

Solanum weeds as hosts for *Phthorimaea operculella*: implications for resistance management of genetically modified potatoes (*Solanum tuberosum*)

M. M. DAVIDSON

A. J. CONNER

New Zealand Institute for Crop & Food
Research Limited
Private Bag 4704
Christchurch, New Zealand
email: connert@crop.cri.nz

Abstract Foliage from *Solanum* species found within or near potato fields in New Zealand was compared to potato (*Solanum tuberosum*) foliage for its ability to support growth of larvae of *Phthorimaea operculella* (potato tuber moth, PTM). *Solanum* species included *S. laciniatum* (poroporo), *S. physalifolium* (hairy nightshade), *S. chenopodioides* (velvety nightshade), *S. nigrum* (black nightshade), and *S. dulcamara* (bittersweet). In detached leaf bioassays larval growth was greater on velvety nightshade and poroporo than on foliage from the potato cultivar, 'Iwa', in the first year, but only larval growth on poroporo foliage was greater in the second year ($P < 0.05$). Larval growth on bittersweet foliage was similar to larvae on 'Iwa' foliage. No larvae survived on black nightshade beyond 5 days in both years. From a field survey, no PTM larval mines were found on any foliage of hairy, velvety, or black nightshade plants in or within 100 m of a field trial of transgenic PTM-resistant potato plants from a survey undertaken in the third summer. Over the entire survey, an average of 0.18 mines/plant were recorded on poroporo plants, all of which were $< 200 \text{ mm}^2$, compared to an average of 3.8 mines/non-transgenic potato plants (volunteer potato plants $> 100 \text{ m}$ from the field trial and non-transgenic potato plants of the cultivars 'Iwa', 'Red Rascal', and

breeding line 2390, used as experimental controls within the field trial). This study demonstrated that, under no-choice conditions, PTM larvae could grow on alternative *Solanum* species. However, PTM exhibited a clear preference for oviposition and development on potato plants in the field.

Keywords *Solanum* species; potato tuber moth; nightshade; bittersweet; poroporo; insect-resistant; transgenic potatoes; *Solanum tuberosum*; refuges

INTRODUCTION

Phthorimaea operculella (Zeller), potato tuber moth (PTM), is an oligophagous insect found wherever potatoes (*Solanum tuberosum* L.) grow. It has attained pest status on potato crops in regions with a distinct dry season (Rothschild 1986). PTM larvae mine into the foliage and tubers of potato plants, which provide the insects with a degree of protection from insecticide sprays.

Potato plants have been genetically engineered with *cry* genes to confer resistance to this insect pest (Jansens et al. 1995; Li et al. 1999; Davidson et al. 2002). One of the major concerns over the use of this technology is the potential of target pests to become resistant to transgenic plants (Gould 1988; Roush 1994; Frutos et al. 1999), consequently, resistance management strategies have been suggested. The most widely adopted strategy for commercially deployed transgenic crops at present involves the use of highly resistant plants (causing $> 95\%$ mortality of heterozygotes) coupled with spatial refuges (Tang et al. 2001). Refuges are areas of non-transgenic plants established either within transgenic crops (plant mixtures) or in separate areas at close proximity to transgenic crops. One of the assumptions made regarding plant mixtures is that insect larvae rarely move between transgenic and non-transgenic plants. If such movement occurs then there may be greater potential for heterozygote individuals to recover from sublethal doses of the toxin (Mallet & Porter 1992).

The high dose/refuge strategy assumes that resistance is recessive (Tabashnik et al. 1997) and rare (Andow & Alstad 1998). It also assumes that susceptible individuals from refuges will mate with any resistant individuals from transgenic crops and the resulting progeny will also be susceptible to the transgenic crops (Roush 1996). The presence of weed species that are potential alternative hosts for the target insect could, therefore, act as an unintentional refuge, within and surrounding potato crops. PTM that developed on such alternative plants could increase the population of susceptible individuals available to mate with any resistant individuals from transgenic plants. Conversely, if PTM larvae move from transgenic plants to alternative host plants within a crop, the effect of the toxin would be reduced, potentially allowing more heterozygote individuals to survive.

