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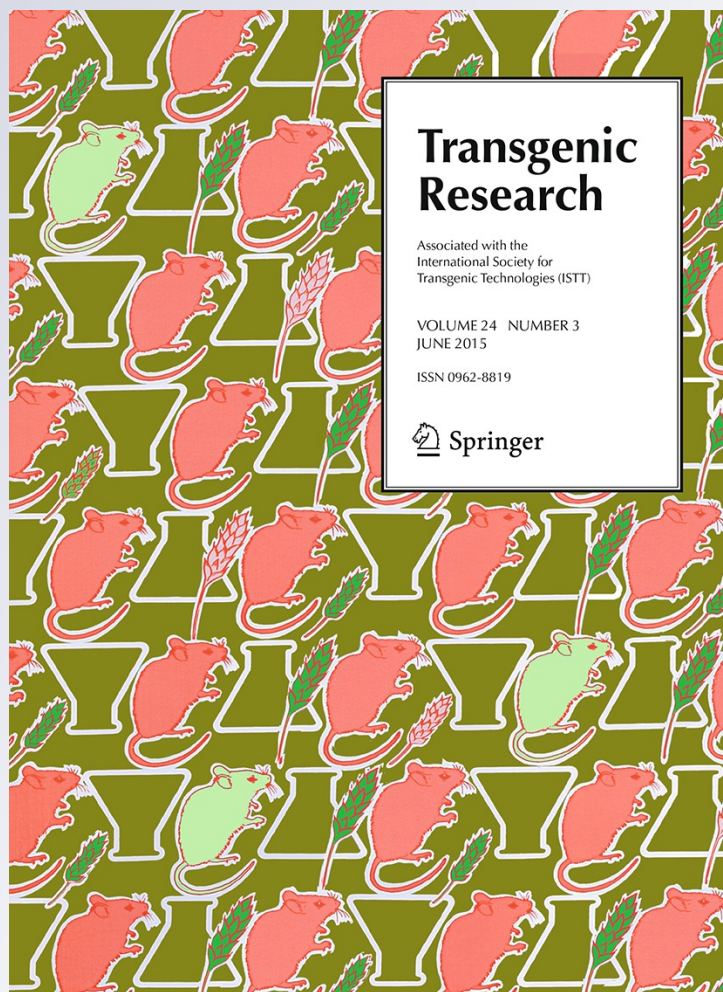
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The effect of *Bt*-transgene introgression on plant growth and reproduction in wild *Brassica juncea*

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Abstract This study aims to investigate the relative plant growth and reproduction of insect-resistant and susceptible plants following the introgression of an insect-resistance *Bt*-transgene from *Brassica napus*, oilseed rape, to wild *Brassica juncea*. The second backcrossed generation (BC₂) from a single backcross family was grown in pure and mixed stands of *Bt*-transgenic and non-transgenic siblings under two insect treatments. Various proportions of *Bt*-transgenic plants were employed in mixed stands to study the interaction between resistant and susceptible plants. In the pure stands, *Bt*-transgenic BC₂ plants performed better than non-transgenic plants with or without insect treatments. In mixed stands, *Bt*-

transgenic BC₂ plants produced fewer seeds than their non-*Bt* counterparts at low proportions of *Bt*-transgenic BC₂ plants in the absence of insects. Reproductive allocation of non-transgenic plants marginally increased with increasing proportions of *Bt*-transgenic plants under herbivore pressure, which resulted in increased total biomass and seed production per stand. The results showed that the growth of non-transgenic plants was protected by *Bt*-transgenic plants under herbivore pressure. The *Bt*-transgene might not be advantageous in mixed stands of backcrossed hybrids; thus transgene introgression would not be facilitated when herbivorous insects are not present. However, a relatively large initial population of *Bt*-transgenic plants might result in transgene persistence when target herbivores are present.

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Introduction

The introgression of transgenes from crops to their uncultivated or weedy relatives should be evaluated as a component of the risk assessment for commercial release of transgenic crops (Darmency 1994; Ellstrand et al. 2013; Kwit et al. 2011; Stewart et al. 2003). Transgenes introgressed from the transgenic crops to their relatives might increase the fitness of transgene-

