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Impact of herbivory and pollination on performance and competitive ability of oilseed rape transformed for pollen beetle resistance

Inger Åhman[1*], Anna Lehrman[2], Barbara Ekbom[2]

[1]Faculty of Landscape Planning, Horticulture and Agricultural Science, Swedish

University of Agricultural Sciences, P.O. Box 104, SE 230 53 Alnarp, Sweden,

[2]Department of Ecology, Swedish University of Agricultural Sciences, P.O. Box

7044, SE 750 07 Uppsala, Sweden

*Author for correspondence.

E-mail: inger.ahman@ltj.slu.se

Phone: +46 40 415240, Fax: +46 40 462166

Abstract

Competitive ability of transgenic oilseed rape transformed with a pea lectin gene was estimated by comparisons of its performance when grown in a mixture with its non-transgenic counterpart and when grown singly, with and without pollen beetles present. The experiments were carried out in cages, once with bumblebees as pollinators and once without. In the absence of herbivory but with the presence of bumblebees, singly grown plant lines without lectin generally performed better than lines with lectin. Pollen beetles affected plant growth and reproduction, but there were no consistent differences between the lectin and non-lectin plant lines indicating that the transgenic trait did not protect plants from pest attack. Herbivory reduced the number of seeds when bumblebees were present. In the absence of bumblebees, however, plants produced more seeds with pollen beetles than without, indicating that some pollination was carried out by the beetles. Efficient pollination affected the competitive abilities of the lines; lectin lines were more competitive with bumblebees present and the reverse was true when bumblebees were absent. In the presence of bumblebees, lectin lines gained from being grown mixed with its non-transgenic counterpart. Because the transgenic plants expressed pea lectin in developing pollen it is possible that pollen quality in those plants was reduced, which may explain why the lectin lines had an advantage over non-lectin lines when exchange of pollen between the two plant types was facilitated by bumblebees.

Introduction

Brassica oilseeds are important sources of vegetable oil used for food, lubricants, and increasingly also as a substitute for fossil fuels (Dusser 2007). Unfortunately, oilseed cultivation is limited by insect pest problems (Lamb 1989; Ekbom 1995; Alford et al. 2003), which are primarily solved by insecticide applications. The key pests differ between geographic regions but many of them are coleopterans such as flea beetles, weevils, and pollen beetles. In northern Europe, pollen beetles, particularly *Meligethes aeneus* Fab. (Coleoptera, Nitidulidae), are severe pests.

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Host plant resistance is one alternative to pesticide use. However, traditional breeding using rapeseed relatives as resistance sources has not shown much promise with respect to plant resistance to *M. aeneus* (Åhman 1993). Transgenic resistance offers new opportunities for plant breeding, with *Bacillus thuringiensis* (Bt) toxins as a successful example. Genes for Bt toxins have been transformed into several crops that are grown commercially (Shelton et al. 2002). A Bt toxin that affects lepidopteran insects; for example the diamondback moth, *Plutella xylostella* L., and the cabbage looper, *Trichoplusia ni* Hubner; has been introduced into oilseed rape by gene transformation (Stewart et al. 1996; Halfhill et al. 2005). Other types of resistance to insects have also been transformed into *Brassica* oilseeds, for example proteinase inhibitors which have been tested against coleopterans and lepidopterans in *Brassica napus* L. (Bonadé-Bottino 1993; Bonadé-Bottino et al. 1999; De Leo et al. 2001), and lectin from onion (*Allium cepa* L.) expressed in transgenic Indian mustard, *Brassica juncea* (L.) Czern., for the purpose of controlling mustard aphid, *Lipaphis erysimi* (Kaltenbach) (Hossain et al. 2006).

For improved resistance to pollen beetles, a pea lectin gene was selected for transformation of *B. napus* (Melander et al. 2003). The transgenic plants expressing this gene in developing pollen reduced larval growth (Melander et al. 2003), juvenile survival rate (Lehrman et al. 2008), and egg size in *M. aeneus* (Lehrman et al. 2007). No cultivars of oilseed rape with any of these transgenic insect resistance traits are currently grown commercially. One reason is that there might be environmental risks with such crops, which need to be addressed before the transgenic crops are adopted in agriculture (Wolfenberger and Phifer 2000; Dale et al. 2002; Conner et al. 2003; Sanvido et al. 2006).

