

The presence of *Bt*-transgenic oilseed rape in wild mustard populations affects plant growth

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Received: 17 February 2015 / Accepted: 28 August 2015 / Published online: 3 September 2015
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Abstract The adventitious presence of transgenic plants in wild plant populations is of ecological and regulatory concern, but the consequences of adventitious presence are not well understood. Here, we introduced *Bacillus thuringiensis CryIAc* (*Bt*)-transgenic oilseed rape (*Bt* OSR, *Brassica napus*) with various frequencies into wild mustard (*Brassica juncea*) populations. We sought to better understand the adventitious presence of this transgenic insecticidal crop in a wild-relative plant population. We assessed the factors of competition, resource availability and diamondback moth (*Plutella xylostella*) infestation on plant population dynamics. As expected, *Bt* OSR performed better than wild mustard in mixed populations under herbivore attack in habitats with enough resources, whereas wild mustard had higher fitness when *Bt* OSR was rarer in habitats

with limited resources. Results suggest that the presence of insect-resistant transgenic plants could decrease the growth of wild mustard and *Bt* OSR plants and their populations, especially under high herbivore pressure.

Keywords *Bt* content · Herbivory · Interspecies competition · Oilseed rape · Population yield · Wild mustard

Introduction

Adventitious presence of transgenic crops outside of cultivation is of interest from both ecological and regulatory perspectives (Darmency 1994; Schafer et al. 2011; Marquardt et al. 2012; Ellstrand et al. 2013; Liu et al. 2013b; Santa-Maria et al. 2014), because of the concern that genetically modified (GM) plants might interbreed or outcompete native plants, including GM plants' wild relatives. Numerous studies have focused on gene flow and introgression via pollen dispersal (Stewart et al. 2003; Warwick et al. 2008; Ellstrand et al. 2013; Liu et al. 2013b), which might result, in rare cases, in transgene persistence in the environment.

In addition, transgenes could persist inside or outside agronomic environments due to seed movement during the transport and processing of transgenic crops (D'Hertefeldt et al. 2008; Reuter et al. 2008; Schafer et al. 2011; van Heerwaarden et al. 2012; Liu

Electronic supplementary material The online version of this article (doi:10.1007/s11248-015-9903-7) contains supplementary material, which is available to authorized users.

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et al. 2013b). For example, Devos et al. (2012) observed transgenic oilseed rape (*Brassica napus*) plants at major ports and along roadsides and railways and proposed that these plants were the result of inadvertent transgenic seed spill during transportation. Seed movement can occur through not only space but also time because seed banks of dormant seeds can accumulate for several years in the soil after crop seed loss at harvest. Herbicide-tolerant seedlings of oilseed rape emerged 10 years after a field trial was conducted, which confirmed the long-term persistence of transgenic seeds in the seed bank in Sweden (D’Hertefeldt et al. 2008). Seed-mediated gene flow leads to a much wider diffusion of transgenes than pollen dispersal alone (van Heerwaarden et al. 2012), and escaped populations can contribute to the spread of transgenes outside of cultivation. For example, reports from Canada, Japan, Great Britain, France, Australia and the United States have confirmed the persistence of feral *B. napus* populations or erstwhile non-engineered populations expressing biotech traits (Crawley and Brown 1995; Aono et al. 2006; Warwick et al. 2008; Schafer et al. 2011).

Introgression into landraces and the effect on genetic diversity is also of concern (Kwit et al. 2011; Vargas-Parada 2014). GM plants might have a competitive advantage when fitness-related transgenes (e.g. conferring insect resistance) are expressed (Stewart et al. 1996; Ramachandran et al. 2000; Snow et al. 2003; Vacher et al. 2004; Liu et al. 2013a), and the resistant plants might suppress the growth of susceptible conspecifics or congeners in mixed populations. For example, Ramachandran et al. (2000) found that insect-resistant transgenic *B. napus* had a competitive advantage in mixed stands with susceptible individuals. To better understand transgene-specific effects, most of these studies focused on comparisons between transgenic plants to their non-transgenic isogenic counterparts in the greenhouse and/or field. However, few studies have considered the combined effects of transgene and species interactions to determine the relative competitive abilities between GM plants and their wild relatives, in addition to the dynamics of wild populations with the presence of GM plants through the direct and indirect effects of selection pressures (Crawley and Brown 2004; Londo et al. 2011; Liu et al. 2013a).

Transgenic OSR ranks fourth among all GM crop species in global cultivation area (James 2012). Wild

brown mustard (*Brassica juncea*) is a weedy plant in agricultural systems in China and is closely related to OSR. The population dynamics of wild brown mustard, thus, could potentially be affected by *Bt* transgene introgression. It is likely that *Bt*-transgenic OSR could persist in natural habitats, particularly under conditions of high plant density and high herbivore pressure (Vacher et al. 2004; Damgaard and Kjaer 2009).

