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INTEGRATED PEST MANAGEMENT

Intraspecific Competition of an Insect-Resistant Transgenic Canola in Seed Mixtures

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ABSTRACT

Seed mixtures are recommended as a strategy to minimize or avoid having insects develop resistance to insect-resistant transgenic crops. The objective of this study was to evaluate a canola, *Brassica napus* L., transgenic for a *Bacillus thuringiensis cryIAc* gene for its resistance against diamondback moth, *Plutella xylostella* L., and for its competitive ability with nontransgenic canola in seed mixtures. Transgenic and nontransgenic canola were planted either as pure stands or in mixtures of 75:25, 50:50, and 25:75 in plastic trays in greenhouse experiments or in field experiments at three locations during the 1996 through 1998 field seasons. The trays and plots were either infested with diamondback moth neonates or left without any insect infestation. In diamondback moth-infested treatments, transgenic plants had low levels of damage both as a pure stand and in mixtures. Nontransgenic plants in diamondback moth-infested trays and plots suffered high levels of defoliation and produced less biomass and seed yield compared with transgenic plants. Relative crowding coefficient (RCC), a measure of competition between the two plant types, ranged from 0.6 to 1.1 in plots where there was no diamondback moth infestation and 1.1 to 12.8 in plots where there was diamondback moth infestation. No competitive advantage was observed for either plant type in seed mixtures when there was no diamondback moth infestation. Transgenic canola because of its high level of resistance was competitively superior in seed mixtures when there was diamondback moth infestation.

INSECT-RESISTANT TRANSGENIC CROPS containing different toxin-producing genes from the bacteria, *Bacillus thuringiensis* Berliner, are being cultivated on a large scale. The possibility of insects adapting to these trans-

genic crops is considered a major threat to the durability of these crops (van Rie, 1991; Gould, 1994; McGaughey, 1994). Refugia in the form of susceptible (nontransgenic) plants to prevent exposure of some portion of target insect population to the toxin are currently considered the best strategy to minimize or avoid insect adaptation to transgenic crops (Gould, 1988; McGaughey and Whalon, 1992; Tabashnik, 1994a). Also, some experimental evidence is available suggesting that refugia would delay insect adaptation (Liu and Tabashnik, 1997). Refugia could be provided either by growing susceptible plants in separate sections of the field or adjacent to the resistant (transgenic) plants as seed mixtures.

Seed mixtures are easy to adopt (Hokkanen and Wearing, 1995); however, in a seed mixture there is the possibility that the target insect would initially develop on nontransgenic plants and later move to transgenic plants. Because later growth stages of the target insect may better tolerate the toxin of a transgenic plant, insect movement from nontransgenic to transgenic plants might cause economic damage to the transgenic plants (Mallet and Porter, 1992; Tabashnik, 1994b). Ideally, transgenic plants should not suffer any economic damage from attack by any stage of the target insect. For a seed mixture to be economically successful, transgenic plants in seed mixtures must have levels of insect resistance equivalent to those in pure stands of transgenic plants. The first objective of this study was to evaluate the resistance of transgenic canola, *Brassica napus* L. containing a *cryIAc* gene of *B. thuringiensis* in seed mixtures against the target insect diamondback moth, *Plutella xylostella* L.

It is often argued that crop plants with improved

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resistance would be agronomically less fit because of the pleiotropic effects associated with the resistance genes (Coley et al., 1985; Bergelson and Purrington, 1996). For example, transgenic *Arabidopsis thaliana* plants with resistance to the herbicide, chlorosulfuron, were less productive compared with the nontransgenic plants (Bergelson et al., 1996; Purrington and Bergelson, 1997). This reduced fitness of transgenic plants was attributed to the improved resistance to herbicides rather than to changes associated with plant transformation and regeneration. Furthermore, genetic transformation could affect other characters of the transgenic plant in addition to the targeted character of the transformation process. Negative impacts of the transformation process have been associated with the poor performance of a few transgenic cotton lines (Jenkins et al., 1997). Thus, there are two possibilities that could lower the performance of insect-resistant transgenic plants: (i) metabolic costs associated with the improved resistance, and (ii) negative impact of the transformation process.