recipient plants, particularly if transgenes confer selective advantage (Haygood et al. 2003; Mikkelsen et al. 1996; Moon et al. 2007; Snow et al. 1999; Vacher et al. 2004; Wang et al. 2013). Increased fitness effects of transgenes have been shown in several studies on interspecific hybrids and their descendants (Mason et al. 2003; Moon et al. 2007; Snow et al. 1999, 2003). Since insecticide-coding transgenes are assumed to have a selective advantage in nature, these pesticidal plants are appropriate to investigate with regards to their potential impacts on plant populations and communities (Chapman and Burke 2006; Hooftman et al. 2008). However, it does not necessarily follow that altered fitness will affect population dynamics because plant fitness will vary depending upon the environmental context that limits the ability of plants to reproduce. This is particularly the case when considering the presence of inter- and intra-specific competitors, herbivores and pathogens, and abiotic conditions (Crawley and Brown 1995; 2004; Wilkinson and Tepfer 2009). If specific herbivores, for example, are susceptible to control by an insect resistance protein encoded by a transgene, and these defoliating insects have an impact on the fecundity of this plant species, then introgression of this transgenic trait could lead to ecological release of plant populations when they are seed limited (Crawley and Brown 1995, 2004).

Should transgene introgression occur from crops into the genomes of wild relatives, a wild population would be generated that would contain both susceptible and resistant genotypes whose relative frequencies depend on selective advantages of the transgene (Campbell and Snow 2007; Hooftman et al. 2007). The dynamics of populations comprised of mixed resistant and susceptible plants might be complex. Genetic background, resource availability, herbivore presence and the relative abundance of resistant plants have all been demonstrated to affect population dynamics (Verkaar 1987). There have been few studies on the dynamics of such mixed plant populations. Beside intrinsic plant performances when grown alone, we were curious to know whether plant interaction alters reproductive and biomass allocation among mixtures of transgenic and non-transgenic plants. One concern is the possible conversion of a pure susceptible population to a pure resistant population in the absence or presence of insects (e.g. Ramachandran et al. 2000), in which case pure resistant populations might trigger the occurrence

of resistant insects because of the continuous selection pressure in both the crops and wild populations. However, the existence of a stable intermediate stage could be possible for a mixed population if there is some protection of non-transgenic plants by transgenic plants. This protection from transgenic plants is known as the “halo effect” that was predicted on theoretical grounds by Alstad and Andow (1996) and has already been observed (e.g. Ramachandran et al. 1998).

Bt-transgenic oilseed rape (*Brassica napus*), although not yet commercialized, has become a favorite system for studying the ecological consequence of gene flow (e.g. Stewart et al. 2003). Wild mustard (*B. juncea*), a widespread weed in Chinese agricultural system, can easily hybridize with oilseed rape (*B. napus*) and hybrids produce viable progeny (Liu et al. 2010; Song et al. 2010). Although pollen viability was low in F₁ hybrids (28–34 %, Arnold and Hodges 1995; Liu et al. 2010), male fertility recovered to 90 % viability at the first backcross with *B. juncea*, posing increased risk of subsequent gene flow and transmission of the transgene (Arnold and Hodges 1995). In addition, the characteristics of the seeds produced by the hybrid are similar to those of the wild parent in seed shedding, seed size and germinability, which warrants similar dispersal in space and time as for a normal wild plant (Di et al. 2009). Therefore, introgression of the *Bt* transgene could potentially affect the dynamics of wild mustard. In this study, a second backcross generation was obtained between transgenic insect-resistant *B. napus* and wild *B. juncea*, with the latter as the recurrent parent, and was employed to assess whether there was a fitness advantage offered by the *Bt* transgene to wild populations of *B. juncea*. Most experimental studies on fitness effects of transgenes have investigated F₁ hybrids that might display either heterosis, a transient condition that may overestimate the probability of persistence of crop genes within wild populations (Arnold and Hodges 1995; Lexer et al. 2003), or outbreeding depression and near sterility, a transient condition that may underestimate the probability of persistence (Burke and Arnold 2001). Such interacting effects are reduced in advanced generations of backcrossed progeny, which are more appropriate generations to estimate benefits and costs of transgene introgression in wild populations (Campbell and Snow 2007; Hooftman et al. 2008). Thus, we compared transgenic and non-transgenic backcrossed progenies

from the same backcross family in a field experiment with and without herbivore treatments. The main topic in this study is to test whether any interaction between resistant and susceptible plants affect the transgene spread in a population. We manipulated the transgenic plant proportion to assess its effect on individual fitness components and population growth.