The host range of PTM extends to 60 other plant species, most of which (52) belong to the Solanaceae (genus *Solanum* and closely related genera) (Das & Raman 1994). Some of these plants can occur as weeds within potato crops in New Zealand, so there is potential for such species to be used by PTM as alternative hosts. *Solanum* spp. that have been described as hosts (feeding damage observed in the field, greenhouse, or laboratory), and are weeds in New Zealand include *S. nigrum* L. (black nightshade), *S. mauritianum* Scop. (woolly nightshade), *S. dulcamara* L. (bittersweet), and both poroporo species (*S. aviculare* Forst. f., *S. laciniatum* Ait.) (Das & Raman 1994). In the North Island of New Zealand the *Solanum* spp. commonly present in and around potato crops include *S. nigrum*, *S. mauritianum*, and *S. aviculare*. In the South Island of New Zealand species include *S. laciniatum*, *S. physalifolium* Rusby (hairy nightshade), *S. chenopodioides* Lam. (velvety nightshade), *S. dulcamara*, and *S. nigrum*. The purpose of the present study was to determine whether *Solanum* spp. commonly found within and around potato crops in the South Island of New Zealand are hosts for PTM and whether they could provide a refuge for PTM.

MATERIALS AND METHODS

Laboratory experiments

In April 1999 and March 2000 foliage from *Solanum* spp. growing in a field near Lincoln, Canterbury, was compared to potato (*Solanum tuberosum* 'Iwa') for

its ability to support PTM larvae. The *Solanum* spp. assayed in 1999 were poroporo (*S. laciniatum*), hairy nightshade, velvety nightshade, and black nightshade. In 2000, foliage from bittersweet was included in the bioassays in addition to the *Solanum* spp. used in 1999.

Neonate PTM larvae used in the laboratory bioassays were collected from a colony. The colony had been established in 1998 from a wild population from Pukekohe (North Island, New Zealand) and was maintained using the method described by Davidson et al. (2002). Briefly, this involved placing neonate larvae on 6–8 (>100 g each) potato tubers punctured to facilitate larval entry, in a 29 × 20 × 13 cm transparent plastic container, with 600 ml of Vermiculite covering the base. The containers were covered with fine Terylene mesh, and when adults had emerged filter paper moistened with a 5% sugar solution was placed on top of the mesh on which females laid eggs through the mesh. The egg-laden filter papers were placed into a sealed plastic container until egg eclosion. All containers were kept in a controlled temperature room (22 ± 3°C) under a photoperiod of 16 h light:8 h dark.

Ten neonate larvae were weighed together because their individual weights did not register on a 5-place balance. The 10 larvae were then placed on young, fully expanded leaves that had been excised from plants growing in the field, near Lincoln (New Zealand) c. 2–3 g fresh weight. The leaves were held in a 350 ml plastic container, the bottom of which was lined with filter paper (Whatman® No. 1, 50 mm). There were three containers for each *Solanum* species, and these were covered and kept in a controlled temperature room at 22 ± 3°C under a 16 h light:8 h dark photoperiod. In 1999, leaves were replaced every 4 days and the surviving larvae were weighed individually after 11 days on the foliage. In 2000, leaves were replaced every 3 days and final larval weights were taken after 9 days. A relative growth index (RGI) was calculated as:
$$RGI = \frac{\log_e(\text{final weight} - \text{mean initial weight})}{\text{No. days before final weighing}}$$

Data from both years were analysed together using residual maximum likelihood (REML) methods (Gilmour et al. 1995). In addition to comparing RGI differences between species, this method also allowed an assessment of whether there were any statistically significant contributions to the variability in the data (variance components) between years and containers within a given year. REML was used because the unequal number of surviving larvae for each species made the data

unbalanced when all the variance components were included. Overall differences between the years, species, and years/species interaction were tested as part of the REML analysis with a Wald statistic. All analyses were carried out with GenStat (GenStat 2002).

