparable to the seed yield in the field. At a final plant density of 40 plants per square metre, seed production per plant ranges between 8 and 14 g DM per plant yielding 320 to 560 g m^{-2} (32 to 56 dt ha^{-1}).

Fig. 5 shows a comparison of the leaf shedding and the leaf greenness in the four cultivars over time. The cultivars A and V had somewhat higher initial SPAD values than the other two cultivars, indicating that there were slight differences in the chlorophyll concentrations between different cultivars. Results of two-way ANOVAs indicating the existence of significant differences between the cultivars and cultivar * treatment interactions are shown in Tab. 2. Chlorophyll degradation and probably the onset of senescence in the spring occurred earlier in A than in the other cultivars despite its higher initial SPAD values. At the same time, cultivars V and E had some-

what higher initial SPAD values in the plants that were treated with ammonia in the previous autumn. However, significant cultivar * treatment interactions were only observed on 28 April. A highly significant effect of the warming treatments was found for the leaf mass that was shed during winter and the final cumulative leaf mass (see first and last observation 61 and 143 DOY in Fig. 5). In the warm treatment cultivars A and L shed more biomass during the winter than in the other treatments, but only in cultivar A the strong leaf loss during the winter could not be compensated in the following spring (see Fig. 5). Furthermore, highly significant cultivar differences and a strong cultivar * treatment interaction were observed in final shoot length. While cultivar E reduced shoot length upon advanced growth by elevated autumn temperatures and ammonia concentrations, cultivar L produced taller plants in both of the advancing treatments and cultivar A in the ELVT+A treatment.

Significant cultivar differences and effects of the elevated temperatures and ammonia concentrations were also observed in the onset and duration of flowering, while a significant cultivar * treatment interaction across the treatments was only found in the onset of flowering. Plants of cultivar V grown at elevated autumn temperatures flowered three days earlier than those grown at ambient temperatures. Nevertheless, differences in the onset of flowering and changed flowering windows did not explain differences in final seed output. While significant cultivar differences were found for seed mass, with cultivar L being the most productive, significant treatment effects were found for elevated temperatures and elevated ammonia. Elevated autumn temperatures reduced seed mass, but elevated ammonia compensated this negative effect. Also the growth traits stem mass, harvest index and oil yield showed opposite responses, with higher values when plants were subjected to a combination of elevated temperatures and elevated concentrations of ammonia.

Interestingly, thousand seed mass was unaffected by the autumn treatments, while ANOVA across all treatments revealed significant cultivar differences with cultivar A having the smallest seeds. Pod numbers and weights of pod walls (siliques) were unaffected by the treatments and did not significantly differ between cultivars, suggesting that these traits had not been changed much (or selected for) during the breeding history. Nevertheless, the harvest index (Fig. 4) showed highly significant cultivar differences with the cultivars E and L having the highest and cultivar V the lowest values. Adverse effects of the autumn treatments on the harvest index are primarily due to the growth enhancement of the vegetative plant parts at the expense of the seeds. Finally, the seed oil contents appeared to be unaffected by the elevated temperatures and ammonia concentrations supplied in the previous autumn. However, significant cultivar differences were observed, with cultivar L having the highest oil contents and the tallest cultivar V the lowest. When addressing relationships between the oil contents and other traits across treatments and cultivars a slight negative relationship ($R^2 = 0.49$) was identified between the mean oil contents and the seed N concentrations. The existence of a negative relationship between these variables and a trade-off between the synthesis of oils and proteins has also been found in other studies (refer to the review of RATHKE et al., 2006). Also the SPAD values recorded on 16 April 2009 showed a slightly negative relationship ($R^2 = 0.15$) to the oil contents that were determined at the end of the season. It therefore seems that plants with a high spring chlorophyll and presumably N status produce seeds with lower oil content.