Seed mixtures add a new dimension to the use of transgenic plants; will the transgenic plants outcompete nontransgenic plants when grown in seed mixtures? In a recent study, no differences in competitive interactions were noticed when transgenic canola with a herbicide resistance gene was grown in mixtures with nontransgenic counterpart or with barley (Fredshavn et al., 1995). However, intraspecific competition has not been studied for transgenic crops with insect-resistant gene(s), which is of prime importance because of the possibility of using seed mixtures for insect-resistant transgenic crops. Thus, the second objective of this study was to examine intraspecific competition between transgenic and nontransgenic canola plants in seed mixtures with and without insect pressure.

MATERIALS AND METHODS

Plants and Insects

Canola cultivar Oscar transformed with a synthetic *cryIAc* gene (line O52-6; referred to as transgenic or Bt canola) (Stewart et al., 1996) and nontransgenic Oscar (referred to as nontransgenic or NBt canola) were tested for diamondback moth resistance and intraspecific competition in seed mixtures. Transgenic Oscar synthesized 238 ± 29 ng of CryIAc protein per gram of total extractable protein (Ramachandran et al., 1998a) and conferred high levels of resistance against diamondback moth under laboratory conditions (Stewart et al., 1996) and as a pure stand under field conditions (Ramachandran et al., 1998b). Diamondback moth eggs (obtained from Abbott Laboratories, Chicago, IL) were hatched at room temperature, and freshly hatched neonates were used in artificial infestations.

Greenhouse Experiments

A pure stand of transgenic and nontransgenic canola and mixtures of both at 75:25, 50:50, and 25:75 were planted in plastic trays (40 by 25 by 15 cm) filled with Craven's potting soil mix (Commerce, GA). Each tray contained a total of 16 plants in four rows (each row contained four plants) with equal spacing between the plants. Transgenic plants were marked with white stakes for identification purposes. Transgenic and nontransgenic plants were alternated within a row

in 50:50 mixtures. In 25:75 and 75:25 mixtures 25% plant types were planted at one per row. The experiment replicated four times was arranged in a split-plot design with diamondback moth and no diamondback moth infestation as main plots and different ratios of transgenic and nontransgenic plants as the subplots. In insect infestation treatments, 20 d after plant germination each tray was infested with approximately 350 diamondback moth neonates mixed with corn grits with a bazooka applicator (Wiseman et al. 1980). Ten days after insect infestation the percentage defoliation of transgenic and nontransgenic plants was recorded and plants were cut at the base from both insect-infested and uninfested trays. Plants were dried in an oven at 75°C, and final dry weight was measured.

Field Experiments

Field experiments were conducted at the Univ. of Georgia, Horticultural Farm, Watkinsville, during the 1996–1997 season and at the Bledsoe Research Farm, Griffin, GA, and Gibbs Research Farm, Tifton, GA, during the 1997–1998 season. Fields were prepared by chisel plowing followed by disking. Lime and fertilizers were added to achieve a moderate level of fertility. Preemergence herbicide trifluralin (Treflan 4EC) was applied at the rate of 0.56 kg a.i. ha⁻¹. Weeds were removed by hand as they emerged. All other normal agronomic practices for the crop were performed.

Sixteen metal nails (6 cm long) were fixed on to a wooden board (145 by 20 cm) at 15-cm intervals in two rows (15 cm apart). The wooden board laden with nails was pressed firmly in the soil to make 64 holes plot⁻¹ for planting seeds. This method ensured that the plants within each plot were equally spaced. Each hole was hand-planted with two seeds of the required genotype on 10 Oct. 1996 at Watkinsville, on 7 Oct. 1997 at Griffin, and on 18 Nov. 1997 at Tifton. In 50:50 mixture, transgenic and nontransgenic plants were planted alternately within a row. In 25:75 and 75:25 mixtures, 25% plant types were uniformly arranged as every fourth plant. A spacing of 1 m was provided on all sides of each plot to minimize interaction among the plots. Ten days after planting, the plants were thinned to one per hole and gaps were filled with new seeds.

Treatments in the field experiments were similar to greenhouse experiments. In insect-infested plots, each plot was infested 30 d after plant germination with approximately 3000 diamondback moth neonates mixed with corn grits using a bazooka applicator (Wiseman et al., 1980) three times at weekly intervals for a total of 9000 neonates. Two weeks after final infestation, the percent defoliation of transgenic and nontransgenic plants was visually estimated. When the plants attained physiological maturity (pods were golden brown in color), stems were cut at the base and dried in an oven at 75°C. After recording the final biomass of transgenic and nontransgenic plants from each plot, the seed was threshed and cleaned using an air blast seed cleaner (Allan Machine Co., IA), and weight was recorded.