Field survey

Over the growing season of 2001/02, *Solanum* spp. within and/or surrounding a field trial of transgenic potato plants resistant to PTM (Environmental Risk Management Authority approval GMF98008) were surveyed for occupied PTM larval mines. The plants examined included the black, hairy, and velvety nightshades, poroporo (*S. laciniatum*), and non-transgenic potato plants. Black and hairy nightshades were found within the trial as well as in the surrounding area, whereas velvety nightshade and poroporo were found within 100 m, but not within the trial. Non-transgenic potato plants were of the cultivars 'Red Rascal' and 'Iwa', as well as the breeding line 2390 ('Urenika' × V431-1) and volunteer potato plants located 100–150 m from the trial. The volunteer potato plants were from an area previously used for evaluating lines from a potato breeding programme and maintaining a potato germplasm collection. Thirty randomly chosen plants of each species, or non-transgenic potato category were examined for larval mines between 4 March and 10 April 2002, with the exception of hairy nightshade, when only four plants were found within the field trial on 4 March. Individual plants were only examined on one occasion. On all

dates plants with a similar amount of foliage biomass to the potato plants in the field trial were surveyed. The size of the mines was recorded using five categories: ≤25, 25–100, 100–200, 200–500, and ≥500 mm². Male PTM activity was monitored in the vicinity of where the field survey was undertaken over the course of the summer using a delta pheromone trap (DeSIRE®, HortResearch, Auckland, New Zealand).

RESULTS

Laboratory experiments

The RGIs of PTM larvae differed between the *Solanum* spp. (Wald statistic = 33.18, d.f. = 4, $\chi^2 P < 0.001$; Table 1). In 1999, the RGI of larvae fed foliage from velvety nightshade and poroporo plants was higher than those fed potato foliage. However, there was a statistically significant interaction between species and year (Wald statistic = 12.59, d.f. = 2, $\chi^2 P = 0.002$). The RGI of larvae fed velvety nightshade, poroporo, or potato ('Iwa') foliage in 2000 were respectively 57.8, 82.1, and 94.2% that of larvae on these plants in 1999. Larval growth on excised bittersweet foliage (RGI = 0.37) was similar to larval growth on excised potato foliage (RGI = 0.32) in 2000. No larvae survived beyond 5 days on black nightshade foliage in either 1999 or 2000. Larval growth was slightly reduced on hairy nightshade foliage (0.30) in 1999 compared to larval growth on potato foliage (0.34), and in 2000 no

Table 1 Mean relative growth indices (RGI) of potato tuber moth (*Phthorimaea operculella*) larvae fed foliage from different *Solanum* species.

<i>Solanum</i> species	1999 RGI	2000 RGI
Potato (<i>S. tuberosum</i>) Iwa	0.34	0.32
Hairy nightshade (<i>S. physalifolium</i>)	0.30	0*
Black nightshade (<i>S. nigrum</i>)	0*	0*
Velvety nightshade (<i>S. chenopodioides</i>)	0.47	0.27
Poroporo (<i>S. laciniatum</i>)	0.47	0.39
Bittersweet (<i>S. dulcamara</i>) [†]	–	0.37
Mean LSD [‡]	0.07	

*No larvae survived beyond 5 days, data not included in the analysis.

[†]Bittersweet was tested in 2000 only.

[‡]d.f. = 100. Each comparison had a slightly different LSD (least significant difference between two means), so this is the mean of all possible LSDs.

