Phytoprotection

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Résumé de l'article

Une étude de deux saisons au Centre de recherches sur la pomme de terre, Fredericton, Nouveau-Brunswick, a démontré que les adultes du doryphore de la pomme de terre (*Leptinotarsa decemlineata*) susceptibles au *Bacillus thuringiensis* (Bt) avaient un temps de résidence quantifiable dans une parcelle de pomme de terre Bt-transgénique. Cinquante pour cent des doryphores susceptibles au Bt relâchés dans une parcelle de pommes de terre Russet Burbank transgéniques (NewLeaf TM) ont été recapturés de 4 à 7 jours plus tard tandis que la même proportion de recapture des doryphores relâchés dans la parcelle témoin non-transgénique a eu lieu de 7 à 11 jours après le lâcher. En dépit de la toxicité des plantes, les doryphores ont survécu pendant un laps de temps appréciable (jusqu'à 31 jours) dans la parcelle de pommes de terre transgéniques. De 25 à 30 % des doryphores relâchés dans la parcelle de pommes de terre Bt-transgéniques furent recapturés sur des plants-pièges ou dans des pièges-fosse en dehors de cette parcelle, ce qui démontre un niveau élevé de dispersion. La durée du temps de résidence et le haut niveau de dispersion pourraient permettre le développement d'une population de doryphores résistante à la pomme de terre Bt-transgénique. Nos résultats sont utiles pour le développement d'une stratégie de déploiement de la pomme de terre Bt-transgénique qui réduit les risques de résistance au Bt chez le doryphore de la pomme de terre.

The effect of Bt-transgenic potatoes on the movement of the Colorado potato beetle [Coleoptera : Chrysomelidae]

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A two-year field study conducted at the Potato Research Centre in Fredericton, New Brunswick, demonstrated a quantifiable residency time for *Bacillus thuringiensis* (Bt) susceptible adult Colorado potato beetles (*Leptinotarsa decemlineata*) in a Bt-transgenic potato field. Fifty percent of Bt-susceptible beetles released in a transgenic Russet Burbank (NewLeaf™) potato plot were recaptured 4 to 7 days after release compared to 7 to 11 days for beetles released in a non-transgenic control plot. Beetles survived for long periods of time (up to 31 days) on transgenic potatoes in spite of the crop's toxicity. A significant number of beetles dispersed from transgenic plots. Twenty-five and 30% of the beetles released in the Bt-transgenic plot were recaptured in border rows or trench traps outside the plot indicating a high level of dispersal activity. The long residency time and the high level of dispersal activity could increase the chances of development of a Bt-resistant beetle population. These findings are important to the development of a deployment strategy that will reduce the risk of build-up of Bt resistance in the beetle population.

[Effet des pommes de terre Bt-transgéniques sur le mouvement du doryphore de la pomme de terre [Coleoptera : Chrysomelidae]]

Une étude de deux saisons au Centre de recherches sur la pomme de terre, Fredericton, Nouveau-Brunswick, a démontré que les adultes du doryphore de la pomme de terre (*Leptinotarsa decemlineata*) susceptibles au *Bacillus thuringiensis* (Bt) avaient un temps de résidence quantifiable dans une parcelle de pomme de terre Bt-transgénique. Cinquante pour cent des doryphores susceptibles au Bt relâchés dans une parcelle de pommes de terre Russet Burbank transgéniques (NewLeaf ™) ont été recapturés de 4 à 7 jours plus tard tandis que la même proportion de recapture des doryphores relâchés dans la parcelle témoin non-transgénique a eu lieu de 7 à 11 jours après le lâcher. En dépit de la toxicité des plantes, les doryphores ont survécu pendant un laps de temps appréciable (jusqu'à 31 jours) dans la parcelle de pommes de terre transgéniques. De 25 à 30 % des doryphores

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relâchés dans la parcelle de pommes de terre Bt-transgéniques furent recapturés sur des plants-pièges ou dans des pièges-fosse en dehors de cette parcelle, ce qui démontre un niveau élevé de dispersion. La durée du temps de résidence et le haut niveau de dispersion pourraient permettre le développement d'une population de doryphores résistante à la pomme de terre Bt-transgénique. Nos résultats sont utiles pour le développement d'une stratégie de déploiement de la pomme de terre Bt-transgénique qui réduit les risques de résistance au Bt chez le doryphore de la pomme de terre.