Although not exclusive for transgenic plants, one of the anticipated risks with transgenic resistance to herbivores is that the introduced trait will confer a

competitive advantage. This might result in displacement of other plants in natural plant communities and increased weed problems in agriculture. *B. napus* is a common crop in temperate regions and can interbreed with some other sympatric wild plant species, among them the wild type of turnip rape, *Brassica rapa* L. (Jörgensen and Andersen 1994; Scheffler and Dale 1994). Both species occur outside farmed fields on disturbed land (Pessel et al. 2001) and as weeds on agricultural land (Lutman 1993; Jörgensen and Andersen 1994). However, for the spread of a transgene, natural selection is far more important than rate of gene flow (Chapman and Burke 2006; Hails and Morley 2005). It is therefore important to study whether there is a competitive advantage for rapeseed plants with improved resistance to pests. Stewart et al. (1997) studied Bt oilseed rape and the diamondback moth and found increased fitness in the transgenic plants. Generally this moth is less severe as a pest compared to the pollen beetle, for which up to 70% seed yield reductions in spring oilseed rape have been reported (Nilsson 1987). Adult pollen beetles feed not only on pollen but also on flower buds. Such feeding causes bud abortions leading to stalks without pods (silique), though podless or blind stalks also occur in the absence of beetles (Williams 1978). Other effects of pollen beetle feeding are stunted plants and lost apical dominance, resulting in side shoot development and pod production shifting from main to side shoots (Tatchell 1983; Nilsson 1988; Åhman 1993). This results in uneven seed maturation and reduced seed production (Nilsson 1987). Pollen beetle larvae mainly feed on developing pollen and they normally cause less severe damage than adults (Williams and Free 1978).

In order to study the efficacy of the pea lectin as a pollen beetle resistance factor and assess other effects of the transgenic character, non-segregating lines of both lectin-containing and lines free of this lectin were developed and evaluated (Åhman et al. 2006). Two high-lectin lines, one intermediate-level and two non-lectin lines were chosen for our analyses. These lines were selected to be similar

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with respect to plant phenology and seed yields. The present study addresses the following questions:

1. Do the lectin and non-lectin plant lines grow and reproduce equally well, in the absence of pollen beetles?
2. How do pollen beetles influence plant growth and reproduction?
3. Does pollen beetle herbivory influence lectin and non-lectin plants differently?
4. Do plant types differ in their performance when grown on their own compared with when grown in mixtures of one lectin and one non-lectin line?
5. Are the competitive abilities of lectin and non-lectin plant lines different, with and without pollen beetles?

Although *B. napus* is capable of self-pollination, in still air and in the absence of pollinators, seed set is lower than when wind and insects are present (Free 1993). Because our experiments were carried out in cages in greenhouses, auto-pollination would be expected to be low. Therefore two experiments were performed, one with and one without pollinators (bumblebees of the species *Bombus terrestris* L.).

Materials and Methods

Plant lines

Three transgenic lines and two non-transgenic lines of spring oilseed rape (*B. napus,* cv. Westar) were tested. The anthers of the transgenic lines T-17 and T-132 contained 0.6 and 0.7% lectin respectively (of total soluble protein) and T-83 0.2%. The two non-lectin lines, C-101 and C-112, had lost their lectin genes via segregation in an earlier generation and subsequently did not contain any pea lectin (Lehrman et al. 2007). All the lines were produced from microspores by doubled haploid technique (Åhman et al. 2006). The plants used in our

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experiments were offspring from the third generation of doubled haploid plants, selfed in each propagation stage. Experimental seeds were produced the year before each experiment in separate batches, but with seeds from the same mother plant.

Experimental design

Two cage experiments were performed from beginning of May to late August, one in 2004 and one in 2005. The experimental setup was the same in both years except for the addition of bumblebees in 2005. Two drones per cage were released on five occasions, distributed over 16 days during flowering. Pollinators were purchased from Econova Predator AB.