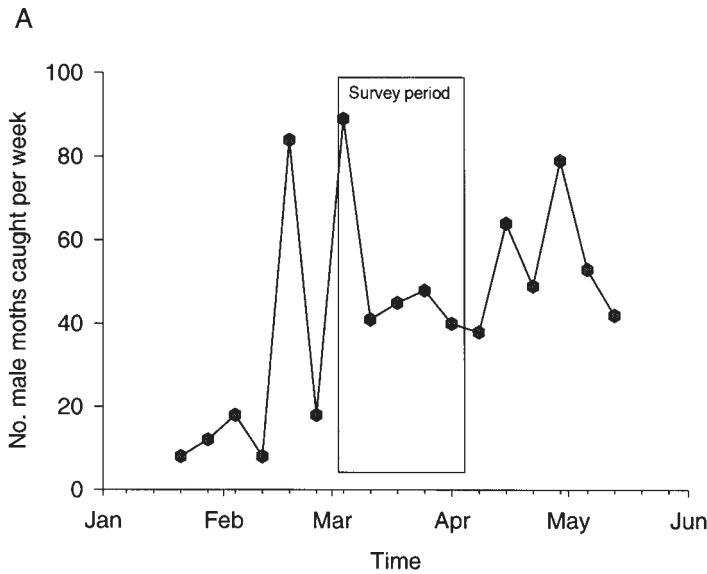
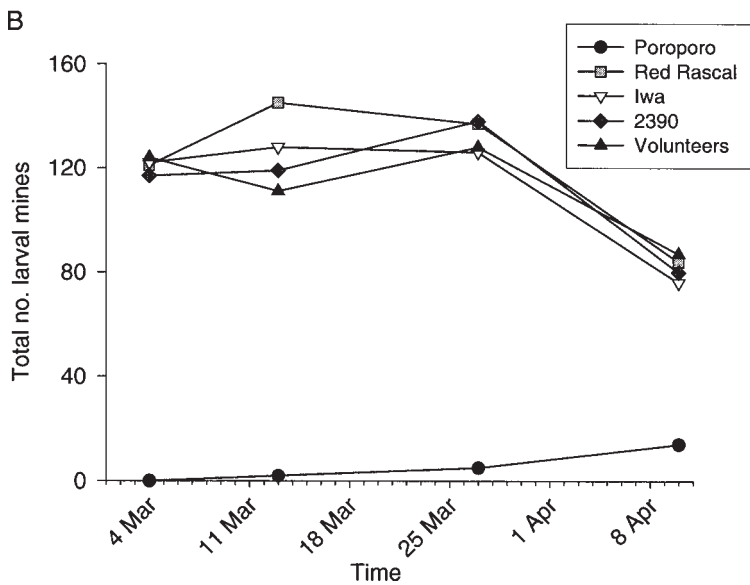


Fig. 1 A, Number of male potato tuber moths (*Phthorimaea operculella*) caught in the pheromone trap over the growing season of 2001/02; and B, the total number of potato tuber moth larval mines recorded on poroporo (*Solanum laciniatum*) and potato plants (*S. tuberosum*) over the survey period. ($n = 30$ plants for each species/category for each sample date.)



larvae survived beyond 5 days on foliage from this species.

Field survey

The greatest number of male PTM were caught in the pheromone trap the week before (25 February–4 March) the start of the field survey (4 March), with more than 80 moths caught during this time (Fig. 1A). Over the entire survey period no mines were

observed on any of the nightshade plants, with mines on only 11% of the poroporo plants (Table 2). In contrast, virtually all potato plants (>85%) had at least one mine (Table 2). On individual survey dates the maximum number of mines found on poroporo plants was 12, on the final sampling day, whereas a large number of mines (80–150) were found on the potato plants on all sampling dates throughout the survey (Fig. 1B).

Fig. 2 Total number of potato tuber moth (*Phthorimaea operculella*) larval mines recorded for different mine size categories from plants of poroporo (*Solanum laciniatum*) and potato (*S. tuberosum*). ($n = 120$ plants for each species/category for combined sample dates.)

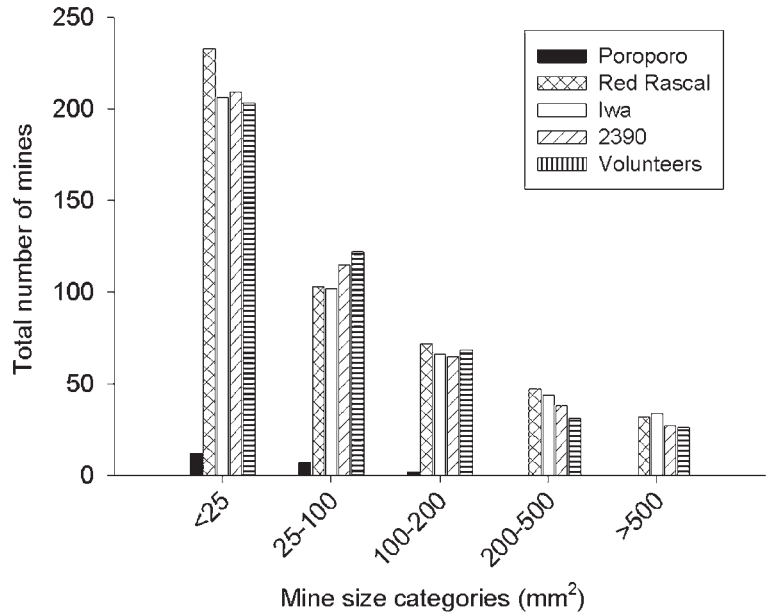


Table 2 Frequency of plants with occupied potato tuber moth (*Phthorimaea operculella*) mines from a field survey of different *Solanum* species.