Materials and methods

Plants

Seeds of wild brown mustard (*Brassica juncea*, $2n = 36$, AABB) originating from a local field collection (Nanjing, China) were provided by Prof. Sheng Qiang, Nanjing Agricultural University (NAU). Transgenic oilseed rape (*B. napus* cv. Westar, $2n = 38$, AACC) was transformed with a pSAM12 plasmid containing genetically linked *gfp* (encoding a green fluorescent protein) and *Bt Cry1Ac* cassettes (GT) that are regulated by independent CaMV 35S promoters (Halfhill et al. 2001). The third generation of the progenies of a single transgenic event (GT1) contained a single transgene insertion or multiple copies at a single locus (Halfhill et al. 2001) and was used as the backcrossing parent in the form of the pollen donor (Fig. 1) used in this current study. F₁ hybrids were formed via hand crossing of 10 wild mustard plants with the pollen from more than 10 *Bt*-transgenic *B. napus* after emasculating the stamens of the maternal wild plants. BC₁ seeds were obtained through pollinating emasculated flowers of 10 mustard plants by pollen from 20 hybrids. *Bt*-transgenic BC₁ plants were identified by GFP fluorescence (Shen et al.

2006). BC₂ generation was obtained by crossing a single wild mustard and a single *Bt*-transgenic BC₁ plant. This created a single BC₂ family with limited expected genetic variation for the purposes of isolating the effects of the *Bt*-insecticidal transgene and affiliated linkage effects in the *B. juncea* genetic background. These BC₂ plants were screened as *Bt*-transgenic or non-transgenic progenies by the presence of GFP fluorescence (Fig. 1) and denoted as *Bt*-transgenic BC₂ (trBC₂) and non-transgenic BC₂ (ntrBC₂) plants, respectively. The *Bt*-transgenic plants of this BC₂ generation had an average Bt Cry 1Ac protein synthesis level of 741.4 ng g⁻¹ FW at the four-to-five leaf stage ranging upwards to 1,366 ng g⁻¹ FW at flowering stage (Lei et al. 2011).

Experimental design

Since seed size can affect subsequent plant growth in these plants (Wei and Darmency 2008), the selection of homogeneous seed size for experiments was intended to assure a similar initial condition for plant growth in this study. Medium-sized seeds (1.2 mm < seed diameter < 1.6 mm), which was the predominant class of BC₂ seeds, were germinated in a greenhouse in Beijing on 19 April 2010. Seedlings at the four-leaf stage were screened with a GFP fluorescence meter (see Millwood et al. 2003; Shen et al. 2006) and labeled as *Bt*-transgenic BC₂ (trBC₂) or non-transgenic BC₂ (ntrBC₂). In total, 176 and 200 seedlings were detected as trBC₂ and ntrBC₂, respectively, which fits with the expected 1:1 ratio of a backcross ($\chi^2 = 1.53$, $P = 0.21$). The seedlings were then transplanted in cages protected by 0.3 mm mesh nylon-net (0.5 m × 0.5 m width × 1.5 m height) in the experimental field of the Institute of Botany, Chinese Academy of Sciences (Xiangshan, Beijing, China, E116°12', N39°59'). Eight plants were planted on a 0.3 m diameter circle within each cage. The two types of plants (trBC₂ and ntrBC₂) were cultivated in pure stands (0 and 100 % of trBC₂) or in mixtures containing 25, 50, 75 % of trBC₂ (proportions P₀, P₂₅, P₅₀, P₇₅ and P₁₀₀, respectively). In mixed stands, one type of plant was planted beside the other type in the cages of P₅₀, and one beside two or three in the cages of P₂₅ and P₇₅, respectively. The five proportion types were randomly distributed in each of four blocks, which formed totally 40 cages in the field. Within a block, five cages chosen at random served as control without insects and five cages were infested with insects.

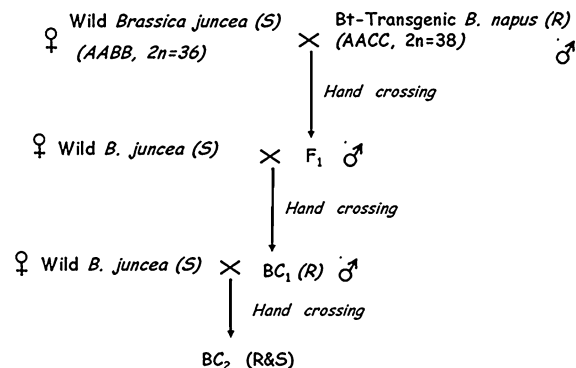


Fig. 1 Plant hybridization scheme for the production of insect-resistance (R) or susceptible (S) plants

Plants were harvested when most of siliques were mature and therefore no seeds were lost. The seeds were harvested by individual plants, wherein for each plant, the siliques were placed in one labeled paper bag and the remaining aboveground biomass was placed in another bag. Seeds were threshed by hand after air drying and the remaining biomass was dried at 80 °C for 48 h. Biomass was weighed for each plant. The seed number per plant was tallied using a seed counter (Contador, Pfeuffer, Germany), and seed mass was weighed. Reproductive allocation per plant was calculated by dividing seed mass by biomass per plant. Thousand-seed weight and reproduction allocation (seed mass/biomass) were estimated.