In each year there were 66 plant cages; 11 in each of six greenhouse chambers. The chambers had cooling facilities keeping the temperature between 18 and 22°C, and were located in pairs in each of three greenhouses. In each pair, one chamber was designated for cages with pollen beetles and one without. In each chamber, the cages were positioned on two 6.7 m long tables, five cages on one and six on the other with 30 cm between cages. The 11 treatments included five cages with each of the five plant lines grown singly and six cages where each of the three transgenic lines (with lectin) were mixed with each of the two control lines (without lectin). In the mixtures, one lectin and one non-lectin line were alternated in every other plant position. For each greenhouse, the position of the 11 treatments was randomized, but within a greenhouse the treatments (with and without beetles) had the same layout in the two chambers.

Cage setup

Test plants were grown from seeds in soil beds (70 x 70 cm), 60 l of Weibulls planting soil "Kronmull" with 10% Leca (Light Expanded Clay Aggregate); N-

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P-K 180-90-195 g m^{-3}) in 7 rows of 12 plants per bed. A mould with pegs was used to make holes in which the seeds were placed. Two seeds were sown at each plant position and the plant stand was thinned at cotyledon stage to obtain a single plant in each position. Cages consisted of metal frames (70 x 70 x 120 cm) covered by Econet T (0.15 x 0.35 mm mesh size, 85% direct and 79% diffuse light transmission, 40% ventilation reduction; www.ludvigsvensson.com). The bottoms of the cages were sealed with soil. A "sleeve" (24 x 50 cm) in the front was used for inspection and management of the cages. Plants were watered through the net when required.

Pollen beetles were collected outdoors on spring flowers, or from oilseed rape, soon after leaving overwintering sites, and kept cool in containers with moist sand until release in the cages. *Meligethes aeneus* is the predominating pollen beetle species in this geographical region (Nilsson 1988) and species determination in parallel studies (Lehrman et al. 2007; Lehrman et al. 2008) indicate that no other *Meligethes* species was used in the present study. To mimic a natural successive invasion of beetles, they were introduced twice; first 89 beetles per cage and then another 56 beetles one week later when the first flowers had opened. This level of 1.7 beetles per plant is within the recommended economic threshold for insecticide application on spring oilseed rape at intermediate to late bud stage in Sweden (1-3 beetles) (Anon. 2007). When the new generation of adult pollen beetles began to emerge from the soil, the cage walls were lifted to half the cage height and yellow sticky traps were used to catch beetles in the chamber. This was to reduce the probability that new-generation beetles might feed on test plants. In addition, soil treatments with *Steinernema feltiae* nematodes to control sciarids may have reduced the number of emerging beetles. We used *Aphidius colemani* wasps to control aphids and *Amblyseius cucumeris* mites to control thrips. Biological control agents were purchased from Econova Predator AB.

When most of the pods were ripe, but before the seeds on the main stem started to shed, the central 18 plants in each cage (three rows of six plants) were cut at the base and placed individually in perforated plastic bags. The plants were examined for length of the main stem, number of side shoots. Both the main stem and the side shoots were then examined for number of pods with and without signs of seeds (referred to as "empty" but includes young developing pods) and stalks without pods (blind stalks). From this information we calculated the number of potential pods (= pods with seeds + empty pods + blind stalks), percentage blind stalks, percentage pods and pods with seeds on the main stem, and total number of pods with seeds. Fifteen plant variables were acquired in this way. After air drying and storage at room temperature, seeds were hand threshed, counted and weighed for each plant. Mean number of seeds per pod, mean weight per seed, yield per plant, and yield per plant with seeds could then be calculated. This gave five additional plant variables and a total of 20 measurements per plant.

Germination test

Germination rates of the harvested seeds were tested after 6-7 months storage at room temperature. Seeds from nine plants were pooled to one sample and two samples were collected per cage (from every other plant of the 6 central ones in the three middle rows). In the case of mixed lines, every other plant belonged to the same line and made up one sample. When possible, 100 seeds were randomly selected and tested. In 2004, 68 samples had less than 100 seeds; of these, 22 samples with fewer than 12 seeds were excluded from the test. In 2005, there was only one sample out of 132 where seed numbers were lower than 100 (28 seeds).

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Seeds were germinated in sand (mean particle size 0.5 mm; Fyleverken no. 50)

mixed with water (2 l of water per 25 kg sand). The seeds were placed evenly on

a layer of ca. 1 cm (0.25 l) compressed sand in a plastic dish (height 4 cm,

diameter 18 cm). Seeds were covered with another 0.25 l of compressed sand

and the dish was enclosed in a plastic bag to maintain moisture. The dishes were

placed on a greenhouse table (temperature 20-25°C; daylight supplemented with

400W HQIE lamps 18 h per day) in six blocks representing the six greenhouse

chambers from which seeds were harvested. Daily recordings of emerging

seedlings started three days after sowing and ended after 10 days. At each

recording time the dishes were moved one step to reduce position effects.