<i>Solanum</i> species	Frequency of plants with mines (%) [*]
Black nightshade (<i>S. nigrum</i>)	
Within field trial	0
Surrounding field trial	0
Hairy nightshade (<i>S. physalifolium</i>)	
Within field trial	0
Surrounding field trial	0
Velvety nightshade (<i>S. chenopodioides</i>)	
Surrounding field trial	0
Poroporo (<i>S. laciniatum</i>)	
Surrounding field trial	11
Potato (<i>S. tuberosum</i>)	
Iwa	96
Red Rascal	99
2390	91
Volunteers	85

^{*} $n = 120$ plants except for hairy nightshade within the field trial where $n = 94$.

Over the entire survey, an average of 3.8 mines/non-transgenic potato plants, compared to an average of 0.18 mines/plant were recorded on poroporo plants. All 21 mines found on the poroporo plants were <200 mm² (Fig. 2), and 57% of these were <25 mm², whereas the occupied mines found on the

potato plants fell within all size categories. A similar number of mines was found in foliage of each potato cultivar/category for each mine size category. The total number of occupied mines observed on potato plants for each mine size category decreased with increasing mine size.

DISCUSSION

This study demonstrated that, under no-choice conditions, PTM larvae can grow on some alternative *Solanum* host species. The RGI of larvae fed potato or poroporo foliage were similar over both years. However, RGI of larvae fed hairy or velvety nightshade foliage differed markedly, with no larvae surviving beyond 5 days on the hairy nightshade and RGI of larvae on velvety nightshade foliage <60% that of larvae from the previous year. The large variation in RGI of larvae fed hairy or velvety nightshade foliage between years was perhaps in part the result of the different environmental conditions between the field sites used each year, which could influence the nutritional quality of foliage for larval growth in these two species.

The results from the field survey in the present study illustrated the strong preference of PTM for oviposition and development on potato plants (Table 2; Fig. 1, 2). A similar conclusion was drawn from surveys in the north of the North Island of New Zealand (Cameron et al. 1997), where no PTM larval mines were observed on black or woolly nightshade in a field trial. All of the potato plants examined by Cameron et al. (1997) were mined, with an average of 8.6 mines/plant, whereas 48% of poroporo (*S. aviculare*) plants had an average of 2.8 mines/plant.

Poroporo (both species, Foot 1976), bittersweet (Picard 1913, cited in Gomaa et al. 1978), black nightshade and woolly nightshade (Cunningham 1969) have been classified as hosts of PTM from observations of PTM mine damage in the field or laboratory. No mines were found on black nightshade plants in the present study or by Cameron et al. (1997) in either the laboratory or the field. However, Cunningham (1969) recorded mines in the pith of stems from black nightshade plants. Leaves of woolly nightshade were severely infested in the laboratory (Cunningham 1969), but there are no records of it being infested in the field. The current study is the first to record velvety nightshade as a host for PTM in the laboratory. However, as with woolly nightshade (Cameron et al. 1997), no PTM mines were found on velvety nightshade plants in the field in the current study.

The size of the mine reflects the size of the mining larvae (Jansens et al. 1995). Larvae that develop into pupae produce larger mines (>200 mm²). The absence of mines >200 mm² on poroporo (*S. laciniatum*) in the present field survey suggests that PTM were unable to fully develop on this species. In order to reconcile the differences between detached leaf bioassays and field survey results in

the current study, future work could involve caged plant experiments to determine oviposition preference and larval survival on *Solanum* weeds.

Das & Raman (1994) concluded that PTM could oviposit and develop on alternative host plants only in the absence of potato plants, a conclusion also supported by the results of the present study. Consequently the management of transgenic potato crops resistant to PTM will require the incorporation of refuges using non-transgenic potato plants because alternative *Solanum* species will not provide naturally occurring refuges.

ACKNOWLEDGMENTS

We thank Jill Reader for technical assistance, Ruth Butler for statistical analyses, and Graham Walker, Dr Richard Falloon, and Dr David Teulon for helpful advice on the manuscript. Melanie Davidson received financial assistance through a Technology in Industry Fellowship from the Foundation for Research Science and Technology with Lincoln University and Alex McDonald (Merchants) Ltd.