Herbivory treatment

Various insects have been used to apply herbivore pressure in previous studies using *Bt* transgenic *Brassicac*s including *Helicoverpa armigera* (Le et al. 2007), *Helicoverpa zea* (Halfhill et al. 2002; Ramachandran et al. 1998), *Pieris rapae* (Letourneau and Hagen 2009), *Pieris brassicae* (Damgaard and Kjaer 2009), *Plutella xylostella* (Halfhill et al. 2005; Londo et al. 2011; Mason et al. 2003; Ramachandran et al. 1998, 2000), *Trichoplusia ni* (Vacher et al. 2004) and natural lepidopteran communities (Halfhill et al. 2005; Letourneau and Hagen 2009). Cotton bollworm (*H. armigera*) was used here to mimic the herbivory stress resulting from polyphagous insect pests. *H. armigera* is a pest of major importance in most areas where it occurs, damaging a wide variety of food, fiber, oilseed and horticultural crops, including cabbage in China and mustard in India. Farmers in China have grown oilseed rape near cotton fields to attract bollworm away from cotton fields (Li et al. 1998). Similarly, mustard is used in India as a trap crop to reduce the incidence of *H. armigera* in chickpea (Trivedi and Ahuja 2011). *H. armigera* was reported to be sensitive to transgenic oilseed rape expressing the Cry1Ac insecticidal protein (Le et al. 2007).

Based on preliminary observations on leaf damage from various dosing of larvae per plant, 30 *H. armigera* larvae per plant were applied to confer relevant herbivore stress on the plants. On May 28, when the first flower opened and approximately 25 % of plants had flower buds (81 out of 320 plants), 30 third-instar larvae of cotton bollworm were applied

onto each plant in each of the cages to be infected. Such an advanced stage of development was chosen to avoid the possibility of early-season damage-induced resistance (Agrawal 1999). The larvae are very mobile so that the herbivores moved freely among plants. The larvae were killed after 7 days' incubation by insecticide (4.5 % alphacypermethrin in 1,000-fold dilution, 8 l in total were applied to the 40 cages).

Statistical analysis

The mean values of trBC₂ and ntrBC₂ plants per cage were used for computation. The homogeneity of variances was tested with a Bartlett test. GLM-ANOVA ($Y \sim I + T + P + B + I \times T + I \times P$) was employed to detect the effects of insect (I), plant type (T) and proportion of trBC₂ (P) as fixed factors, and blocks (B, not a studied factor) on plant growth and reproduction. Contrasts were used to detect significant differences between trBC₂ and ntrBC₂ plants according to their proportion (P₀, P₂₅, P₅₀, P₇₅ and P₁₀₀). A linear regression function ($Y = a + b \times X$) was used to analyze the measured variables (Y) in terms of the proportion (X) of trBC₂. The total cage production (biomass and seeds) was then evaluated as the sum of the contributions of all transgenic and non-transgenic plants in each cage and data were fitted to linear regression ($Y = a + b \times X$). All statistics were conducted in R.

Results

Growth and reproduction of trBC₂ and ntrBC₂ plants

Data from the two ntrBC₂ plants in one cage of P₇₅ are missing, and one ntrBC₂ plant in one cage of P₅₀ inexplicably produced 7 times more seeds than the average, so data of those two cages were excluded from the analysis. On the whole, the insect herbivory affected plant biomass but not the number and weight of seeds, which resulted in significantly different reproductive allocation (Table 1). The two plant types, trBC₂ and ntrBC₂, did not differ significantly in any of the plant growth and reproduction characters when all factors were considered (Table 1). The plant type proportion had a marginal effect (P bordering

Table 1 F values of GLM-ANOVA for BC₂ plants characteristics in stands with different proportions (*P*: 0, 25, 50, 75 and 100 %) of plant types (T: trBC₂ and ntrBC₂) under insect and non-insect pressure (I): * *P* < 0.05; ** *P* < 0.01