Proportion of germinated seeds and days when 50 and 75% of the seeds had

germinated were recorded. An estimate of plant line fitness was calculated as

viable seeds per plant (percent germination x mean no. of seeds per plant). This

variable was used when calculating an RCC-index (relative crowding

coefficient) (cf. Harper 1977; Ramachandran et al. 2000) for competition effects

on viable seed production. The index for transgenic lines (T) was calculated as:

no. of viable seeds line T in mix with line C / no. of viable seeds line C in mix with T
no. of viable seeds line T grown singly / no. of viable seeds line C grown singly

and for control lines (C) as:

no. of viable seeds line C in mix with line T / no. of viable seeds line T in mix with C
no. of viable seeds line C grown singly / no. of viable seeds line T grown singly

Statistical analyses

The basic experimental design for the two years was the same, but because

pollinators were added to the cages in 2005 the years were analyzed separately.

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For all 24 plant variables (20 measurements taken at harvest and 4 germination measurements; percentage germination, days to 50% and 70% germination, and number of viable seeds), means based on recordings from the nine selected plants, fewer if plants had died, were calculated from the two samples taken in each cage and used in the subsequent statistical analyses. All data were normally distributed and parametric statistics were applied using SAS statistical package for Windows.

To answer our first question differences in performance between lines with and without lectin were analysed by using data from cages without beetles. First all the plant lines were tested by one factor ANOVA and then data were grouped by plant type (lines with or without pea lectin) and analysed using a *t*-test as there were only two groups. Separate tests were performed for data from cages with one plant line and from cages with two lines.

In order to analyse how pollen beetles affected plants, data for all plant lines grown with and without herbivory in single line cages from 2004 and 2005 were used. Differences between cages with and without pollen beetles were tested using a *t*-test.

A two factor ANOVA using data from single line cages, with plant line and herbivory as factors, was used to test for a possible differential effect of herbivory among the plant lines. Then plant lines exposed to herbivory were compared by a one way ANOVA, and lectin versus non-lectin lines by a *t*-test.

Differences in performance between lines grown singly or in mixture were analysed by using data from cages without beetles (2005 with pollinators) grouped by plant type (lines with or without pea lectin) and analysed using a *t*-test. Separate tests were performed for data from lines with and without lectin.

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Competitive abilities (using the natural logarithm of the index described above) of the lines with and without pollen beetle herbivory were tested in an ANOVA.

Results

Do the lectin and non-lectin plant lines grow and reproduce equally well, in the absence of pollen beetles?

There were no significant differences among plant lines in 2004, but with bumblebees (2005) as pollinators some differences were found between the two groups of lines with and without lectin. On the whole the non-lectin plants performed better than the lectin lines with higher seed weight and yield but with no significant difference in number of viable seeds (Table 1; 1st and 2nd column).

Comparing individual lines, 15 variables showed significant differences between lines in 2005 (data not shown). There were, however, no significant differences among the lectin lines, but only between the two non-lectin lines for 5 of the 15 variables.

How do pollen beetles influence plant growth and reproduction?

Herbivory influenced plant responses, and there were significant differences between cages with and without pollen beetles for several plant growth variables (Table 2). The effects of pollen beetles were somewhat different for the two years. Still, there was a common set of variables that differed significantly in both years; plants exposed to herbivory had lower number of potential and empty pods, lower percent pods and pods with seeds on the main stem and a higher number of side shoots (Table 2).

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More variables showed significant effects of pollen beetles when pollinators were present in 2005 (Table 2); beetle infested plants had a shorter main stem with a lower number of pods with seeds and more blind stalks. Pollen beetle exposed plants also had side shoots with more potential pods, pods with and without seeds and also more blind stalks. Plants in cages with pollen beetles had fewer seeds; though the seed weight was higher their viability was lower.

In 2004, when there was no proper pollinator in the cages, exposure to pollen beetles was positive for some plant performance variables; exposure resulted in higher yield and faster germination (Table 2).