Transgene persistence generally depends on complex factors, such as the expression of the transgene, plant characteristics and interactions among plant species, herbivores, and other ecological factors. Our intent for the present study was to simulate the escape of a *Bt*-transgenic crop in a population of its wild relatives. Here we used various percentages of *Bt*-transgenic oilseed rape (*Bt* OSR) in wild brown mustard (*B. juncea*) stands. This study aimed to investigate (1) the effects of *Bt* expression, the target herbivore (*Plutella xylostella*), interspecies competition, and resource availability on the presence of *Bt* OSR in wild mustard populations, (2) the consequences of this presence, including individual plant growth and reproduction of wild mustard and *Bt* OSR and population yield.

Materials and methods

Plants

Seeds of wild brown mustard (*B. juncea*, $2n = 36$, AABB) originating from a local field collection (Nanjing, China) were provided by Prof. S. Qiang, Nanjing Agricultural University (NAU). Oilseed rape (*B. napus* cv. Westar, $2n = 38$, AACC) was previously transformed with a pSAM12 plasmid containing genetically linked *gfp* (encoding a green fluorescent protein) and *Bt CryIAC* cassettes (GT) that were regulated by independent CaMV 35S promoters (Halfhill et al. 2001). Transgenic *B. napus* plants used in the experiments were from an advanced (T4) generation and homozygous for the transgenes.

Greenhouse experiment

Seeds of *Bt*-transgenic OSR and wild brown mustard were sown in Jiffy-7 pots in a greenhouse (natural light, 25–26 °C) on November 18, 2011. Seedlings were transplanted in pots after 1 week. Five plant densities were tested using different sized pots:

diameters and heights of pots ranged from 40 and 36 cm (low density, L), 33 cm and 29 cm (median low density, ML), 30 cm and 27 cm (median density, M), 19 cm and 16 cm (median high density, MH), and 16 cm and 15 cm (high density, H). In each, three *Bt* OSR plants and one mustard plant were arranged on four corners square. Each pot was replicated eight times, so that there were 40 pots in total. One half of the pots were infested with insects and the remainder of the pots had no insect infestation as controls. Five second-instar larvae of diamondback moth (DBM, *P. xylostella* L.) were applied onto each plant on December 20. To prevent insects from escaping, each pot was enclosed by a 0.3 mm mesh nylon net covering each pot to 1.5 m in height. Pots without insects were also similarly enclosed. Pots were arranged randomly, and the positions of pots were changed each week in order to minimize edge effects.

Insects were collected at the pupae stage on January 2, 2012. Several larvae were also found and collected at that time. Then, nylon nets were removed, but the four support poles were left and bound with ropes to keep the density fixed. Damaged leaves of plants under herbivory were assigned to one of three ratings: <5, 5–30 and >30 % of damaged leaf area for *Bt* OSR; <10, 10–60 and >60 % of damaged leaf area for mustard.

Since the collected larvae were progenies and the number was low (17 in total), they were omitted in the statistical analysis. Survival rate per pot was calculated by dividing the sum of collected cocoons by the 20 DBM larvae infested on plants. Dry weight per cocoon was calculated after freeze-drying for 48 h.

The date to first flowering was recorded and plant height at flowering (PHF) was measured. Siliques were harvested simultaneously for both species when most of them were brown and mature, as to mimic the farmers' situation at harvest. The number of siliques, aborted flowers without siliques and the remainder of flowers in bloom were tallied, and the sum of these was calculated as total flower number. Flower abortion rate was calculated by dividing the number of aborted flowers by total flower number. Plant height at harvest time (PHH) was also measured. The number of branches and seeds per plant was calculated, and seed weight per plant was tallied after air drying for 5 days. Above-ground biomass per plant was measured after 48 h in an 80 °C oven.

The concentration of Cry1Ac protein in the insects and leaves of *Bt* OSR was measured using a Bt Cry

1Ab/Ac ELISA (Agidia EnviroLogix, USA) from samples after they were dried at 4 °C for 48 h.

Field experiment

Seeds of *Bt*-transgenic *Bt* OSR and wild brown mustard were sown in Jiffy-7 pots on April 16, 2012, and 2 weeks later, 36 seedlings were transplanted in six rows by six columns per cage (2 m width × 2 m length × 2 m height, protected by a 0.5 mm mesh nylon net). The experiment was performed amongst 30 cages in total laid out in five rows by six columns, with 2 m distance between cages. The 30 cages were grouped into three blocks. Each block included two columns of cages, in which insects were applied to one column of cages, whereas the other column was not infested as a control (Liu et al. 2015a). Two second-instar larvae of DBM were applied onto each plant on May 19. To simulate increasing proportions of insect-resistant plants in a wild population, five different proportions of *Bt* OSR were included in cages in a replacement series: 0 (P₀), 25 % (P₂₅), 50 % (P₅₀), 75 % (P₇₅), and 100 % of *Bt* OSR (P₁₀₀). The five proportions were placed randomly in a column. Plant types (*Bt* OSR vs. mustard) were placed randomly within each cage, while ensuring that the same proportion existed for the 16 plants in the center and for the 20 plants at the border.