	<i>df</i>	Plant height	Biomass	Seed number	Seed mass	TSW	RA
Insect (I)	1	2.82	4.67*	0.98	0.17	1.28	10.42**
Plant type (T)	1	0.29	0.37	0.25	0.09	0.21	0.76
Proportion (P)	4	1.68	0.98	1.23	1.31	1.84	2.53
Block	3	1.07	0.94	0.67	0.46	1.43	0.37
I × T	1	0.92	3.08	1.72	1.98	0.01	0.00
I × P	4	0.53	0.46	0.52	1.15	2.86*	1.20
Residual	46						

TSW thousand-seed weight, RA reproductive allocation

0.05) for reproductive allocation. There were no interactions observed between the presence of insects and plant type (I × T). Thousand-seed weight was the only character affected by the interaction between the presence of insects and the proportion of trBC₂ versus ntrBC₂ (I × P).

TrBC₂ plants performed better than ntrBC₂ plants in the cages containing a single plant type, regardless of the insect treatment. TrBC₂ plants produced twofold more seeds, seed mass and reproductive allocation than ntrBC₂ plants in the absence of insects (*P* < 0.05, contrast test, Fig. 2); no significant difference was found in biomass and thousand-seed weight. Under insect stress, trBC₂ plants produced twofold more biomass, 3.4-fold more seeds (*P* < 0.05, contrast test) and 3.0-fold more seed mass than ntrBC₂ plants (*P* = 0.06, contrast test); no difference was found in thousand-seed weight and reproductive allocation (Fig. 2).

Variable results were observed in cages of mixed plant types in the absence of insects, including the case in which significant differences were observed between trBC₂ and ntrBC₂ (at *P*₂₅, *P* < 0.05, Fig. 2). In cages of mixed plant types under insect stress, no difference was observed between trBC₂ and ntrBC₂ (Fig. 2).

Regression analysis showed no significant trend in the no-insect treatment. Under herbivore pressure, the linear regressions of trBC₂ in terms of proportion of trBC₂ plants were not significant (*P* > 0.05). Regression of the seed number ($Y = 954 + 2,000 \times X$, $R^2 = 0.19$, *P* = 0.09), seed mass ($Y = 1.06 + 2.38 \times X$, $R^2 = 0.19$, *P* = 0.09) and reproductive allocation ($Y = 0.21 + 0.19 \times X$, $R^2 = 0.23$, *P* = 0.06, Fig. 2) of ntrBC₂ was marginally significant and tends to show upcoming increased performances.

Productivity per cage

Under insect pressure, the total cage vegetative and reproductive biomass increased linearly with increased proportions of trBC₂ ($Y = 27.1 + 28.4 \times X$, $R^2 = 0.21$, *P* = 0.04 for biomass; $Y = 5,705 + 11,925 \times X$, $R^2 = 0.22$, *P* = 0.04 for seed number, Fig. 3). No such trend was observed in absence of insects.

Discussion

Individual plant performance without selection pressure

The performance of *Bt*-transgenic and non-transgenic BC₂ siblings were tested in pure and mixed stands. The results show increased biomass and seed production in pure stands of *Bt*-transgenic BC₂ in the no-insect treatment compared to the non-transgenic BC₂ plants. Such increased fitness was unexpected since the insecticidal *Bt CryIAc* transgene is neutral under no or low insect pressure, as observed in *Bt*-crops: maize (Ma and Subedi 2005), cotton (McPherson and MacRae 2009) and oilseed rape (Ramachandran et al. 2000). However, a similar trend was observed between *Bt*-transgenic and non-transgenic BC₂ plants backcrossed to wild *B. rapa* using the same line of transgenic *B. napus* used in our study, when grown in pure stands (Halfhill et al. 2005). Linkage effects caused by the introgression of a transgenic event into a block of crop genes could account for this effect, either the result of *B. napus* genes that are well adapted to arable fields or that of gene interactions with those of