Conclusion

The pot-grown plants from present experiments produced comparable final shoot and seed mass as plants grown in the field. Also
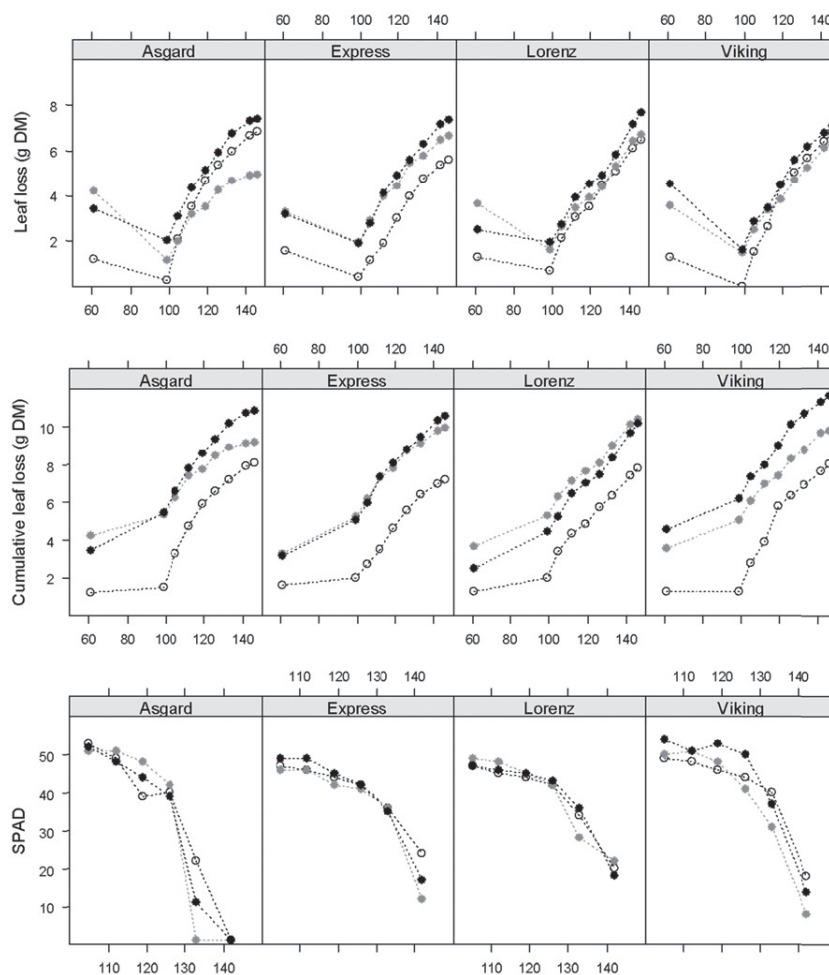
Fig. 5: Development of leaf loss during winter and spring (above), cumulative leaf loss (centre) and leaf greenness (below) in four OSR cultivars subjected to warming treatments in the previous autumn. X-axis represents time in days of the year (DOY). Treatments refer to AMB (○), ELVT (●) and ELVT+A (●). For the existence of significant treatment effects refer to Tab. 2. Note that the final cumulative leaf loss represents the dry mass of all leaves that were produced by a plant from sowing to harvest.

SCHULTE AUF'M ERLEY et al. (2007) found that pots-in-greenhouse experiments are a simple but suited tool to study the performance of different OSR cultivars under a variable N supply. When sown late, plants of the tested four OSR cultivars strongly increased pre-winter shoot mass under elevated temperature (+180%) and even more so under elevated ammonia concentrations (+270%). Relative shoot mass stimulations were lower when plants were sown earlier. The results indicate that OSR was able to utilise gaseous ammonia to produce significantly more biomass than the untreated plants. Still we are not sure how much of the ammonia-N that was absorbed in the autumn was lost later on due to leaf shedding or due to outgassing. Although the different treatments were ceased before the winter, spring growth of the plants remained different in the three treatments. Final shoot mass was still 13% ($p<0.05$) and seed mass 16% ($p<0.05$) higher in the plants that were fumigated with ammonia in the previous autumn. In contrast, the plants that were subjected to higher autumn temperatures had a 5% (ns) lower shoot mass and a 13% ($p<0.05$) lower seed mass than those grown under ambient temperatures. The findings show that pre-winter growth enhancement has discernible effects on the performance of plants in the following spring. Reasons are the loss of resources due to the shedding of leaf mass in the winter. Nevertheless, the effects of autumn growth advancement by elevated temperatures and the leaf loss due to winter frost will be of minor importance as compared to late frost events eventually occurring in spring. Furthermore, we suggest that growth

advancement due to atmospheric ammonia will probably not play a significant role at ambient concentrations, which are normally lower than 50 μg m^{-3}.