Plants were harvested simultaneously for both species when siliques were brown starting from July 20 to 25. The number of branches and seed weight per plant were calculated. Above-ground biomass was collected and oven-dried at 80 °C for 48 h and biomass was tallied per plant. Reproductive allocation was calculated by dividing seed weight by the sum of biomass and seed weight.

Statistical analysis

For the greenhouse experiment, a one-way analysis of variance (ANOVA) was employed to test the difference between insect *Bt*-content and insect weight and survival rate in plant densities. The pot volume at each density was calculated to conduct linear regressions for insect weight and survival rate. A two-way ANOVA ($Y \sim I \times D$) was used to test the effects of insect infestation (I) and density (D) on *Bt*-content of *Bt* OSR and leaf damage ratings of *Bt* OSR and mustard. The average value of three *Bt* OSR plants per pot was calculated before conducting ANOVA. A

three-way ANOVA ($Y \sim I \times D \times T$) was used for the effects of insect infestation, density and plant types (T, OSR vs. mustard) on plant growth and reproduction. Since there were significant difference between the two plant types (see results), a two-way ANOVA was also used to test whether insect infestation and density affected of *Bt* OSR and mustard separately. The mean values of all *Bt* OSR and mustard plant data per cage in the field experiment were used for statistical analysis. Split-plot ANOVA [$Y \sim I \times P \times T + \text{Error}(\text{block}/I/P)$] was employed to test the effects of insect infestation, proportion of *Bt* OSR plants (P) and plant types for the field experiment. The data were *log*-transformed to ensure a normal distribution of residuals whenever appropriate. A Tukey's HSD test was used for multiple comparisons among percentages of *Bt* OSR plants.

For replacement series analysis, the relative crowding coefficient (RCC) was calculated according to the equation in Ramachandran et al. (2000):

$$\text{RCC} = \left(\sum (R_{T_i}/S_{T_i})/3 \right) / (R_{T_{100}}/S_{T_0})$$

where R_{T_i} and S_{T_i} are the biomass for *Bt* OSR and mustard respectively at percentage T_i (T_{25} , T_{50} and T_{75}) per unit area in the plot center. An RCC value of 1 indicates equal competitiveness between OSR and mustard plants. $\text{RCC} > 1$ indicates that *Bt* OSR plants are more competitive than mustard plants and vice versa. A *t* test was performed to determine whether the RCC values significantly deviated from 1.

Biomass and full seed weight data were analyzed using a per-plot mixed ANOVA, with blocks as a random factor and infection and percentage of *Bt* OSR plants as fixed factors, using the sum of the data of all plants per plot, i.e. the whole plot yield. All statistical analyses were conducted in R software (R Development Core Team 2008).

Results

Greenhouse experiment

Insect growth

Thirty-three percent of diamondback moth (*DBM*, *P. xylostella* L.) larvae survived in pots planted with *Bt* OSR and wild mustard. There were significant

differences found among the five densities, and the highest insect survivorship was found in the lowest density (Fig. 1a). Insect weight per capita in low density was higher than in high densities (Fig. 1b). There were in total 132 cocoons collected in all 20 pots and 17 larvae were collected among 12 pots. The cocoons from 75 % of pots (15 out of 20) contained a measureable amount of *Bt* protein, and larvae from 42 % of pots (5 out of 12) contained *Bt* protein. The content of *Bt* protein in larvae ($2.39 \mu\text{g g}^{-1}$ dry weight on average) was significantly higher than in cocoons ($0.09 \mu\text{g g}^{-1}$ dry weight on average). The content of *Bt* protein in cocoons was not significantly different among five densities ($F_{4,15} = 0.79$, $P = 0.55$; Fig. 1c).

Individual plant growth

Significantly more *Bt* protein was found in *Bt* OSR when plants were DBM-infested ($F_{1,30} = 5.65$, $P = 0.024$). There was $4.47 \mu\text{g g}^{-1}$ of *Bt* protein in dry leaves in the absence of insects and $6.41 \mu\text{g g}^{-1}$ of *Bt* protein in dry leaves in the presence of insects. Plant density did not affect the *Bt* content in *Bt* OSR ($F_{4,30} = 1.36$, $P = 0.27$; Fig. 1d).

Mustard plants were severely damaged in DBM treatments, wherein 67 % of leaves were damaged over 60 % of the leaf surface area (Supplementary Fig. 1A). There were more leaves with >60 % damaged area in high density than in low density plots (Supplementary Fig. 1A). *Bt* OSR leaves suffered little defoliation by DBM. Approximately half the leaves were not damaged at all and the damaged leaf area was under 5 % (Supplementary Fig. 1B). Plant density did not impact insect feeding on *Bt* OSR leaves.