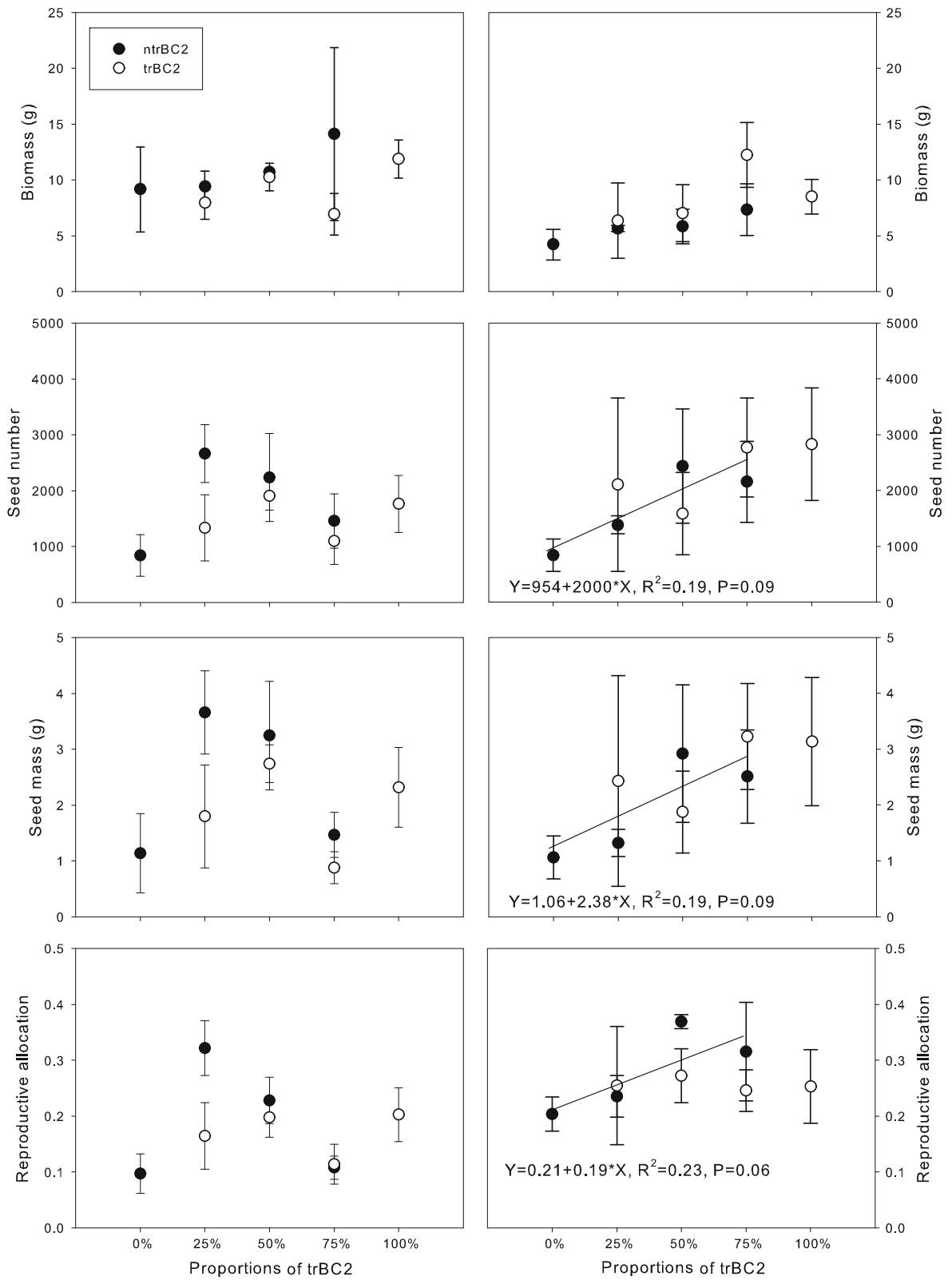


Fig. 2 Plant biomass, seed mass and number and reproductive allocation of *Bt*-transgenic BC₂ (trBC₂; empty symbols) and non-transgenic BC₂ (ntrBC₂; filled symbols) plants at varied proportions of trBC₂ without (left column) and with (right column) insect treatment. Data shown are means from individual plants. Standard errors are indicated by vertical line. Lines are regression equations calculated for ntrBC₂ with $P < 0.10$