INTRODUCTION

The Colorado potato beetle (*Leptinotarsa decemlineata* (Say)) [Coleoptera : Chrysomelidae] is one of the most important insect pests on potato (*Solanum tuberosum* L.) in North America. The beetle has developed resistance to most conventional insecticides used against it (Georghiou 1986). Some strains of the soil bacterium *Bacillus thuringiensis* Berliner (Bt) produce a protein that is toxic to the Colorado potato beetle when ingested. Commercial preparations of the protein have been used to effectively control the Colorado potato beetle (Ferro *et al.* 1993). However, this natural insecticide has a short residual activity (Ferro *et al.* 1993) and low efficacy against adults and large larvae (Zehnder and Gelernter 1989). The insertion of the gene (CryIIIA) responsible for the production of the protein into the potato plant extended the persistence and the activity of the toxin. NewLeaf™ expresses a high concentration of the protein throughout the life of the plant making it effective against adults and large larvae (Perlak *et al.* 1993). However, the Colorado potato beetle may develop field resistance to Bt and the NewLeaf™ potato. As a result of selection in the laboratory, Whalon *et al.* (1993) produced a Bt-resistant strain of Colorado potato beetle. Second and older instars and adults of this strain were more tolerant to exposure to the Bt-transgenic potato than a susceptible strain (Wierenga *et al.* 1996). Several options for the design of a deployment strategy for Bt-transgenic potato were proposed to reduce the risk of Bt-resistance development in field populations (Gould 1998). With a transgenic plant producing a high dose of toxin, the deployment strategy should allow a high level of mating among pest genotypes (resistant and susceptible) while avoiding movement of feeding stages between toxic and non-toxic plants. Resistance management strategies have to integrate not only the genetic characteristics of the insect population but also its ecology.

Adult Colorado potato beetles disperse within and between potato fields (Dively *et al.* 1998; Follett *et al.* 1993, 1996; French *et al.* 1993; Weisz *et al.* 1996). A better understanding of the level of movement of the beetle in Bt-transgenic potato fields and between Bt-transgenic and other potato fields is necessary in order to determine the most effective resistance management strategy (Follett *et al.* 1993; Gould 1998; Mallet and Porter 1992). It was proposed that the transgenic potato crop resistant to Colorado potato beetle should be accompanied by a non-transgenic refuge near by (Gould 1998) to reduce the build-up of resistance in the insect population. The concept of refuge implies that Bt-resistant beetles would mate with Bt-susceptible beetles either in the refuge or the Bt-transgenic field. The level of gene flow between Bt-susceptible and Bt-resistant Colorado potato beetle populations determines directly the rate at which resistance to Bt will develop.

The objective of this study was to measure and contrast the movement activity of Bt-susceptible Colorado potato beetle adults on Bt-transgenic and non-transgenic potatoes. Only Bt-susceptible beetles were studied here since a Bt-resistant population of beetles was not available. If Bt ingestion reduces significantly the level of movement of Bt-susceptible beetles, mating between Bt-resistant and Bt-susceptible beetles will be less likely in a Bt-transgenic field.

MATERIALS AND METHODS

Beetle movement

Field experiments were conducted at the Potato Research Centre of Agriculture and Agri-Food Canada in Fredericton, New Brunswick, during the summers 1994 and 1995. Two plots, a Bt-transgenic Russet Burbank (Newleaf™) plot and a control plot (Russet Burbank), each consisting of fifteen 14 m rows (1 m apart), were planted side by side in the same field, on 16 June 1994 and 14 June 1995. Each plot was surrounded by a plastic trench (75 cm wide, 60 cm deep), to capture beetles walking out of the plots and to prevent beetles from walking into the plots (Boiteau *et al.* 1994). Each plot was also surrounded by four rows of Russet Burbank potatoes planted outside the trench. The function of these four rows of potatoes was to retain beetles that left the plot by flight. The field was bordered on the east side by a strip (around 5 m wide) of large trees, on the west and south sides by meadow and on the north side by a road.