Data Analysis

A *t*-test was performed within each seed mixture to identify differences in the percent defoliation between transgenic and nontransgenic plants. Percent defoliation data from Watkinsville and Griffin trials were combined and a single analysis was performed. An analysis of variance using general linear models procedure was performed to identify significant differences ($P < 0.05$) in biomass and seed production between main plot treatments and also to identify interaction between main plot and subplots (SAS Inst., 1985). When there was a significant interaction between main plot and subplots, the

Table 1. Mean (± SE) percent defoliation of transgenic (Bt) and nontransgenic (NBt) canola plants grown in different mixtures by diamondback moth infestations in the greenhouse, Watkinsville, and Griffin field experiments.

Proportion of Bt: NBt	Defoliation					
	Greenhouse experiment			Mean of Watkinsville and Griffin		
	Bt	NBt	P value	Bt	NBt	P value
	%			%		
0:100	—	38.2 ± 4.3	—	—	22.6 ± 7.1	—
25:75	1.8 ± 0.6	29.5 ± 3.5	0.003	0.6 ± 0.2	17.5 ± 5.5	0.004
50:50	1.8 ± 1.0	33.8 ± 3.8	0.003	0.2 ± 0.1	6.6 ± 3.3	0.016
75:25	1.3 ± 0.8	25.5 ± 3.4	0.009	0.2 ± 0.1	23.9 ± 7.5	0.004
100:0	1.0 ± 0.6	—	—	0.2 ± 0.2	—	—

subplot means were separated using least significant difference (LSD) within each main plot treatment. Because, there were no significant interaction between main plot and subplots in the Tifton trial main plot treatments were combined and a single analysis was performed. Data were analyzed after appropriate transformations.

Competition between two plant species (I and J) within a mixture (50:50) could be identified using the RCC, which is

represented as [(mean production of I at 50:50)/(mean production of J at 50:50)]/[(mean production of I at 0:100)/(mean production of J at 100:0)] (Harper, 1977). An RCC of 1 indicates that there is no competitive advantage for I or J. An RCC > 1 indicates I is more competitive than J and an RCC < 1 indicates J is more competitive than I. The above formula was modified for our analysis as per Novak et al. (1993), to include all mixed plantings from our experiments. The revised formula for RCC was

$$\frac{((\text{var}_{\text{bt}}^{25:75}/\text{var}_{\text{nbt}}^{25:75}) + (\text{var}_{\text{bt}}^{50:50}/\text{var}_{\text{nbt}}^{50:50}) + (\text{var}_{\text{bt}}^{75:25}/\text{var}_{\text{nbt}}^{75:25})/3)}{(\text{var}_{\text{bt}}^{0:100}/\text{var}_{\text{nbt}}^{100:0})}$$

where $\text{var}_{\text{bt}}^{\text{m:n}}$ is the variable (biomass or seed weight) of transgenic plants in m:n mixture and $\text{var}_{\text{nbt}}^{\text{m:n}}$ is the variable (biomass or seed weight) of nontransgenic plants in m:n mixture. RCC values of >1 would indicate transgenic plants are more competitive than nontransgenic plants and an RCC of <1 would indicate the reverse. A *t*-test was performed to identify whether the RCC values were significantly different from 1.

RESULTS

Transgenic canola plants showed high levels of resistance against diamondback moth both as a pure stand and in mixtures with nontransgenic canola. Nontransgenic plants were significantly more defoliated in all mixed stands with transgenic plants. But, no appreciable level of defoliation occurred for either plant type in the Tifton trial (data not presented). Transgenic plants were defoliated <2% in greenhouse and Watkinsville and Griffin field experiments (Table 1).

Significant reduction in total biomass occurred between main plot treatments in the greenhouse (df = 1, 3; *F* = 66.73; *P* < 0.004), Watkinsville (*F* = 9.98, *P* < 0.05), Griffin (*F* = 24.43, *P* < 0.016), and Tifton (*F* = 12.47, *P* < 0.039) trials. Diamondback moth infestation also reduced the seed weight in the Watkinsville (*F* = 9.23, *P* < 0.05) and Griffin (*F* = 13.77, *P* < 0.034) trials. However, no differences were detected in the seed yield between main plot treatments in the Tifton (*F* = 1.42, *P* > 0.32) trial.