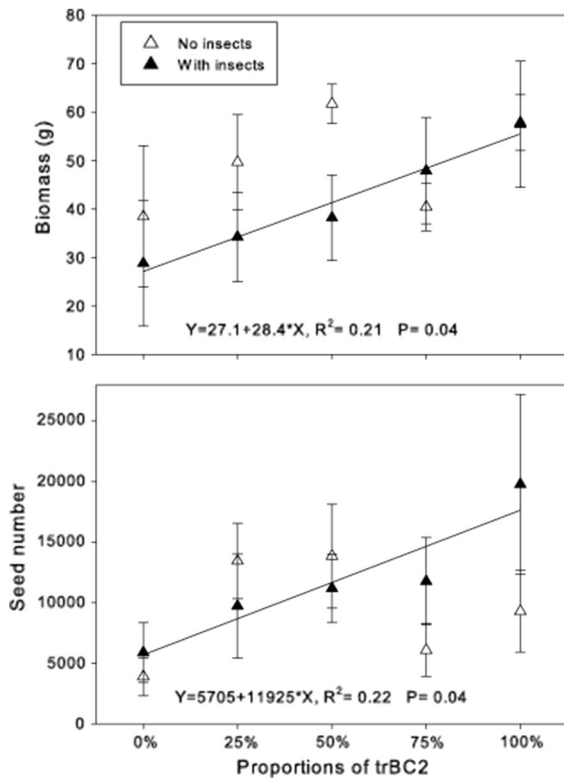


Fig. 3 Total cage productivity with varied proportions of non-transgenic BC₂ (ntrBC₂) and *Bt*-transgenic BC₂ (trBC₂) plants with insect treatment (filled symbols) or no insect treatment (empty symbols). Standard errors are indicated by vertical line. Lines are significant regression equations calculated in presence of insects

the introgressed wild species (Rose et al. 2009; Stewart et al. 2003). Both *Bt*-transgenic and non-transgenic BC₂ siblings shared the similar average genetic background for most chromosomes with the exception of an undetermined block of genes (perhaps hundreds of crop genes) adjacent to the insertion point of the transgenic construct which are not present in the non-transgenic BC₂ plants, which can severely affect plant growth (Uwimana et al. 2012). The possible linkage effect observed here is certainly due to pure chance; such a linkage effect would be different in

plants that had another insertion site. In contrast, the presence of crop genes could make the *Bt*-transgenic BC₂ less competitive when grown alongside more weedy counterparts (Rose et al. 2009; Stewart et al. 2003). The growth of ntrBC₂ plants varied under the absence of herbivory due to the variable competition pressure at different proportion of trBC₂ plants as plant interactions might affect seed production and vegetative growth (Suter 2009). In addition, susceptible plants may perform differently between the absence and presence of selective stress (Finckh and Mundt 1996). Indeed, in mixed stands (P₂₅, P₅₀, P₇₅) without insects, non-transgenic BC₂ had higher biomass and seed production than its *Bt*-transgenic counterpart, although the difference was not always significant. Therefore, the result suggests that, in years without insect attack, the spread of *Bt*-transgenic plants could be decreased by relatively low reproductive values relative to wild counterpart plants. This would contribute to a possible equilibrium between *Bt*-transgenic and non-transgenic plants at certain transgenic proportions in an herbivore-free or a discontinuous selection environment.

Individual plant performances under selection pressure

As expected, the *Bt*-transgenic BC₂ plants performed better than the non-transgenic BC₂ plants in pure stands under moderate-to-high insect pressure (Letourneau and Hagen 2009; Moon et al. 2007), and the difference of biomass and seed number and weight was accentuated with respect to the performance without insects. In contrast the reproductive rate and the individual seed weight did not vary. The productivity of non-transgenic BC₂ showed a marginal trend to increase with proportion of *Bt*-transgenic BC₂ in the mixed stands, which could be the result of two non-exclusive phenomena. Firstly, herbivory could induce plants to modify resource allocation (Miller et al. 2006), and certain studies showed no damaging effects of herbivory because of compensatory growth (Boalt and Lehtilä 2007; Hawkes and Sullivan 2001). Plasticity of reproductive investment of plants could be regarded as an adaptive strategy, which depends on growth conditions (e.g., competition and herbivory) and genotype (Pagán et al. 2009) and plant species effects (Rogers and Siemann 2002). For instance, in a purslane (*Portulaca oleracea*) competition study,

stress conditions increased investment in seed production (Friess and Maillet 1996). This phenomenon could have been a contributing factor in the non-transgenic BC₂ plants doubling their reproductive allocation, resulting in stabilized reproduction.

Secondly, another phenomenon could be the protection of non-transgenic plants by *Bt*-transgenic plants, which is known as the “halo effect,” as observed in other studies (Hutchison et al. 2010; Ramachandran et al. 1998; Wu et al. 2008). The plant density in our study allowed leaves among plants to touch one another, which could enable free movement of caterpillars within the cage, which included larvae movement from non-transgenic to *Bt*-transgenic plants. However caterpillars seldom moved in the opposite direction. After caterpillars feed on *Bt* Cry1Ac toxin-producing plants, their health would be compromised (Ramachandran et al. 1998), which might reduce subsequent damage on non-transgenic BC₂. Although heavily damaged and less damaged leaves were observed on non-*Bt* and *Bt* plants respectively (personal observation), the fitness advantage of transgenic plants might have been offset by the “halo effect” when insects were present, which resulted in little difference of seed production between *Bt*-transgenic BC₂ and non-transgenic BC₂ plants. Consequently, the relative advantage of *Bt*-transgenic plants in a wild habitat would not be fixed, even if insect pressure were constant. Hence, the halo effect would be expected to manage transgene frequency in a wild population up to an equilibrium point and so allow stable co-occurrence of transgenic insect-protected and non-transgenic insect-susceptible plants in the same population.