The first date of flower for mustard and *Bt* OSR was 64 and 31 days, respectively, in the greenhouse, and the flowering times overlapped over 35 days. Wild mustard had higher fitness parameters than *Bt* OSR (three-way ANOVA, $P < 0.05$; Table 1), except of PHF, RAF and seed weight. There were significant interactions between insects and plant type for PHH, biomass, flower number and floescence date, silique number and seed number (three-way ANOVA, $P < 0.05$). Significant interactions were observed between density and plant type for branch number, PHH, biomass, flower number, silique number and seed number (three-way ANOVA, $P < 0.05$). There

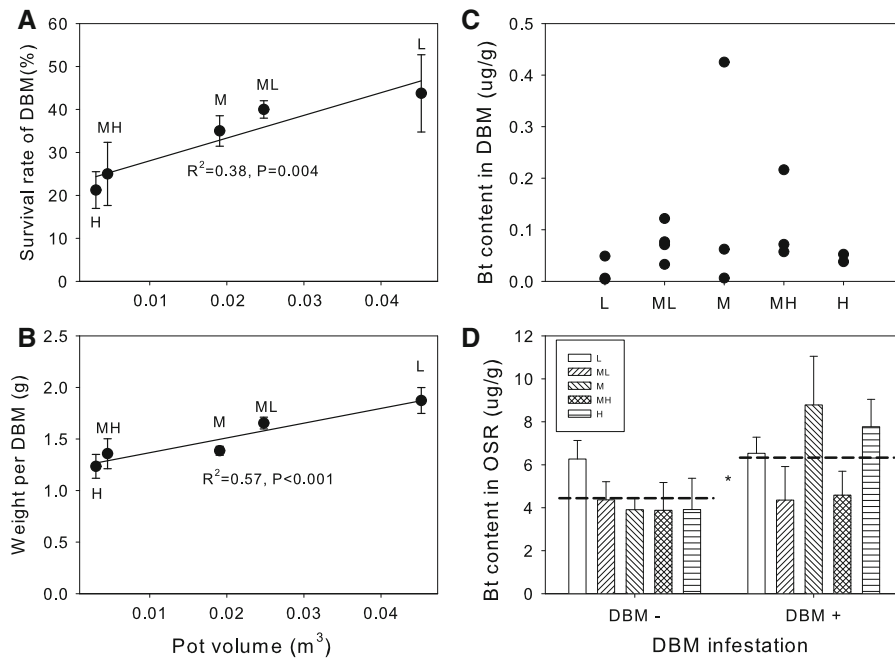


Fig. 1 Diamondback moth (DBM) growth and *Bt*-transgenic oilseed rape (*Bt* OSR) *Bt* content in the greenhouse. **a** Mean survival rate of diamondback moth, **b** mean weight per diamondback moth; *different letters above bars* indicate the significant difference based on HSD test, **c** each point represents the *Bt* Cry content in diamondback moth per pot, **d** *Bt* content in

OSR, *dotted lines* indicate the mean value of the group. *L, ML, M, MH* and *H*, from low to high plant densities with different pot volume. *Asterisk* indicates a significant difference between groups with and without DBM at $P < 0.05$ from two-way ANOVA. *Vertical bars* indicate SE

Table 1 Effects of the presence of *Bt* OSR in wild mustard populations on fitness-related traits in *Bt* OSR in wild mustard plants under no insect (insect−) versus insect pressure (insect+) in the greenhouse

	Mustard		Bt OSR	
	Insect−	Insect+	Insect−	Insect+
Flower date	34.0 ± 2.5	41.8 ± 2.2	58.0 ± 2.3	50.8 ± 3.6
PHF	68.0 ± 4.2	84.6 ± 6.0	73.1 ± 3.3	71.8 ± 5.3
No. of branches	3.5 ± 0.3	3.6 ± 0.4	2.3 ± 0.3	2.9 ± 0.5
No. of flowers	812.4 ± 147.8	552.0 ± 95.0	41.2 ± 14.0	129.4 ± 49.3
PHH	121.4 ± 6.2	115.8 ± 9.2	68.6 ± 10.3	89.7 ± 12.9
Biomass (g)	11.7 ± 1.9	10.0 ± 1.7	2.0 ± 0.4	4.2 ± 1.3
Rate of aborted flowers	63.5 ± 2.6	62.1 ± 2.1	65.7 ± 2.0	70.2 ± 1.9
No. of siliques	246.7 ± 38.2	197.1 ± 37.8	12.9 ± 4.2	33.1 ± 11.9
Seed number	1326 ± 211	1341 ± 274	107 ± 39	223 ± 77
Seed weight (g)	1.1 ± 0.2	1.0 ± 0.2	0.2 ± 0.1	0.5 ± 0.2

Bold numbers indicate significant difference ($P < 0.05$; ANOVA) between insect− and insect+ for mustard or *Bt* OSR respectively *PHF* plant height at first flowering, *PHH* plant height at harvest

was a significant difference between *Bt* OSR and mustard plants in low densities (one-way ANOVA, $P < 0.05$), but the difference was not significant in

high densities ($P > 0.05$). There was no significant interaction for plant characteristics between insect, density and plant type, except for biomass.