Significant main plot and subplot interactions were observed for biomass in greenhouse (df = 4, 24; *F* = 25.98; *P* < 0.001), Watkinsville (*F* = 4.50, *P* < 0.007), Griffin (*F* = 2.78, *P* < 0.05) trials and for seed weight in Watkinsville (*F* = 4.41, *P* < 0.008) trial. No significant main plot and subplot interactions were recorded at Tifton location for either the biomass (*F* = 0.47, *P* > 0.76) or seed weight (*F* = 0.43, *P* > 0.78). Thus, main

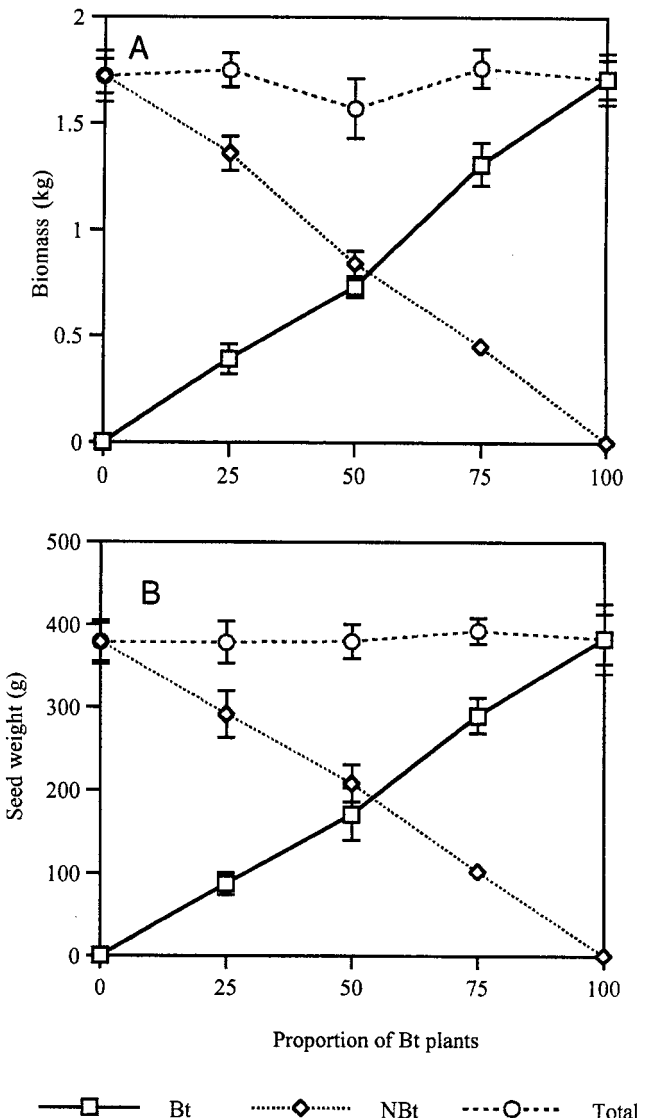


Fig. 1. (A) Biomass and (B) seed production of transgenic and nontransgenic canola plants in different seeded mixtures in a Tifton, GA, experiment.

plot treatments were combined to identify differences among subplots for the Tifton trial. The combined analysis exhibited no significant differences among pure stand and mixtures of transgenic and nontransgenic plants in the biomass production ($df = 4, 32; F = 0.82; P > 0.52$) and seed yield ($F = 0.10, P > 0.98$) (Fig. 1A and 1B).

When there was no diamondback moth infestation, no significant differences occurred in the total biomass among pure and mixed stands of transgenic and nontransgenic plants in the greenhouse, Watkinsville, and Griffin trials (Fig. 2A, 2B, and 2C) and also for seed production at Watkinsville and Griffin trials (Fig. 3A and 3B).

Generally, for insect-infested plots total biomass and seed production increased as the proportion of transgenic plants increased in the mixture. Within diamondback moth-infested plots, biomass production was significantly different among mixed plantings in the greenhouse, Watkinsville, and Griffin experiments (Fig. 4A, 4B, and 4C) and also seed yield in Watkinsville and Griffin trials (Fig. 5A and 5B).

The RCC values were not significantly different from 1 when there was no diamondback moth infestation except for biomass in the Watkinsville trial (Table 2). When diamondback moth caused defoliation (greenhouse, Watkinsville, and Griffin trials), RCC values were significantly greater than 1. Infested plots in the Tifton trial had very little defoliation and RCC values were not significantly greater than 1.