Cage productivity with varied proportions of *Bt*-transgenic BC₂ plants

The total plant productivity per plot linearly increased with the proportion of *Bt*-transgenic plants under insect stress: both biomass and seed production doubled from 0 to 100 % *Bt*-transgenic BC₂. This suggests that the whole cage population was increasingly protected by *Bt*-transgenic plants as *Bt*-transgene frequency increased, while no such a trend was observed without insect pressure. Although increased “individual fitness” does not necessarily give rise to increased invasiveness (Crawley et al. 1993; Vacher et al. 2004), enhanced seed production could increase

the probability of population spread to neighboring habitats particularly when the population is seed limited (Verkaar 1987). Few studies have focused on the dynamics of insect-resistant plants in susceptible populations, although some studies showed that herbivory strongly affected population dynamics of plants (e.g. Letourneau and Hagen 2009; Palmisano and Fox 1997). In experiments simulating insect-susceptible plants by mechanical wounding, the increase of healthy plants in a population of damaged plants did not increase the population productivity but resulted in a maximum seed output when healthy plants dominated the population, albeit not completely (i.e. 75 % of healthy plants) (Liu et al. 2013), and there was no “halo effect” observed. Although the spread of the insect resistance transgene could be limited within the population because of the halo effect, it is likely to rapidly spread throughout adjacent and connected populations because of the higher population productivity. When herbivorous insects are abundant on large-scale agronomic crop cultivation, the occurrence of such insect infestations is less concentrated in wild habitats, which could also hamper the spread of the insect resistant transgene in and among wild populations, as observed in the case of virus-resistance genes in squash (Fuchs et al. 2004a, b). Similarly, Laughlin et al. (2009) showed that a virus-resistance transgene and conventional crop genes can increase the fecundity of wild plants under virus pressure only.

Although wild mustard easily hybridizes with oilseed rape and produces viable progeny (Liu et al. 2010; Song et al. 2010), BC₁ offspring is seldom produced (e.g. Frello et al. 1995). Selectively advantageous traits in nature under correlated selection should sustain dispersal. In addition, Liu et al. (2010) found that the *B. napus*-like BC₁ plants produced with canola pollen are very likely to be established in the nature, which represents an additional bridge for introgression.

The plant interaction dynamics observed in this present study would not lead to the transgene becoming fixed in the backcross population, and suggests co-occurrence of resistant and susceptible plants might occur in introgressed wild *B. juncea* populations. Hooftman et al. (2007) demonstrated the likelihood of co-occurrence of wild *Lactuca serriola* and crop-wild relative hybrids using a stochastic model, which is, again, more likely when a population is composed of a wide variety of genotypes. *Bt*-transgene introgressed

weedy populations could have higher fitness because insect-resistant crops could reduce the target herbivore pressure in wild populations, but in turn such reduced insect pressure would hamper the spread of insect-resistance transgene in wild populations (Meier et al. 2013; Yang et al. 2011, 2012). The prediction of the co-occurrence of *Bt*-transgenic and non-transgenic plants in the wild should be tested by further comparisons of advanced generations or establishing permanent populations over years in various habitats, and modeling their fate and allele frequencies in wild populations in the field according to the effective estimate of insect pressure both in crop and wild habitats.

In conclusion, if insecticidal transgenes are introgressed into wild species, the co-occurrence of *Bt*-transgenic and non-transgenic plants in the wild would likely occur variably according to habitats, effective insect pressure within crop and wild stands, outcrossing rate, heterosis breakdown of hybrids, relative fitness and proportion of both types of plant (Hooftman et al. 2008). Insect resistance is a likely selective advantage in nature. However, in mixed stands, this advantage might not benefit plants with a *Bt*-transgene since they could provide indirect protection to nearby non-transgenic plants via the halo effect. This effect decreases the likelihood of population conversion to that of pure resistance, i.e., a fixed homozygous *Bt* gene in the population. However, there is a chance that the reproductive effort of the entire population could increase, which might render the hybrids and their descendants to increase their competitive advantage and, perhaps, become troublesome as an agronomic weed or an invader in wild habitats if insect pressure was high.

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