Date to first flower was later in herbivore-treated plots for mustard plants, and these plants also produced fewer flowers and less siliques and had increased plant height at florescence (PHF), but PHH, branch number, biomass and the rate of aborted flowers and seed weight was unaffected (Table 1). For *Bt* OSR, insect infestation significantly increased biomass, but had no significant effects on other plant characteristics (Table 1). In high density plots, most plant morphological measurements were significantly less (two-way ANOVA, $P < 0.05$), except for the flowering date and the amount of aborted flowers (two-way ANOVA, $P > 0.05$). The combined effects of herbivory damage and plant density on plant growth and reproduction were additive.

Plant yield by pot

In herbivore treatments there were significant differences observed for whole pot biomass (two-way mixed ANOVA, $F_{1,27} = 5.01$, $P = 0.03$) and seed number ($F_{1,27} = 4.03$, $P = 0.05$), including all *Bt* OSR and mustard plants. The pot yield was significantly different among plant densities ($F_{4,27} = 452$, $P < 0.001$ for total biomass; $F_{4,27} = 22.9$, $P < 0.001$ for total seed number). There were significant linear trends for biomass ($Y \sim 5.36 - 0.77 \times X$, $R^2 = 0.89$, $P < 0.001$ without insects; $Y \sim 5.34 - 0.79 \times X$, $R^2 = 0.89$, $P < 0.001$ with insects; log-transformed) and seed number ($Y \sim 10.22 - 0.85 \times X$, $R^2 = 0.87$, $P < 0.001$ without insects; $Y \sim 11.46 - 1.51 \times X$, $R^2 = 0.64$, $P < 0.001$ with insects; log-transformed) against the plant density, irrespective of the presence of insects. There was no significant interaction between insect and plant density for the total biomass (two-way mixed ANOVA, $F_{4,27} = 0.40$, $P = 0.81$) and seed number (two-way mixed ANOVA, $F_{4,27} = 2.26$, $P = 0.09$).

Field experiment

Individual plant growth

The DBM application treatment did not affect plant branch number, biomass and reproductive allocation of wild mustard, but there was increased seed weight (Table 1). Branch number, biomass, seed weight and reproductive allocation were significantly different among the proportions of *Bt* OSR (Table 2). There

was a significant interaction between insect infestation and the proportion of *Bt* OSR for biomass and seed weight but not for branch number and reproductive allocation. Biomass and seed weight of *Bt* OSR significantly decreased with increasing proportions of *Bt* OSR, irrespective of insects, but not for branch number and RA (Supplementary Table 1; Fig. 2). For mustard, no linear trend was observed, except for seed weight which showed a decreasing trend (Supplementary Table 1; Fig. 2).

Interspecies competition

Bt OSR plants produced more branches, higher biomass and seed weight but lower reproductive allocation than mustard plants did (Table 2; split-plot ANOVA, $P < 0.05$). There was no significant interaction between plant type and insects and proportion of *Bt* OSR for all measured plant characteristics (split-plot ANOVA, $P > 0.05$).

The mean value of RCC was 3.72 and 6.12 in the absence and presence of insects, respectively. The RCC values significantly deviated from 1 ($P < 0.05$, *t* test) for biomass, irrespective of the presence of insects.

Plot yield

The presence of insects did not affect the total biomass of all plants per cage, but there was increased total seed weight in the presence of insects (Supplementary Table 2). The analysis showed significant differences among pure and mixed cages with different proportions of *Bt* OSR. There was significant interaction between insect and proportion for total seed weight, but not for total biomass. Total biomass increased linearly with increasing proportions of *Bt* OSR in the presence of insects ($Y \sim 530 + 158 \times X$, $R^2 = 0.53$, $P = 0.002$), whereas seed weight decreased with the increased proportions of *Bt* OSR in the presence of insects ($Y \sim 239 - 39.2 \times X$, $R^2 = 0.51$, $P = 0.003$; Fig. 3). A contrast test showed that total biomass in cages of pure mustard plants was significantly lower than that in other cages ($t = -5.65$, $P < 0.001$), whereas the total seed weight was higher ($t = 9.28$, $P < 0.001$). There was no linear trend observed for total biomass and seed weight in the absence of insects (Fig. 3).