Does pollen beetle herbivory influence lectin and non-lectin plants differently?

Herbivory appeared to influence both plant groups in similar ways. This conclusion is based on the fact that almost no significant interactions between herbivory and plant lines were found for the 24 plant variables investigated. When data from single line cages were analyzed considering both herbivory and plant lines as factors in an ANOVA, no significant interactions were found for 2004. For 2005 there were only four variables that showed significant interactions between herbivory and plant line; pods on the main stem ($p = 0.004$), pods with seeds on the main stem ($p = 0.04$), number of side shoots ($p = 0.02$), and percent blind stalks on side shoots ($p = 0.03$).

Few variables differed significantly among plants lines when exposed to herbivory; for 2004 total number of pods with seeds ($p = 0.04$), total seed weight ($p = 0.005$) and for 2005 number of pods with seeds on the main stem ($p = 0.04$; one factor ANOVA). Between lectin and non-lectin plants the only significant

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difference was a higher number of pods with seeds on the main stem on non-
lectin plants compared to lectin plants (p = 0.03; *t*-test).

Do plant types differ in their performance when grown on their own compared
with when grown in mixtures of one lectin and one non-lectin line?

Plants with no pea lectin generally performed better than those with lectin when
grown by themselves: Number of pods with seeds, both on the main stem and the
side shoots, were significantly higher in the non-lectin plants when compared to
plants with lectin (Table 1; 1st and 2nd column). The same was true for mean seed
weight and yield. Proportion of blind stalks was significantly higher on side
shoots of lectin plants.

In contrast to the treatment with single lines, lectin plants performed better than
non-lectin plants in cages with the mixture of the two; here lectin lines had
significantly more pods with seeds on side shoots and more seeds per pod and
plant than the non-lectin lines (Table 1; 3rd and 4th column). Number of viable
seeds was also higher, but time until 75% germinated seeds was longer.

Lectin plants grown together with non-lectin plants performed better than when
grown in monoculture. When lectin plants grown singly were compared to the
same line grown in mixture, singly grown lectin plants had main stems with
significantly fewer pods with seeds, fewer potential pods, and higher percent
blind stalks and altogether fewer seeds, lower mean seed weight, fewer pods with
seeds, lower seed yield, number of viable seeds, and longer time to 50%
germinated seeds (Table 1; 1st and 3rd column).

Conversely, non-lectin plants performed somewhat better when grown in
monoculture than when mixed with lectin plants. Number of pods with seeds on

side shoots, and number of potential and empty pods on main shoots, was significantly higher when grown singly than in mixture (Table 1; 2nd and 4th column). However, time to 75% germinated seeds was longer in the single line treatment for non-lectin plants.

Are the competitive abilities of lectin and non-lectin plant lines different, with and without pollen beetles?

Competitive ability was analysed using the index for competition effects on viable seed production (RCC-index) as a response variable in a one-factor ANOVA with plant line as the factor. Without herbivory there were no significant differences in competitive abilities among lines for either 2004 ($p = 0.74$) or 2005 ($p = 0.12$). With herbivory opposite results were obtained for the two years. In 2004, when no proper pollinator was present, non-transgenic lines were generally more competitive than transgenic lines ($p = 0.009$) but in 2005, in the presence of bumblebees, transgenics were more competitive than non-transgenics ($p = 0.05$) (Fig. 1).

Discussion

We studied growth and reproduction of transgenic oilseed rape transformed with a pea lectin gene for improved resistance to the pollen beetle. The addition of pea lectin to the plants did not result in any clear advantages compared to non-lectin plants. The results depended to a large extent on pollination regimes, especially when lectin and non-lectin plants were grown together. Lectin plants performed better and were more competitive than non-lectin plants only when non-lectin pollen and an efficient pollinator were present.

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Oilseed rape is normally self-fertile but seed set is favoured by wind and pollinators (Free 1993). It is possible that pollen quality was poorer in lectin lines than in non-lectin lines, which could be a result of the foreign protein expressed in the transgenic oilseed rape pollen. When the two plant types were mixed, bumblebees probably transferred pollen between non-lectin and lectin plants. This appeared to affect the lectin plants positively and the non-lectin ones negatively. Indications of reduced pollen quality in the transgenic plants were also shown by the results from 2004 when no proper pollinator was present; non-lectin lines were more competitive than lectin lines when the beetles were present, probably due to beetle-induced self pollination of the plants as suggested by Burkhardt & von Lengerken (1920). Thus, even though the five lines had been selected to be similar in seed production (Åhman et al. 2006), these extended evaluations indicate that the rapeseed plants with foreign lectin in their pollen have reduced fertilization rate when self-pollinated.