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Tab. 2: Effects of growth enhancement by increased autumn temperatures and elevated concentrations of the gas ammonia in four cultivars of winter oilseed rape. Data refer to the plant performance (phenology, SPAD and biomass) in the following spring with means across four replicates per cultivar in each of the three treatments. Cultivars: 'A' - Asgard, 'E' - Express, 'L' - Lorenz, 'V' - Viking. Results of the ANOVA indicate significant differences between cultivars (C), treatments (T) and interactions between C * T. Significant temperature effects were tested using the comparison between AMB and ELVT, while tests for ammonia effects rested on the comparison between the ELVT and ELVT+A treatments. Significance levels: * $p<0.05$, ** $p<0.01$ and *** $p<0.001$. [a] refers to leaf mass that had been shed during the winter; leaves were collected on 1 March 2009. [b] relates to air dried seeds.

Treatments / Cultivars	AMB (Ambient temperature)				ELVT (Elevated temperature)				ELVT+A (Elevated temperature plus ammonia)				Temp. Effect			NH₃ Effect			Combined Effect		
	A	E	L	V	A	E	L	V	A	E	L	V	Cultivar	Treat.	C*T	Cultivar	Treat.	C*T	Cultivar	Treat.	C*T
Leaf loss during winter (g DM)[a]	1.21	1.58	1.27	1.27	4.21	3.30	3.67	3.56	3.42	3.18	2.50	4.53		***						***	
Onset of Flowering (DOY)	102	102	102	105	100	101	102	102	102	102	103	102	**	**			**	*	*		*
Flowering duration (days)	26	26	25	27	26	25	25	25	25	24	25	27						**	*		
Final shoot length (cm)	143	148	139	155	140	140	150	154	153	130	154	155	*			***		*	***		***
SPAD (16 April 2009)	52.8	46.9	46.7	49.1	50.6	46.0	49.2	50.0	52.3	48.9	46.5	53.5	*			*			**		
SPAD (21 April 2009)	49.1	46.3	45.3	47.5	50.8	46.2	47.7	50.5	48.2	48.6	46.4	51.1	*			*			**		
SPAD (28 April 2009)	39.1	44.3	43.8	45.7	48.5	42.0	45.2	48.1	44.4	44.7	44.9	53.0		*	*	***			**	*	
SPAD (5 May 2009)	40.2	41.8	42.0	43.9	42.0	41.1	42.3	41.3	38.6	42.2	43.3	50.0		*		*		*	*		*
SPAD (13 May 2009)	21.6	35.5	34.2	40.3	1.0	35.7	28.2	30.6	11.2	35.4	36.1	36.6	***	*		***			***		
Leaf mass total (g DM)	8.1	7.2	7.8	8.0	9.2	10.0	10.4	9.8	10.8	10.6	10.2	11.6		***						***	
Stem mass (g DM)	15.8	14.1	17.0	16.8	12.3	12.9	15.8	14.4	15.9	14.1	17.8	14.7	*	**		*	*		**		
Pod number	188	184	188	155	151	169	165	153	185	195	163	163									
Pod walls (g DM)[b]	10.0	9.7	9.8	8.8	8.4	8.9	9.5	8.3	10.2	10.8	9.7	8.5					*				
Seed mass (g DM)[b]	12.7	12.1	13.2	11.7	10.3	11.2	12.0	9.7	12.4	13.2	13.6	10.8				*	*		*		
Shoot mass (g DM)	46.6	43.1	47.8	45.3	40.2	43.0	47.7	42.2	49.3	48.7	51.3	45.6		*			*				
Harvest index	0.27	0.28	0.28	0.26	0.26	0.26	0.25	0.23	0.25	0.27	0.27	0.24	***	***		***	*		***	**	
Thousand seed mass (g)	2.9	2.8	3.2	3.0	3.1	3.3	3.1	2.9	2.8	3.2	3.5	3.0							*		
Seed oil contents (%)	50.2	53.3	53.5	50.5	52.6	52.7	53.6	51.6	50.2	53.0	54.3	50.4	**			***		*	***		
Oil yields (g plant⁻¹)	6.4	6.5	7.1	5.9	5.4	5.9	6.4	5.0	6.2	7.0	7.1	5.4		*		*	*		**		
Seed N concentrations (%)	2.3	2.2	2.0	2.4	2.1	2.2	2.0	2.3	2.2	2.2	1.9	2.3	***			***		*	***		
NUE$_S$ (g seed g⁻¹ available N)	16.8	16.0	17.5	15.4	13.6	14.8	15.9	12.9	16.4	17.5	18.1	14.3		*		*	*		*		
NUE$_O$ (g oil g⁻¹ available N)	8.4	8.6	9.4	7.8	7.2	7.8	8.5	6.6	8.3	9.3	9.4	7.18		*		*	*		**		

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