Table 2 Effects of the presence of *Bt* OSR in wild mustard populations on fitness-related traits in *Bt* OSR in wild mustard plants under no insect (insect−) versus insect pressure (insect+) in the field

	Mustard				Bt OSR			
	No. of branches	Biomass (g)	Seed weight (g)	RA	No. of branches	Biomass (g)	Seed weight (g)	RA
Insect−								
P0	7.0 ± 0.3 ^a	8.4 ± 0.6 ^a	3.0 ± 0.3 ^a	21.7 ± 1.2 ^a				
P25	6.7 ± 0.3 ^a	8.1 ± 0.7 ^a	2.5 ± 0.3 ^a	21.9 ± 1.6 ^a	14.7 ± 0.9 ^a	93.7 ± 10.2 ^a	5.6 ± 1.0 ^a	4.7 ± 0.8 ^a
P50	7.6 ± 1.1 ^a	17.0 ± 5.4 ^a	1.7 ± 0.5 ^a	18.2 ± 3.1 ^a	14.9 ± 0.5 ^a	68.4 ± 5.8 ^{ab}	3.7 ± 0.4 ^a	6.6 ± 1.3 ^a
P75	7.0 ± 0.1 ^a	1.5 ± 0.6 ^b	0.6 ± 0.2 ^b	18.9 ± 0.3 ^a	13.8 ± 0.4 ^a	46.5 ± 2.9 ^{bc}	3.8 ± 0.3 ^a	7.8 ± 0.7 ^a
P100					13.1 ± 0.3 ^a	27.7 ± 1.2 ^c	1.4 ± 0.1 ^b	4.8 ± 0.3 ^a
Insect+								
P0	7.7 ± 0.3 ^a	14.5 ± 1.1 ^a	8.4 ± 0.8 ^a	32.7 ± 1.4 ^a				
P25	6.3 ± 0.3 ^{ab}	5.6 ± 1.0 ^{ab}	2.7 ± 0.4 ^{ab}	31.3 ± 2.2 ^a	16.1 ± 0.4 ^a	106.2 ± 8.2 ^a	6.6 ± 0.9 ^a	5.8 ± 0.9 ^{bc}
P50	4.3 ± 0.7 ^b	7.3 ± 3.4 ^{ab}	1.4 ± 0.6 ^b	17.9 ± 2.8 ^b	13.9 ± 0.5 ^a	55.8 ± 3.7 ^b	3.5 ± 0.6 ^{bc}	6.2 ± 0.9 ^b
P75	4.8 ± 0.7 ^{ab}	4.9 ± 1.4 ^b	2.0 ± 0.6 ^{ab}	33.9 ± 6.3 ^a	15.0 ± 0.3 ^a	44.7 ± 2.7 ^{bc}	4.8 ± 0.4 ^{ab}	10.8 ± 0.8 ^a
P100					14.1 ± 0.3 ^a	33.7 ± 1.5 ^c	1.5 ± 0.2 ^c	3.8 ± 0.3 ^c

Different letters after the means and SE (±) in the same column indicate significant differences according to Tukey’s HSD tests at $P < 0.05$ followed by Bonferroni correction in the same treatment. N = 12 cages

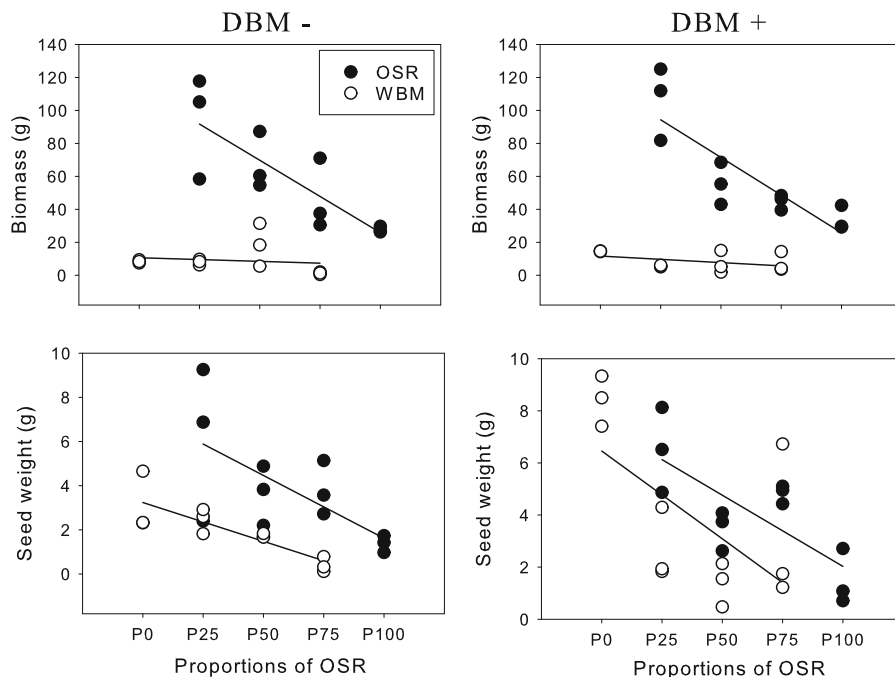


Fig. 2 Trends of biomass and seed weight per plant against the proportions of *Bt* OSR in the field. *WBM* wild brown mustard, *OSR* *Bt*-transgenic oilseed rape; the *left column* labeled *DBM*− indicates the control without diamondback moth; *right column*

labeled *DBM*+ indicates diamondback moth infestation. *Each point* represents the mean value of all OSR or WBM per cage. For parameters of linear regressions please see Supplementary Table 1