It may also be that lectin in pollen negatively affected the bumblebees so that their pollination was less efficient when there was only lectin-containing pollen present in the cage. However, bumblebee males do not forage for pollen but visit flowers to feed on nectar and to rest (Alford 1975). Nectar of the transgenic lines is very unlikely to contain pea lectin since the lectin gene was regulated by a pollen specific promoter (cf. Hong et al. 1997; Melander et al. 2003) and nectar contains very little protein (Malone and Pham-Delègue 2001). Furthermore, a study on honey bees fed pollen from the same plant lines showed no negative effect of lectin pollen on larval performance (Lehrman 2007).

Relatively few differences between plant lines were found when plants were grown in monoculture. In 2004 when no bumblebees were present there were no differences between plant types when grown without herbivory. On the other hand when pollinators were added in 2005 the non-lectin plants performed

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slightly better than lectin plants in some aspects. However, when exposed to herbivory there was virtually no difference between plant types irrespective of pollination regime.

Yield per plant was much higher in 2005 than in 2004, in addition herbivory was generally more negative for plants in 2005. Although the experiments were performed in two different years and this may have influenced plant growth in general, the experimental setup (including temperature control) and materials were identical in the two years. We therefore believe that the contrasting results are caused mainly by pollination efficiency.

Activities by pollen beetles affected rapeseed growth and reproduction and plants compensated for beetles removing flower buds on the main shoot by production of more side shoots (cf. Tatchell 1983; Nilsson 1988; Åhman 1993) in both years. The lower number of seeds per plant in the presence of pollen beetles in 2005 was compensated for by heavier seeds. Such a relationship between seed numbers and seed size has also been found in several pollination studies (Free 1993). Despite the compensatory abilities of oilseed rape there was evidence of a disadvantage to the plant from pollen beetle activities. The reduced percent germination of seeds harvested from cages with beetles in 2005 suggests that seed quality was poorer in beetle-infested plants, possibly a result of less mature seeds on the side shoots (cf. Nilsson 1987). Also, when fitness was calculated by combining percent germination and seed production, it was significantly lower among plants exposed to beetles.

The transgenic lines tested here were developed with the aim of producing oilseed rape resistant to pollen beetles, and it has been shown previously that pea lectin lines reduce net reproductive rate of this rapeseed pest, mainly by affecting larval survival to adult stage (Lehrman et al. 2008). In the present study we

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found no evidence for the transgenic lectin protecting the plants from pollen beetle damage, something which is essential for the economic value to the farmer who grows the crop. On the other hand if such crops were grown on a large scale, the introduced trait might reduce the beetle population attacking the crop the following season. In the absence of herbivory non-lectin plants generally performed better than lectin plants and herbivory influenced all plants in a similar manner. Lectin containing plants only showed a competitive advantage when pollen beetles were present and the probability for cross pollination with non-lectin plants was as high as with lectin-plants. In a field with such a transgenic variety we would expect the probability of pollination with pollen from non-lectin plants to be very low. Because lectin plant performance is only enhanced under a special set of circumstances, we conclude that the invasiveness of lectin plants would not be higher than their non-transgenic counterparts.

Acknowledgements

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Table 1. Growth and reproduction as influenced by presence/absence of lectin and plants grown singly or in mixtures. Means (± standard errors) of variables with significant differences for plants not exposed to pollen beetle herbivory in 2005.

