Development, Characterization & Agronomic Performance of Transgenic Canola Producing a *Bacillis thuringiensis* Toxin for Lepidopteran Control

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INTRODUCTION

The production of canola quality oilseed rape, *Brassica napus* L. in the United States has increased dramatically in the past five years with more than one-half million hectares grown in 1998 (Raymer *et al.*, 1998; Gumpert, 1999). As canola production increases, insect problems associated with the crop are also likely to increase (Lamb, 1989). Insect problems associated with canola may be more serious in the southeastern United States due to the mild climate and presence of crucifer specialists including diamondback moth, *Putella xylostella* (L.) and cabbage looper, *Trichoplusia ni* (Hòbner). Beet armyworm, *Spodoptera exigua* (Hòbner) and corn earworm, *Helicoverpa zea* (Boddie) may increase in importance where canola is grown in warmer regions (Buntin and Raymer, 1994). Transgenic canola cultivars with insecticidal properties will certainly play a major role in integrated pest management strategies for canola pests (All *et al.*, 1999; Evans and Scarisbrick, 1994).

Scientists at The University of Georgia successfully transformed *Brassica napus* L. cultivars 'Oscar' and 'Westar' with a truncated synthetic *Bacillis thuringiensis* (*Bt*) insecticidal crystal protein gene (*cry1Ac*) gene using *Agrobacterium tumefaciens* mediated transformation. A total of 57 independently transformed lines were produced, containing 1 to 12 copies of the transgenes. Expression in transformed lines ranged from 0 to 0.4% *cry* as a percentage of total extractable protein (Stewart *et al.*, 1996).

GREENHOUSE AND FIELD EVALUATIONS OF TRANSGENIC CANOLA

A line with high levels of toxin expression (O52-6; transgenic Oscar) and another with moderate levels of toxin expression (W58-3; transgenic Westar) were tested under greenhouse and field conditions (Ramachandran *et al.*, 1998b). Transgenic lines provided 100% mortality of diamondback moth neonates in greenhouse antibiosis tests at seedling, vegetative, bolting, and flowering stages of the plant (fig. 1). In greenhouse preference assays conducted at vegetative and flowering plant stages, transgenic plants recorded less than 4% of defoliation as compared to up to 30% of defoliation in non-transgenic plants.

In field tests conducted during 1995-1997, plots were artificially infested with diamondback moth neonates (Ramachandran *et al.*, 1998b). Averaged over all trials, non-transgenic plants harbored at least 10 times more of diamondback moth larvae as compared to transgenic plants, and had 41.2% defoliation compared with 3.5% defoliation for transgenic plants. Furthermore, transgenic plants had a better plant stand and produced more pods and seeds at the end of the season than non-transgenic.

Diamondback Moth Larval Movement in Plant Mixtures

No 2nd, 3rd, and 4th instar larvae were able to complete the life cycle when exposed to transgenic plants for 48 h and subsequently transferred to non-transgenic plants. Some larvae survived exposure to transgenic plants for shorter periods indicating that the larvae need to feed on transgenic plants for at least 48 h to acquire a lethal dose of the toxin. However, when transgenic and non-transgenic plants arranged in various ratios in closed cages in greenhouse, larvae were observed to move from the infested plant within 24 h (Table 1). This shows that larvae would move from the transgenic plants before acquiring lethal doses of the toxin. Thus, we conclude that seed mixtures of canola may not be the best strategy to minimize rate of resistance development by diamondback moth against transgenic canola. Instead of seed mixtures, strip planting could be practiced with adequate row spacing between transgenic and non-transgenic plants to avoid larval movement between the rows.

Table 1. Mean ± SE percentage of 2nd, 3rd, 4th instar stages of diamondback moth recovered from infested transgenic and uninfested non-transgenic canola plants, 24 h after infestation1

Larval instar	Percent live larvae recovered		t value*
	On infested transgenic plant	On uninfested non-transgenic plant	
II	71 ± 9	29 ± 9	2.3
III	52 ± 5	48 ± 5	0.5
IV	55 ± 5	45 ± 5	1.1

* No significant differences were recorded in the larval recovery from transgenic and non-transgenic plants. **1** from Ramachandran *et al.* (1998c)

Bt Resistant Diamondback Moth on Transgenic Canola

Diamondback moth has already evolved resistance to sprayable Bt products in various parts of the world. Thus, some resistance of these populations to the transgenic canola is expected. So, we evaluated the survival and development of a Bt resistant diamondback moth strain on transgenic canola. The resistant strain was provided by B. E. Tabashnik, Univ. of Arizona, and was originally collected from the field and intensively selected with Bt sprays in laboratory. The colony expressed > 3000 folds of resistance to Bt sprays.