Discussion

The adventitious presence of insect-resistant transgenic crops in a wild-relative plant population is one of the major concerns in the biosafety assessment of GM crops. In this study we assessed the consequences of adventitious presence in a model crop-weed system. The simulated presence of *Bt*-transgenic oilseed rape in wild mustard populations had effects on plant growth in this study, which varied with biotic and abiotic factors, including diamondback moth (*P. xylostella*) infestation, inter-plant competition, and resource availability.

Herbivory increased *Bt* expression in transgenic OSR in this study, possibly because DBM feeding on leaves stimulated plants to provide high levels of plant defense that was reflected in increased *Bt* content (Agrawal 1998). The Cry1Ac content in the leaves of

Bt OSR was not affected by artificial damage or feeding of larvae (Wei et al. 2005). The *Bt* content in transgenic plants is important to the growth and survival and resistance evolution of herbivores and also to the survival and fitness of the *Bt*-transgenic plants themselves. Whereas a high dose strategy—high *Bt* Cry protein synthesis—is used in commercial transgenic plants, these experimental *Brassica* plants had various levels of expression (Lei et al. 2011; Liu et al. 2014). In mixtures of *Bt* OSR and mustard plants, one out of three diamondback moths (*P. xylostella*) survived and 75 % of them contained low concentrations of Cry proteins. This result can be attributed to the movement of larvae among *Bt* OSR and mustard plants (Liu et al. 2015b). This indicates that perhaps mixed populations of transgenic plants with non-transgenic plants could delay the evolution of resistance (Carrière et al. 2010). Non-*Bt* refuge plants could significantly decrease the speed of resistance selection to *Bt* toxin protein (Tabashnik et al. 2013; Liu et al. 2014). The concentration of *Bt* toxins in transgenic plants has seasonal changes (Showalter et al. 2009), which can affect the evolution of resistance (Carrière et al. 2010). While it was not studied here, after insects feed on *Bt* plants, the *Bt* protein can be transferred through the food chain to natural enemies (Chen et al. 2014). This might have impacts on the presence of *Bt*-transgenic plants in nature because the prevalence of target insects determines the competition advantage of *Bt* plants.

In addition to the effects of insect damage, competition from neighboring plants is known to have negative effects on growth, reproduction and survival of plants, and they are both believed to have potential effects on population dynamics (Damgaard and Kjaer 2009; Liu et al. 2013a). If the neighboring plants are insect-resistant, the herbivory damage of susceptible plants will be aggregated, and resistant neighbors can usurp the resources and thus suppress growth of the susceptible neighbors. Thus, the relative competitive capacity is influenced by herbivory (Weis and Hochberg 2000; Damgaard and Kjaer 2009). High plant densities decreased the growth and survival of target herbivores in this study, whereas other studies found increased numbers of western corn rootworm in plots of high plant densities than in low densities (Marquardt et al. 2012). The presence of insects decreased the growth of mustard but was beneficial to insect-resistant *Bt* OSR plants, which also affects the

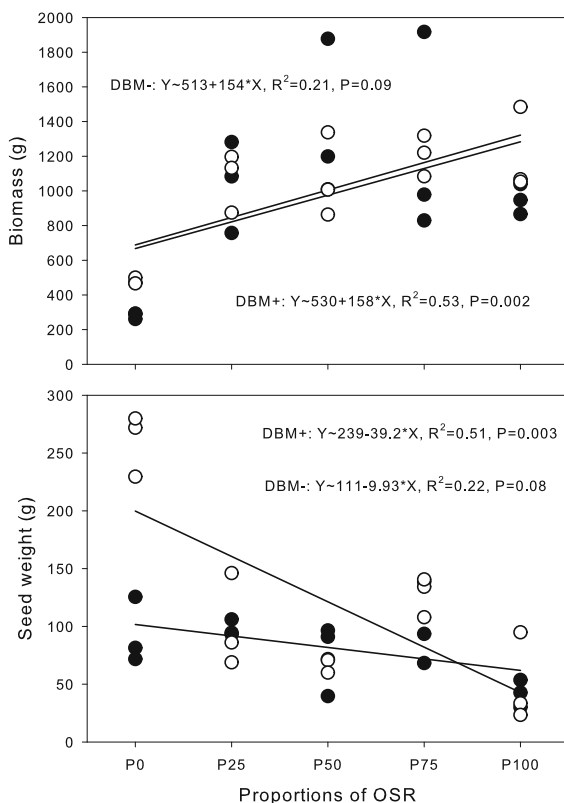


Fig. 3 Trends of total biomass and seed weight per cage against the proportions of *Bt* OSR in the field. White circles represent diamondback moth (DBM+) infestation; black circles represent the treatment without diamondback moth (DBM-). Each point represents the sum value of all plants per cage

competitive interaction between *Bt* OSR and mustard and, subsequently, the presence of *Bt* OSR in nature.