DISCUSSION

The defoliation data from the greenhouse and Watkinsville and Griffin field experiments clearly demonstrate high levels of resistance exhibited by the transgenic canola against diamondback moth both as a pure stand and in mixtures with nontransgenic canola. High level resistance of transgenic canola against diamondback moth in mixtures implies that even if the larvae had moved from nontransgenic to transgenic plants they did not cause great damage to the transgenic plants. In an earlier study, the same transgenic line provided 100% mortality of all larval instars of diamondback moth when fed exclusively on the transgenic line (Ramachandran et al., 1998c). The principle of the seed mixture strategy is that adults emerging from susceptible plants would mate with the adults emerging from the resistant plants thereby the rate of resistance development could be delayed. Although our results suggest that seed mixtures would be feasible from an economic standpoint, it is essential to study the impact of seed mixtures on delaying insect resistance development, the basic purpose of seed mixtures.

Generally, biomass and seed production of both plant types was lower in the Watkinsville trial as compared with the Griffin and Tifton trials. This variation could be attributed to effects of season and location. Low levels of damage to nontransgenic plants in the Tifton trial could be because of intermittent rains after each infestation that might have washed larvae from the

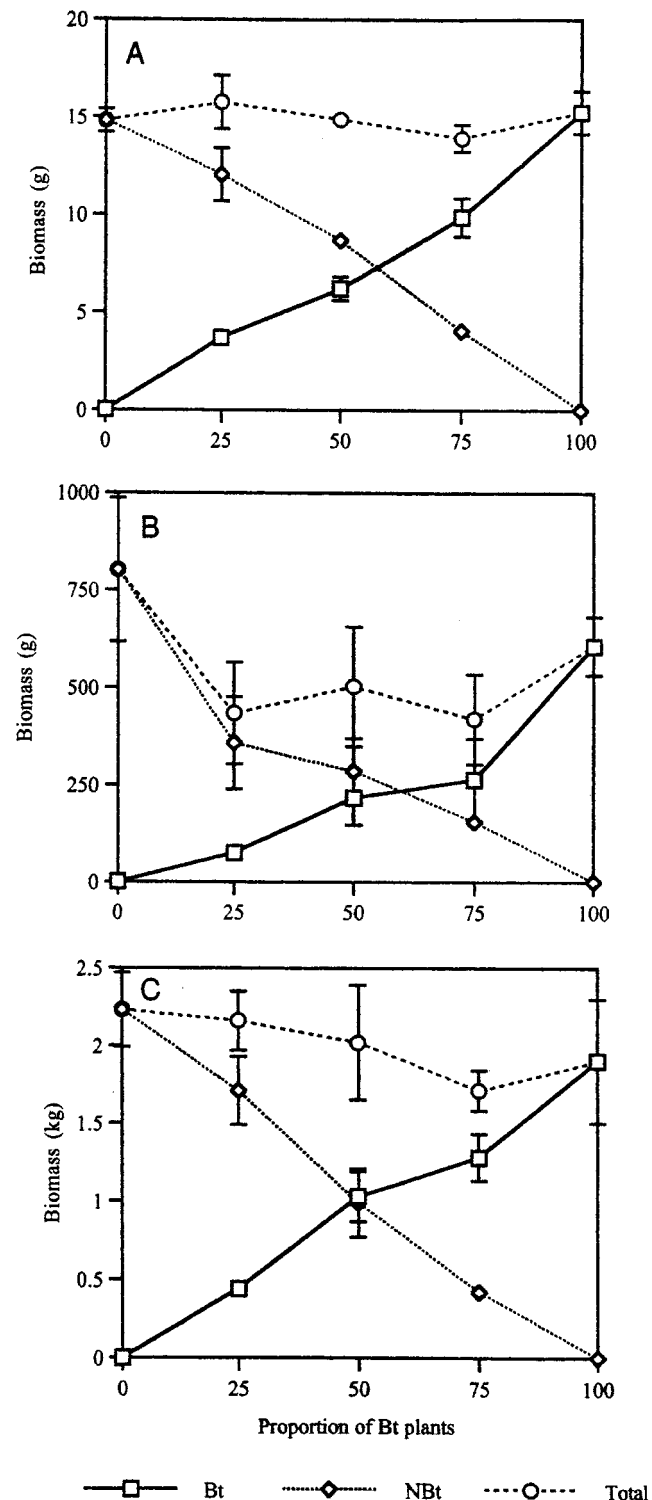


Fig. 2. Biomass production of transgenic and nontransgenic canola plants in different seed mixtures when there was no diamondback moth infestation in (A) greenhouse (B) Watkinsville, GA, and (C) Griffin, GA, experiments.