Adult Colorado potato beetles were collected in fields (not treated with insecticides) at the Potato Research Centre. Beetles were marked by making small holes in the elytra with a dissecting pin (Unruh and Chauvin 1993). This marking technique does not influence the ability of the insect to fly or walk. In 1994, there were three releases of beetles as follows: on 5 July, 200 marked beetles were released in the control plot, on 11 July, 400 were released in each plot and on 18 July, 400 were released in the Bt-transgenic plot, for a total of 600 marked beetles released in the control plot and 800 in the Bt-transgenic plot. There was a single release in 1995 with 775 marked beetles being released in each plot on 6 July. Beetles were released in the centre of the plot in a section containing around 30 plants. The presence of marked beetles was monitored between 6 July and 5 August 1994 (up to 31 d after the first release) and between 7 and 19 July 1995 (up to 13 d after release). In 1994, the control plot was usually monitored twice a wk and the Bt-transgenic plot two or three times per wk. In 1995 both plots were monitored three times a wk. When a plot was monitored, each plant was examined thoroughly. Marked beetles were returned to the plant where they were found. During the 1994 season (up to July 18) and for the entire 1995 season all unmarked adults, egg clutches, and larvae found in the control and Bt-transgenic plots were removed to prevent defoliation by larvae and unmarked adults. There was no oviposition and almost no defoliation in the Bt-transgenic plot while a large number of egg clutches were removed from the control plot. In 1994 the trenches were monitored daily, except for weekends, and the border rows were monitored on the same d as the control or Bt-transgenic plot they surrounded. In 1995, the trenches and border rows were both monitored daily, except for weekends, by walking through them and collecting any beetles seen. Border sampling in 1995 was more frequent but less thorough than in 1994. Beetles found in the borders and in the trenches were taken back to the laboratory where they were checked for marks. None of these beetles were returned to the field. Beetles found in the plot where they were not released were collected and counted as found in the border.

Walking and flying propensity

The propensity of beetles to walk and fly after feeding on field-collected Bt-transgenic and control (Russet Burbank) foliage was evaluated in 1995. Beetles from the overwintering generation and later from the summer generation collected from a potato field at the Potato Research Centre were fed Bt-transgenic or control foliage for 48 h. Potato stalks were placed in 2 L plastic containers with damp vermiculite. Twenty-five beetles were placed on each stalk and then the stalk was covered with a Plexiglas cylinder (30 cm high and 15 cm in diam) with screening at one end. The beetles were held in a screenhouse and the stalks were replaced daily. After 48 h, 50 control and 50 treatment beetles were placed individually in Petri dishes (9 cm) lined with damp filter papers and kept without food for 1.5 h before the test. The tests were conducted in a growth chamber at 22°C under fluorescent light. One hundred Petri dishes each containing one beetle without food were set on a table and observed every 5 min for 1.5 h to determine if the insects were walking. The walking propensity was estimated as the number of times a beetle was observed walking. The experiment was repeated three times for each generation of beetles.

Flying propensity was evaluated immediately after the walking test. Fifty beetles were placed in a 2 L plastic container, with a Styrofoam cone (12.5 cm base diam and 30.5 cm high) placed in its centre. The rim of the plastic container was coated with Fluon to prevent the beetles from walking out. Two containers, one with 50 Bt-transgenic fed and one with 50 control foliage fed beetles, were placed along one wall of a flight chamber. Beetles were marked as having fed on transgenic or control foliage (not with individual numbers) by making a combination of small holes in the elytra with a dissecting pin (Unruh and Chauvin 1993). The flight chamber was 2.4 m x 3.7 m x 2.1 m high with 14 fluorescent lights (two 2.4 m HO tubes per light) at ceiling level and 2 fluorescent lights (76 cm) 33 cm above the cones. The chamber was maintained at 29°C and 70% RH. Beetles were observed for 2 h and the total number of flights was recorded. Beetles initiating flight were immediately returned to the container that they flew from. The experiment was repeated three times for each generation of beetles. The flying propensity was estimated as the number of flights observed from beetles of each treatment.

Statistical analysis

A *Z*-test was used to compare the proportion of beetles recaptured (Zar 1974). A *t*-test ($\alpha = 0.05$) was used to compare the average propensity of walking and flying by beetles fed Bt-transgenic foliage with those of beetles fed control foliage.

RESULTS

Beetle movement

The density of adult Colorado potato beetles decreased consistently through the sampling period in the Bt-transgenic and the control plots. The percentage of beetles recaptured was lower in Bt-transgenic than in control plots both yr (Fig. 1). A recapture rate of 50% was reached 4 to 7 d and 7 to 11 d after releasing beetles in Bt-transgenic and control potatoes respectively. Eighty-eight to 95% of the released beetles were recaptured at least once (Table 1). The percentage of beetles released that were subsequently found dead was significantly higher ($P \leq 0.05$) in the Bt-transgenic plot than in the control plot in 1994 and 1995 (Table 1). A higher proportion of dead beetles were found in the control plot in 1995 than in 1994. A significantly higher proportion of the beetles released in the control plot were recaptured in the border than in the trench both yr. A significantly higher proportion of beetles released in the Bt-transgenic plot were recaptured in the trench than in the border in 1995. Forty-seven percent and 20% of the total beetles released in the control plot were recaptured outside of the plot in 1994 and 1995 respectively. Dispersal (the sum of the percent recapture in border and in trench) from the Bt-transgenic plot was lower than in the control in 1994 (*Z*-test, $P < 0.001$) but higher in 1995 (*Z*-test, $P < 0.001$).