No herbivory - with pollinators

Variable	One plant line			Two plant lines		
	Lectin	No lectin		Lectin	No lectin	
Main stem						
No. of pods with seeds	6.4 ± 0.7^a	10.0 ± 1.0	**	9.4 ± 0.6^b	8.1 ± 0.8	
No. of empty pods	2.5 ± 0.2	3.7 ± 0.4^c	*	2.3 ± 0.2	2.7 ± 0.2^d	
No. of blind stalks	3.9 ± 0.3	5.7 ± 0.7	**	4.8 ± 0.5	4.3 ± 0.4	
% blind stalks	41.9 ± 3.8^a	32.2 ± 1.9		32.3 ± 2.9^b	31.6 ± 2.5	
No. of potential pods	12.9 ± 0.9^a	19.4 ± 1.6^c	***	16.5 ± 1.0^b	15.2 ± 1.1^d	
Side shoots						
No. of pods with seeds	0.7 ± 0.3	2.1 ± 0.7^c	*	1.5 ± 0.5	0.5 ± 0.2^d	*
% blind stalks	67.1 ± 6.0	48.3 ± 5.9	*	58.6 ± 3.9	56.2 ± 2.8	
Seeds						
Total no. of pods with seeds	7.1 ± 0.8^a	11.7 ± 1.6	**	10.9 ± 0.9^b	8.9 ± 0.9	
No. of seeds	115.4 ± 15.8^a	162.7 ± 28.9		180.1 ± 19.3^b	122.4 ± 13.1	*
Mean no. of seeds per pod	14.0 ± 0.8	12.5 ± 1.1		14.8 ± 0.7	12.0 ± 0.6	**
Mean weight per seed (mg)	1.9 ± 0.1^a	2.6 ± 0.2	**	2.3 ± 0.1^b	2.4 ± 0.1	
Yield per plant (g)	0.24 ± 0.04^a	0.40 ± 0.07	*	0.41 ± 0.06^b	0.31 ± 0.04	
Days to 50% germination	4.5 ± 0.1^a	4.3 ± 0.1		4.1 ± 0.1^b	4.2 ± 0.1	
Days to 75% germination	4.8 ± 0.1	4.6 ± 0.1^c		4.7 ± 0.1	4.2 ± 0.1^d	**
No. of viable seeds	109.8 ± 15.4^a	158.9 ± 29.4		174.1 ± 19.4^b	116.9 ± 12.5	*

Significant differences between plants with (T-17, T-83, T-132 pooled) and without lectin (C-101, C-112) are noted as: *t*-test * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Significant differences (*t*-test $p < 0.05$) between plants grown without competition (one plant line) and with competition (two plant lines) are indicated by means in bold text and; a and b for difference between lectin-containing plants, and c and d for difference between non-lectin plants, lectin and non-lectin lines analysed separately.

No significant differences were found for length of main stem, percent seed pods or percent pods on main stem, number of side shoots, number of empty pods on side shoots, number of blind stalks or potential pods on side shoots, yield per plant with seeds (g) or percent germination.

Table 2. The influence of herbivory on plants. Means (± standard errors) of variables for plants exposed to, and protected from pollen beetle herbivory in 2004 and 2005. Data are from all plant lines and single line cages.

Variable	2004 without pollinator			2005 with pollinator		
	Herbivory	**No herbivory**		**Herbivory**	**No herbivory**	
Main stem						
Length (cm)	99.8±1.8	100.6±1.9		**89.3±1.3**	**93.1±1.3**	*
No. of pods with seeds	1.6±0.2	1.5±0.2		**3.8±0.3**	**8.35±0.4**	***
No. of empty pods	**1.9±0.2**	**4.7±0.5**	***	**1.1±0.1**	**2.72±0.1**	***
No. of blind stalks	3.2±0.3	4.6±0.3		**7.9±0.4**	**4.6±0.2**	***
% blind stalks	51.0±2.2	50.8±2.8		**66.0±2.0**	**34.7±1.6**	***
No. of potential pods	**6.7±0.6**	**10.8±0.9**	***	**12.8±0.6**	**15.7±0.6**	***
% seed pods on main stem	**38.1±3.1**	**49.2±3.6**	**	**57.9±2.8**	**93.9±0.9**	***
% pods on main stem	**28.5±2.4**	**40.3±2.9**	**	**55.1±2.7**	**89.6±1.2**	***
Side shoots						
No. of side shoots	**3.9±0.1**	**3.1±0.1**	***	**3.4±0.1**	**1.2±0.1**	***
No. of pods with seeds	2.9±0.3	2.0±0.3		**4.0±0.4**	**1.1±0.2**	***
No. of empty pods	7.9±0.9	7.5±0.8		**2.2±0.2**	**1.0±0.1**	***
No. of blind stalks	5.8±0.6	4.8±0.4		**10.0±0.9**	**2.1±0.3**	***
% blind stalks	35.12±1.7	38.4±2.4		**65.6±1.5**	**56.2±2.8**	**
No. of potential pods	16.5±1.7	14.3±1.4		**16.0±1.5**	**4.3±0.6**	***
Seeds						
Total no. of pods with seeds	4.5±0.4	3.5±0.4		**7.9±0.5**	**9.5±0.5**	**
No. of seeds	**22.1±2.6**	**13.9±2.1**	*	**110.0±7.9**	**143.6±9.7**	**
Mean no. of seeds per pod	**4.4±0.3**	**3.5±0.3**	**	**12.1±0.4**	**13.4±0.4**	*
Mean weight per seed (mg)	3.7±0.1	3.7±0.2		**2.7±0.1**	**2.3±0.1**	***
Yield per plant (g)	**0.08±0.01**	**0.05±0.01**	*	0.30±0.02	0.33±0.03	
Yield per plant with seeds (g)	0.16±0.02	0.13±0.02		0.41±0.03	0.40±0.03	
Days to 50% germination	**4.3±0.1**	**4.6±0.1**	*	4.2±0.1	4.3±0.1	
Days to 75% germination	**5.2±0.1**	**5.7±0.2**	*	4.7±0.1	4.6±0.1	
% germination	69±3.3	75±3.0		**92.3±1.0**	**95. 5±0.6**	**
No. viable seeds	19.0±2.4	14.0±2.2		**103.5±7.7**	**138.2±9.7**	**