plants. Furthermore, delayed planting and cold temperatures coinciding with the infestations also contributed to very low levels of damage to nontransgenic plants in the Tifton trial. Significant differences in biomass production and no differences in seed weight occurred

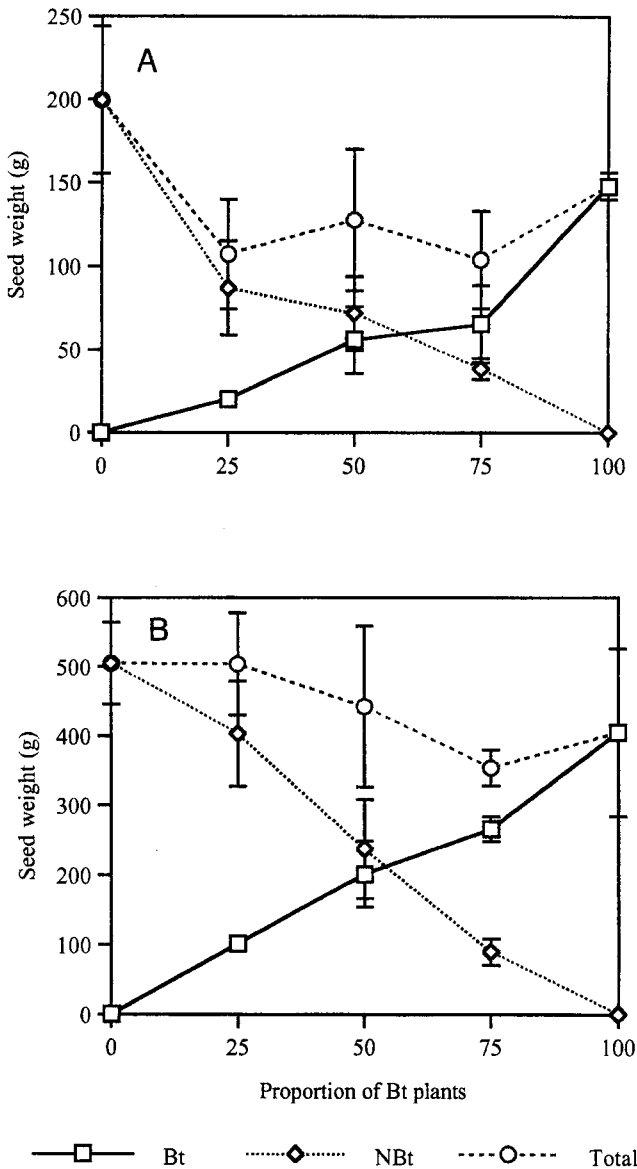


Fig. 3. Seed production of transgenic and nontransgenic canola plants in different seed mixtures when there was no diamondback moth infestation in (A) Watkinsville, GA, and (B) Griffin, GA, experiments.

between plots with and without diamondback moth infestations in the Tifton trial. This result suggests that diamondback larvae were able to damage the plants to some extent to reduce the biomass, but the damage was not sufficient enough to cause reductions in yield.

No differences in biomass and seed weight occurred among mixtures and between pure stands of transgenic and nontransgenic plants in plots where there was no diamondback moth infestation. These results indicate that production of Cry1A(c) toxin in the transgenic line did not measurably impact plant productivity. Also, these results indicate that the process of genetic transformation did not affect agronomic performance of the tested transgenic line. In contrast, *A. thaliana* plants with genetically engineered resistance to the herbicide, chlorosulfuron, produced fewer seeds compared with