Walking and flying propensity

Beetles kept on Bt-transgenic foliage had a lower propensity to walk than beetles kept on control foliage (Table 2). Summer adults walked less than adults from the overwintered generation.

Overwintered adults kept on Bt-transgenic potato foliage flew less than beetles kept on control foliage whereas the summer adults kept on Bt-transgenic potato foliage showed the same flying propensity as the controls (Table 2).

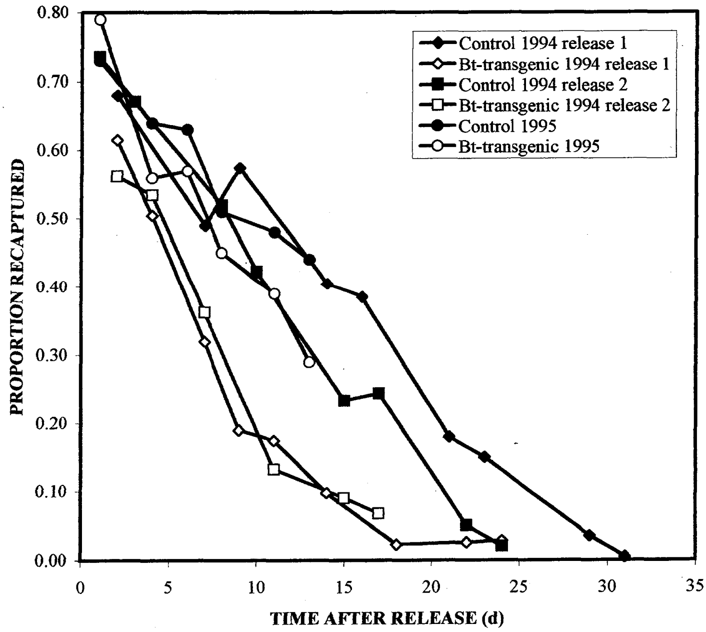

Figure 1. Proportion of released adult Colorado potato beetles recaptured in Bt-transgenic or control potato plots as a function of time after release.

Table 1. Number of the adult Colorado potato beetles individually marked and released in the control or Bt-transgenic potato plots and percentage recaptured dead, in the potato border, in the plastic trench and total percentage recaptured. The total percentage of recaptured includes beetles recaptured at least once in the potato plots

Treatment	Number released	% recaptured			
		Dead[a]	Border[a]	Trench[a]	Total[a]
Control 1994	600	6.6a	29.0a*	17.7a	93.0a
NewLeaf 1994	800	32.0d	11.4bc	14.1a	88.6b
Control 1995	775	11.2b	12.6b*	7.4b	95.2a
NewLeaf 1995	775	16.3c	9.4c*	21.0a	95.4a

[a] Percentages in the same column with the same letter are not statistically different (*Z*-test at $\alpha = 0.05$).

* Statistically different from the percentage recaptured in the trench for the same treatment/yr combination (*Z*-test at $\alpha = 0.05$).

Table 2. Walking and flying propensity of adult Colorado potato beetles fed for 48 hours on Bt-transgenic or control foliage

	Walking[a]		Flying[b]	
	Mean ± SE	N	Mean ± SE	N
Overwintered adults				
Bt-transgenic	5.5 ± 0.5 *	150	23 ± 11 *	3
Control	7.8 ± 0.4	149	60 ± 5	3
Summer adults				
Bt-transgenic	3.3 ± 0.4 *	147	27 ± 7	3
Control	3.9 ± 0.4	150	15 ± 2	3

[a] Average number of observations (out of 18) that the beetle was seen walking.

[b] Average total number of take-offs from three repetitions each with 50 beetles with replacement.

* Statistically different from the control based on a *t*-test at $\alpha = 0.05$.

Control beetles from the summer generation flew markedly less than control beetles from the overwintered generation.