Larval and pupal durations, pupal weights, and adult emergence were similar for both resistant and susceptible strains on non-transgenic canola. Transgenic canola killed all susceptible larvae tested. In contrast, transgenic and non-transgenic canola did not significantly affect larval survival, percentage pupation, pupal weight, and adult emergence of the resistant strain (Ramachandran *et al.*, 1998a). Resistant-strain larvae were able to damage transgenic and non-transgenic plants equally well. There also were no significant differences between transgenic and non-transgenic canola in ovipositional preference by adults and feeding initiation by neonates for either moth strain (Ramachandran *et al.*, 1998a). This suggests that oviposition preference by adults and larval susceptibility is unrelated and that Bt-toxin expression in canola did not alter the oviposition by adults and feeding initiation by neonates. We were not able to demonstrate any adverse effects of Bt-transgenic canola on this *Bt*-resistant strain of diamondback moth, thereby demonstrating that this pest can overcome high levels of resistance exhibited by transgenic plants.

Competitive Ability of Transgenic Canola

A replacement-series study was conducted to examine intraspecific competitive interaction between transgenic and non-transgenic plants in the field at different locations. Plots were either infested with diamondback moth neonates or left without any insect infestation. Transgenic and non-transgenic plants were harvested separately and total biomass and seed weights were recorded. Transgenic plants recorded significantly lower levels of defoliation compared to the non-transgenic plants in all mixtures and in pure stands (Ramachandran *et al.*, 2000). In plots without diamondback moth infestation, relative competitive coefficient (RCC), a measure of competitive interaction between plants, were not significantly different from 1 for both biomass and seed weight indicating no competitive advantage occurred for either plant type in the absence of insect damage. In plots with diamondback moth infestation, RCC values were significantly different from 1 indicating transgenic plants out performed non-transgenic plants implying that transgenic plants were able to better withstand diamondback moth infestation.

Agronomic Performance of Transgenic Canola

Six of the original Oscar and three of the original Westar transformed *Bt* lines with varying levels of *cry* expression were advanced to the T3 generation in the greenhouse using the pedigree method. T3 seed from single T2 generation plants were harvested from greenhouse grown plants. Because seed quantities were limited, a single replicate of each T3 line was compared to multiple replicates of the parental lines (Oscar and Westar) in field trials at two locations. A total of 70 T3 Oscar *Bt* lines and 12 T3 Westar *Bt* lines were compared in each trial. These field evaluations were fall-seeded and grown as a winter crop at Griffin and Tifton, Georgia in 1997-98. Individual plots consisted of seven rows, 3 m in length, and spaced 18 cm apart. Data were collected on date of 50 percent bloom and harvest maturity, plant height, lodging, and seed yield at both locations. Total oil content was determined using NMR on seed collected from the Tifton trial only.

Seed yield for the 70 Oscar *Bt* lines tested ranged from 1248 to 3034 kg ha -1 compared to Oscar, which averaged 2554 kg ha -1. Seed yield of the 12 Westar *Bt* lines tested ranged from 1205 to 1858 kg ha -1 compared to Westar, which averaged 1386 kg ha -1. In spite of the wide range of values, few Oscar *Bt* lines were statistically different from Oscar and none of the Westar *Bt* lines were statistically different from Westar (Raymer *et al.*, 1999). Substantial variability for the other traits measured also occurred, but it is obvious that further testing with more replication will be necessary to detect true differences among these lines.

To gain a more reliable comparison, data was summarized by location and data pooled within T1 families. Four of the six Oscar T1 *Bt* families produced similar seed yields to Oscar at both locations while the O3*Bt* and O63*Bt* families produced statistically lower seed yields than Oscar. All three of the Westar *Bt* families produced seed yields similar to Westar at both locations. Few differences from Oscar or Westar parental lines were observed for total oil content or other traits at either location.

These results suggest that many of the *Bt* lines developed are comparable in agronomic performance to the parental line from which they were derived. We acknowledge that few of these lines are likely to be commercially competitive by the time they could be released due to the rapid progress in cultivar development in most production regions of the world. However, our research clearly demonstrates the feasibility of developing high-yielding, well-adapted canola cultivars with highly effective insecticidal properties for use in controlling lepidopterous pests of canola.

Conclusion

Transgenic canola quality *B. napus* plants expressing cry1Ab toxin provided high levels of resistance to a number of lepidopterous defoliators at all stages of canola development in both greenhouse and field conditions. Larval movement off transgenic plants in a plant mixture before acquiring a lethal dose, demonstrates that a seed mixture strategy could possibly accelerate insect resistance to the transgenic crop and supports the high-dose, pure-planting/refugia strategy that has been adopted for the deployment of insect-resistant transgenic crops (Tabashnik 1994). We also demonstrated that transgenic plants were equally competitive with non-transgenic plants in the absence of insect damage. Furthermore, the transgenic *Bt* lines developed are comparable in agronomic performance to the parental line from which they were derived thereby demonstrating the feasibility of developing canola cultivars with highly effective resistance to lepidopterous pests. However, lepidopterous pest such as diamondback moth are sometimes and in some production regions important pests but rarely of severe pests of canola (Lamb 1996). Thus, the cost of development and deployment of transgenic *Bt*-resistant canola for lepidopteran control may not be economically justified. In addition, the ability of a field derived strain a diamondback moth with resistance to sprayable Bt products to completely overcome the cry1Ab toxin of transgenic canola raises further concern about the commercialization of Bt-transgenic canola for lepidopteran control.

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