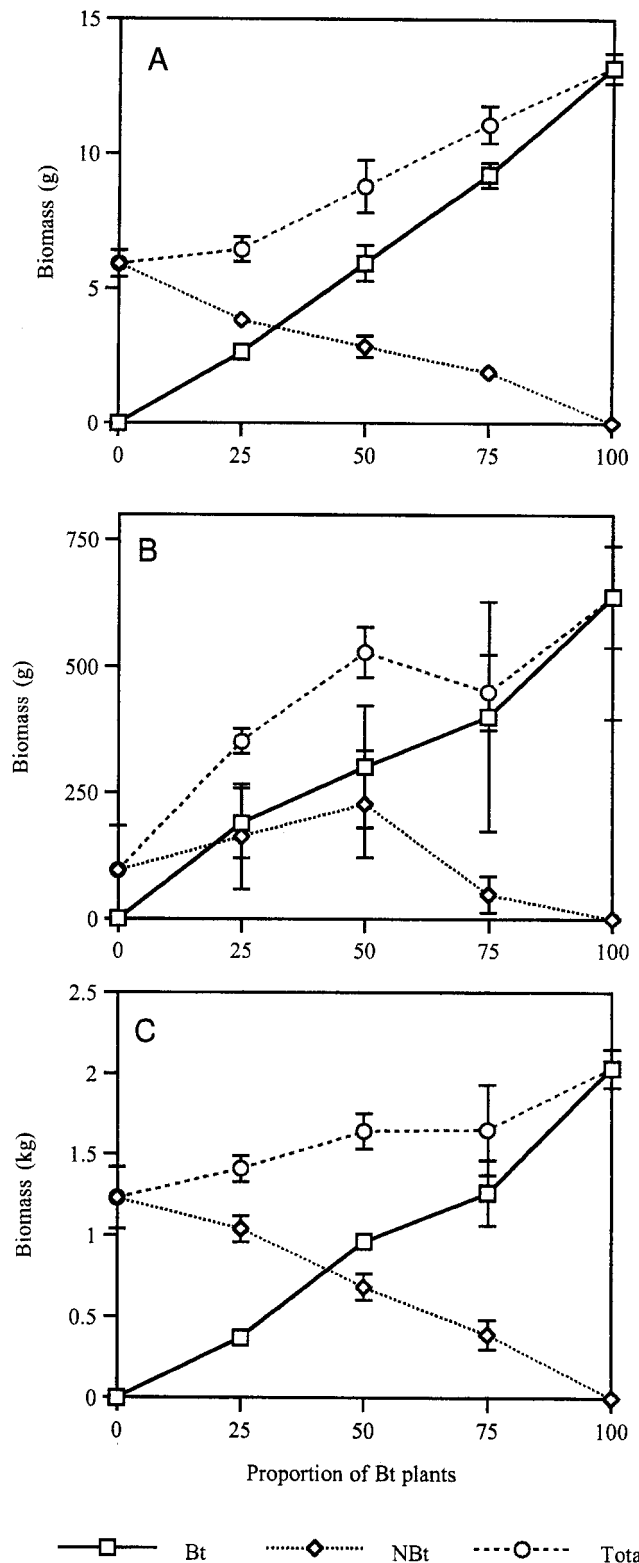


Fig. 4. Biomass production of transgenic and nontransgenic canola plants in different mixtures under diamondback moth infestation in (A) greenhouse (B) Watkinsville, GA, and (C) Griffin, GA, experiments.

the nontransgenic plants (Bergleson et al., 1996; Purington and Bergleson, 1997). Similarly, performance of some insecticidal transgenic cotton lines varied de-