DISCUSSION

The ability of Bt-transgenic Russet Burbank potato (NewLeaf™) to control all foliage feeding stages of the Colorado potato beetle is well established (Perlak *et al.* 1993). As a consequence, the beetles released in the Bt-transgenic potato plots had a negligible impact on plant defoliation. Oviposition did not occur and feeding was inhibited. Dead adult beetles were observed both yr but a large proportion of the insects remained alive in the Bt-transgenic plot for several d. Fifty percent of the released beetles was recovered 4 to 7 d after release in the Bt-transgenic. Thirteen d after release, 10 to 30% of the beetles were still in the Bt-transgenic plot (Fig. 1). This is the first demonstration of a long (in the order of wk) residency time for adult Colorado potato beetles in a Bt-transgenic potato field. A long residency time as well as a high level of dispersal activity by the Bt-susceptible beetles could reduce the chance that Colorado potato beetle would develop resistance to Bt (Caprio and Tabashnik 1992; Gould 1998). A large proportion of colonizing adult beetles could leave the Bt-transgenic plot before experiencing its lethal effect. This would result in a lower selection pressure for resistance to Bt. Later during the season, Bt-susceptible beetles could colonize and spend enough time in Bt-transgenic potato plot to be able to mate with Bt-resistant beetles, reducing the development of a Bt resistant population.

The relatively long residency period for beetles in transgenic potato plots could be attributed to the intake of sublethal doses of the toxin by a proportion of the population placed in the plot. Sublethal concentration of insecticide can either increase (Hurej and Dutcher 1994) or decrease (Boiteau and Osborn 1997) locomotion in insects. The presence of Bt in the food of lepidopteran larvae produces an increase in locomotion (Halcomb *et al.* 1996; Harris *et al.* 1997). Bt-resistant Colorado potato beetles do not move more than susceptible beetles (Whalon *et al.* 1993). In summary, when sublethal effects exist their impact ranges widely and depends on the species and on the toxin. In this case, if we assume that the Colorado potato beetle acquired a sublethal dose of the Bt from transgenic plants, the effect on movement was inconsistent, limited in magnitude, and could have been modulated by other factors. The laboratory tests showed a greater difference in the walking and flying propensity of summer and overwintered adults than between control and transgenic host plants.

Our data indicated that residency time in and emigration out of the Bt-transgenic plot were similar to that of the control plot. The lower proportion of recovery as a function of the time after release in the Bt-transgenic plot could be a result of the higher mortality rate. Emigration was an important cause of the reduction in the adult population observed in both plots. This high level of movement out of the Bt-transgenic plot would impact the selection for resistance to Bt in susceptible beetles colonizing Bt-transgenic fields. The proportion of beetles found dead or recaptured outside the plots did not compensate for the decrease in the marked-beetles population recaptured from the plots, which indicated that an important proportion of insects flew outside the test area. The level of emigration in control plots supports the theory that susceptible beetles could migrate from refuges to Bt-transgenic potato field. Both scenarios, beetles from non-transgenic field colonizing Bt-transgenic field and beetles from Bt-transgenic field colonizing non-transgenic field, are likely to increase the chance of Bt-resistant beetles mating with Bt-susceptible insects. Since no major differences in the level of movement was observed between susceptible beetles in Bt-transgenic and control plot, one can hypothesize that Bt-resistant beetles will behave in a similar way. If this is the case, the high level of movement of Bt-resistant beetles should increase the chance they will leave a Bt-transgenic field, colonize a non-transgenic potato field (or refuge) and mate with Bt-susceptible beetles. On the other hand, the long residency time of the remaining beetles does provide an opportunity for these beetles to mate among themselves thereby propagating the resistance genes. However, this long residency time does also increase the probability of encounters with visiting Bt-susceptible beetles originating from the refuges. In summary, the Colorado potato beetle demonstrated a similar dispersal behaviour on transgenic and non-transgenic potatoes that could be compatible with the recommended strategy for the management of resistance if mutant beetles' dispersal behaviour is the same or greater.

This study provided important information on the level of movement and emigration of Bt-susceptible Colorado potato beetle. This information could be used for the development of a deployment strategy for Bt-transgenic potato. Information on the distance moved by Bt-susceptible beetles could also be useful in developing a deployment strategy. This study provided little information on distance moved by the beetles due to the relatively small size of the plots. More study on the movement of both Bt-susceptible and Bt-resistant Colorado potato beetles in relation to gene flow is required to fully evaluate the impact on resistance build-up to Bt-transgenic potato.

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