Besides herbivores, resource availability and plant density also had impacts on competitive relationships and on the presence of *Bt* OSR. Wild mustard held a competitive advantage in low plant densities in the greenhouse, but this advantage was not observed at high densities. High densities exacerbated the herbivory damage on mustard plants because of interspecies competition from transgenic OSR. The productivity of *Bt* OSR plants was lower than that of mustard plants in the greenhouse experiment, whereas, in the field experiment, transgenic OSR had higher production than mustard plants did in the mixed plots. This result might arise from resource limitations in the greenhouse study that were not evident in the field. As is the case in many crop-weed systems, mustard plants grow faster and flower earlier than *Bt* OSR and can first use resources, including space and light, thus suppressing *Bt* OSR plants. Therefore, harsh habitats with limited resources would likely be unfavorable for the invasion of transgenic plants, which is contrary to the suggestion by Vacher et al. (2004).

As expected, *Bt*-transgenic OSR was a superior competitor relative to mustard in the field study. When herbivory was simulated by leaf removal of wild mustard, insect-resistant plants had a competitive advantage compared to damaged plants (Liu et al. 2013a). Previous studies focused on the hybridization and introgression of the *Bt* transgene from crops into wild species, and found that insect-resistance in the wild had a fitness advantage under moderate or high herbivory pressure (Stewart et al. 1997; Moon et al. 2007). Hybrid and backcross progeny resulted in less productive populations that had attenuated competitive ability, even when they contained introgressed insect-resistant transgenes (Halfhill et al. 2005). The hypothesis that was posited was that introgressed hybrids suffered genetic load of linked crop-adapted alleles, the expression of transgenes and other ecological factors (Stewart et al. 2003). Here, we studied the direct presence of *Bt* OSR in wild mustard populations, one of the major concerns about the adventitious presence of transgenic seeds in natural habitats, and found it was affected by herbivory and interspecies competition that were interfaced with *Bt* expression, resource availability, plant growth characteristics, and the interaction between plant and insect species.

The presence of *Bt*-transgenic OSR had few impacts on mustard plants in the greenhouse with limited resources, whereas it decreased the growth and reproduction of both OSR and mustard plants in the field, with the increased proportion of *Bt* OSR, irrespective of the presence of insects. High intensity of artificial defoliation led to a similar declining trend with increased proportions of *Bt* OSR, but not for a low intensity of defoliation (Liu et al. 2013a). With the increased proportion of *Bt* OSR in mustard populations, the total seed production, including all transgenic OSR and mustard plants, significantly decreased under herbivore treatments, but fitness was not altered in the absence of insects. Variation of plant productivity determines the kinetics of population growth, because populations with lower relative seed production will be less invasive than the original population and potentially has a lower probability for persistence. Thus, the trend of plot yield in this study suggests the presence of *Bt* OSR would not change invasiveness of the host, which might support the coexistence of transgenic plants with wild species (Hall et al. 2000; Vacher et al. 2004; Hooftman et al. 2007).

This study presented a scenario of the presence of *Bt*-transgenic OSR in wild mustard populations, taking into consideration resource availability, interspecies competition and the target herbivore. It seems, however, impossible to completely understand the consequences of the presence of GM plants in nature, considering complex species interaction and ecological factors, including soil disturbance, seed input, and climatic forces (Crawley and Brown 2004). For example, the time of emergence of crop volunteers sometimes affects their competitive ability and other effects in the field (Marquardt et al. 2012). Insects hosted by crops could lead to considerable damage on neighboring wild plants and vice versa (Meier et al. 2013). It is important to consider the consequences of the presence of GM plants outside of agroecosystems in biosafety assessments of GM plants, especially in the case of crops that might establish feral populations outside of cultivation (Schafer et al. 2011; Ellstrand et al. 2013).

Acknowledgments This work was financially supported by the Natural Science Foundation of China (Grant 31200288), the National Environmental Protection Public Welfare Science and Technology Research Program of China (Grant 201309038) and one Project of the State Key Laboratory of Environmental Criteria and Risk Assessment, CRAES. The work was enabled

by an earlier USDA Biotechnology Risk Assessment grant to CNS.

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