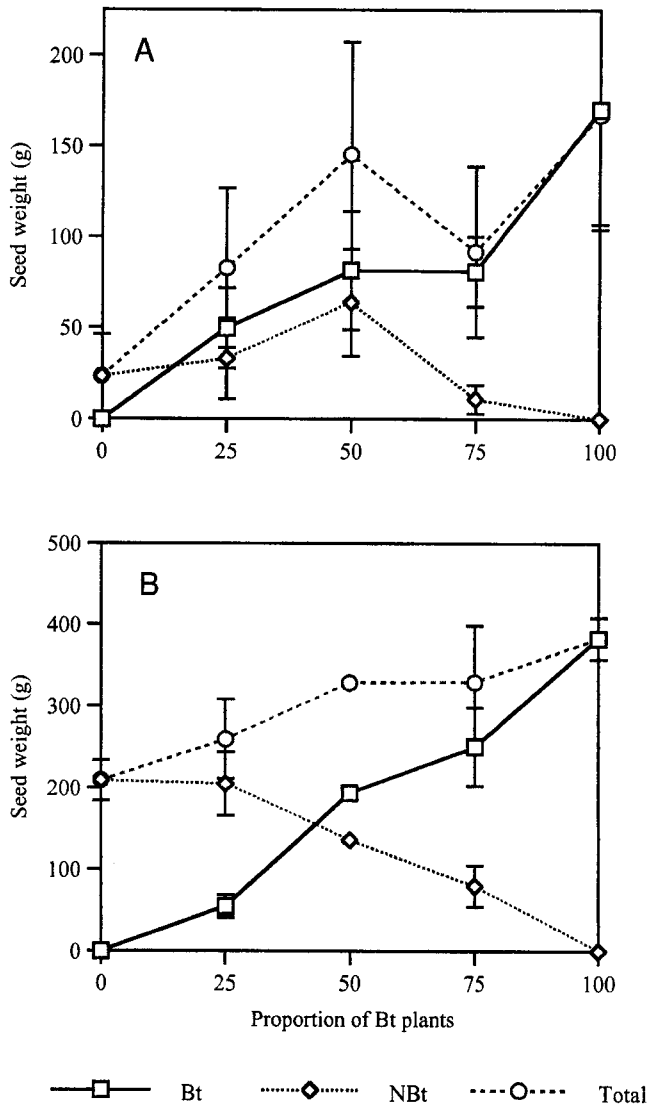


Fig. 5. Seed production of transgenic and nontransgenic canola plants in different seed mixtures under diamondback moth infestation in (A) Watkinsville, GA, and (B) Griffin, GA, experiments.

pending on genetic and environmental factors (Sachs et al., 1998).

The RCC values equivalent to 1 (except for biomass in Watkinsville trial), and straight biomass and seed weight lines for both plant types (Fig. 1A, 1B, 2A, 2C, and 3B) show that there is no competitive advantage for either plant type in plots where there was no diamondback moth infestation. In the Watkinsville trial, germination of transgenic plants was low compared with the nontransgenic plants, which led to more replanting of transgenic seeds. This replanting and delayed germination of transgenic plants could have resulted in the significantly lower biomass production of transgenic plants and consequently curved lines (Fig. 2B and 3A) in the Watkinsville trial. RCC value equivalent to 1 for seed production in Watkinsville trial when there was no diamondback moth infestation indicates that in spite of low biomass production by transgenic plants their yield was equal to that of nontransgenic plants. These

Table 2. Relative crowding coefficient for biomass and seed production of various mixed plantings with and without diamondback moth (DBM) infestation treatments in greenhouse and field experiments.

Experiment	Relative crowding coefficients			
	Biomass		Seed production	
	No DBM infestation	DBM infestation	No DBM infestation	DBM infestation
Greenhouse	0.9 ± 0.1	4.7 ± 0.5*	—	—
Watkinsville	0.6 ± 0.1*	12.8 ± 1.4*	0.6 ± 0.2	12.8 ± 3.1*
Griffin	0.8 ± 0.2	2.3 ± 0.4*	0.8 ± 0.2	2.4 ± 0.5*
Tifton	0.8 ± 0.1	1.4 ± 0.5	1.1 ± 0.3	1.1 ± 0.1

* Indicates values are significantly different from 1 at the $P = 0.05$ level in a t -test.

data along with Tifton trial data (no diamondback moth infestation) suggests that canola plants are able to withstand biomass reduction to a certain extent (either by diamondback moth damage or poor growth) without any significant yield loss. In another study, canola plants were able to withstand more foliar damage as the crop matured without significant yield loss (S. Ramachandran and G.D. Buntin, unpublished data, 1998).

In diamondback moth-infested plots, RCC values of 2.3 to 12.8, which were significantly different from 1 (except for the Tifton trial), indicate that transgenic plants contributed 2.3 to 12.8 times more than the nontransgenic plants to the total biomass and seed weight. Significant RCC values and shape of the curves (Fig. 4A, 4B, 4C, 5A, and 5B) show that transgenic plants were competitively superior compared with nontransgenic plants in diamondback moth-infested plots. Better performance of transgenic plants is due to the high levels of resistance exhibited by the transgenic plants against diamondback moth.

In summary, transgenic canola exhibited high levels of resistance against diamondback moth both as a pure stand and in mixtures with nontransgenic plants. Agronomic performance of transgenic canola plants was comparable to that of the nontransgenic plants, indicating neither any measurable fitness costs associated with the diamondback moth resistance nor any negative impact of the transformation process in the tested transgenic line. When there was no diamondback moth infestation, no competitive advantage occurred for either plant type in seed mixtures. Transgenic plants performed to a better extent when there was diamondback moth infestation because of their high levels of resistance prevented performance-reducing levels of injury by diamondback moth.

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