Significant differences in *t*-tests between plants with and without herbivory are noted next to the means in bold text for each year; * p < 0.05, ** p < 0.01, *** p < 0.001.
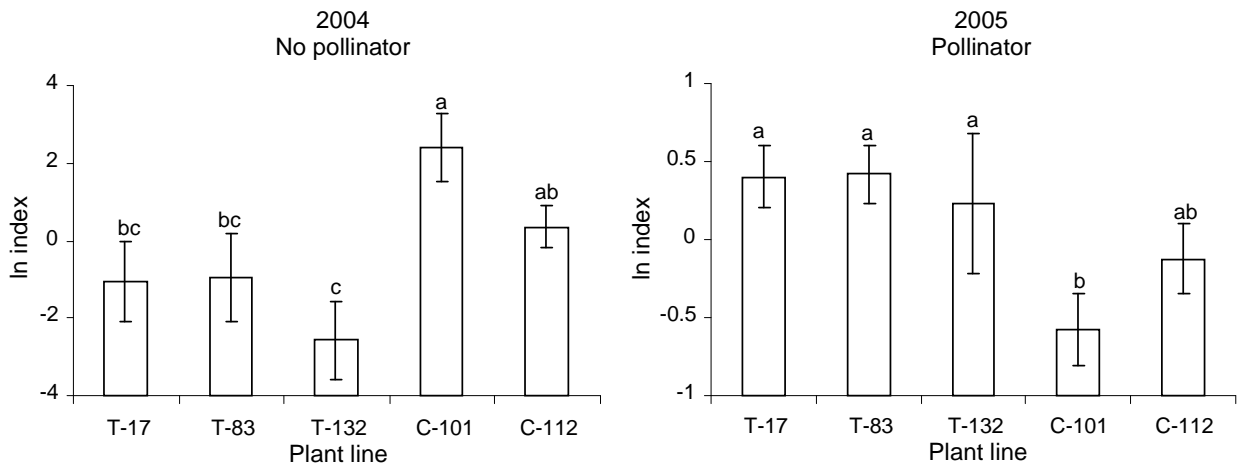
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Figure 1

Figure legend

Fig. 1. Mean competitive abilities of lectin-containing lines (T-17, T-83 and T-132) and control lines without lectin (C-101 and C-112). Competitive ability is measured by an index based on number of viable seeds from T- and C-lines when grown in mixtures in relation to when grown separately (logarithmic values, see text for explanation). The index is calculated using data from 2004 and 2005 with plants exposed to herbivory by pollen beetles, Bumblebees were present as pollinators in 2005. The plant lines are significantly different as shown in an ANOVA (see text). Using a t-test of least squared means (p<0.05) differences were shown among lines. Different letters indicate significant difference between plant lines.