REFERENCES

- Andow, D. A.; Alstad, D. N. 1998: F₂ screen for rare resistance alleles. *Journal of Economic Entomology* 91: 572–578.
- Cameron, P. J.; Gatland, A. M.; Walker, G. P.; Wigley, P. J. 1997: Alternative host plants as refugia for diamondback moth and potato tuber moth. *Proceedings of the 50th New Zealand Weed and Pest Control Conference* 50: 242–246.
- Cunningham, I. C. 1969: Alternative host plants of tobacco leafminer (*Phthorimaea operculella* (Zell.)). *Queensland Journal of Agricultural and Animal Sciences* 26: 107–111.
- Das, G. P.; Raman, K. V. 1994: Alternate hosts of the potato tuber moth, *Phthorimaea operculella* (Zeller). *Crop Protection* 13: 83–86.
- Davidson, M. M.; Jacobs, J. M. E.; Reader, J. K.; Butler, R. C.; Frater, C. M.; Markwick, N. P.; Wratten, S. D.; Conner, A. J. 2002: Development and evaluation of potatoes transgenic for a *cry1Ac9* gene conferring resistance to potato tuber moth. *Journal of the American Society for Horticultural Science* 127: 590–596.
- Foot, M. A. 1976. Susceptibility of twenty potato cultivars to potato moth at Pukekohe: a preliminary assessment. *New Zealand Journal of Experimental Agriculture* 4: 239–242.

- Frutos, R.; Rang, C.; Royer, M. 1999: Managing insect resistance to plants producing *Bacillus thuringiensis* toxins. *Critical Reviews in Biotechnology* 19: 227–276.
- Genstat 2002: Genstat for Windows, sixth edition. Oxford, VSN International Ltd.
- Gilmour, A. R.; Thompson, R.; Cullis, B. R. 1995: Average information REML, an efficient algorithm for variance parameter estimation in linear mixed models. *Biometrics* 51: 1440–1450.
- Gomaa, A. A.; El-Sherif, S.; Hemeida, I. A. 1978: On the biology of potato tuber worm, *Phthorimaea operculella* (Zeller) (Lepidoptera, Gelechiidae). *Zeitschrift für Angewandte Entomologie* 86: 290–294.
- Gould, F. 1988: Evolutionary biology and genetically engineered crops. *BioScience* 38: 26–33.
- Jansens, S.; Cornelissen, M.; de Clercq, R.; Reynaerts, A.; Peferoen, M. 1995: *Phthorimaea operculella* (Lepidoptera: Gelechiidae) resistance in potato by expression of the *Bacillus thuringiensis* CryIA(b) insecticidal crystal protein. *Journal of Economic Entomology* 88: 1469–1476.
- Li, W. B.; Zarka, K. A.; Douches, D. S.; Coombs, J. J.; Pett, W. L.; Grafius, E. J. 1999: Coexpression of potato PVY⁰ coat protein and cryV-Bt genes in potato. *Journal of the American Society for Horticultural Science* 124: 218–223.
- Mallet, J.; Porter, P. 1992: Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy? *Proceedings of the Royal Society of London Series B* 250: 165–69.
- Rothschild, G. H. L. 1986: The potato moth—an adaptable pest of short-term cropping systems. *In: Kitching, R. L. ed. The ecology of exotic animals and plants.* Brisbane, John Wiley & Sons. Pp. 142–162.
- Roush, R. 1994: Managing pests and their resistance to *Bacillus thuringiensis*: can transgenic crops be better than sprays? *Biocontrol Science and Technology* 4: 501–516.
- Roush, R. T. 1996: Can we slow adaptation by pests to insect transgenic crops? *In: Persley, G. J. ed. Biotechnology and integrated pest management.* Wallingford, United Kingdom, CAB International. Pp. 242–263.
- Tabashnik, B. E.; Liu, Y. B.; Finson, N.; Masson, L.; Heckel, D. G. 1997: One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins. *Proceedings of the National Academy of Science, USA* 94: 1640–1644.
- Tang, J. D.; Collins, H. L.; Metz, T. D.; Earle, E. D.; Zhao, J. Z.; Roush, R. T.; Shelton, A. M. 2001: Greenhouse tests on resistance management of Bt transgenic plants using refuge strategies. *Journal of Economic Entomology* 94: 240–247.

