ALTERNATIVE HOST PLANTS AS REFUGIA FOR DIAMONDBACK MOTH AND POTATO TUBER MOTH

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ABSTRACT

Studies of the host range occupancy of diamondback moth (*Plutella xylostella*) and potato tuber moth (*Phthorimaea operculella*) were carried out to determine what proportion of their populations occur in refugia and would therefore not be exposed to brassica or potato crops transformed to include *Bacillus thuringiensis* toxins. The incidence of known host plants and their infestation levels were estimated in surveys of crops, roadside and waste areas and in replicated field trials. Although potato tuber moth was found on Solanaceae other than potato in the field trial, these alternative hosts were rare in vegetable growing areas and therefore were not significant refugia for this pest. Diamondback moth was common on Brassicaceae other than vegetable brassicas, particularly white mustard (*Sinapis alba*), which was the main refuge. Wild radish (*Rhaphanus raphanistrum*) was less preferred but was the most common alternative host plant on roadsides. The area covered by wild radish was approximately 6% of the area planted in commercial brassicas and therefore formed a minor refuge for diamondback moth. These results suggest that to limit the development of resistance, insect resistant transgenic potatoes should be less widely used than insect resistant transgenic brassicas.

Keywords: Solanaceae, Brassicaceae, *Phthorimaea operculella*, *Plutella xylostella*, alternate hosts

INTRODUCTION

Determining the extent and importance of alternative host plant refugia prior to the deployment of genetically transformed crops is a fundamental first step in preventing the development of insect populations that are resistant to the control toxins present in transgenic crops. Pest species with high proportions of their populations in refugia require a fundamentally different approach to resistance prevention than do species effectively confined to a single host species (Tabashnik *et al.* 1991). This study formed the first part of a programme to identify the risk of resistance to transgenic plants and aimed to determine what proportion of the populations of diamondback moth (DBM) (*Plutella xylostella*) and potato tuber moth (PTM) (*Phthorimaea operculella*) occur in refugia and would therefore not be exposed to transformed brassica or potato crops. Current programs to genetically transform plants for crop resistance to insects in New Zealand include the transformation of vegetable brassicas (Christie and Sinclair 1992) and potatoes (Conner *et al.* 1994) to include *Bacillus thuringiensis* (Bt) toxin genes.

The host range of DBM is generally considered to include most of the Brassicaceae, especially vegetable brassicas, forage crops and mustards (Talekar and Shelton 1993). PTM is mostly confined to solanaceous hosts (Das and Raman 1994), although there is considerable variability in the importance of some hosts, particularly tomato, which suggests the existence of races that have different host preferences. The New Zealand hosts of both these insects are listed in Spiller and Wise (1982). Although much of the literature documents infestations on the preferred hosts of these two insects, the role of the alternative host refugia in maintaining populations is unknown.

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METHOD

Survey

A survey was carried out to estimate the abundance of non-vegetable Brassicaceae and Solanaceae (other than potatoes) that may be hosts for DBM or PTM. For those plants that were large enough to be visible without disturbing foliage, an area of approximately 3 km radius (2500 ha) centred on the Pukekohe Research Centre in South Auckland was surveyed. Roadside, fence line and waste areas were examined and the area of potential host plants estimated. The area of vegetable brassicas, white mustard (*Sinapis alba*), wild radish (*Rhaphanus raphanistrum*) and wild turnip (*Brassica rapa*) was marked on aerial maps and estimated graphically. For plants that were difficult to assess because of their small size, shepherd's purse (*Capsella bursa-pastoris*) and twin cress (*Coronopus didymus*) or sparse distribution, hedge mustard (*Sisymbrium officinale*), an intensive survey of the total 4.7 ha area of the Pukekohe Research Centre in South Auckland was carried out to map the area occupied by each plant species. The main stands of these plants were sampled two to six times during the survey in November and December, and two to three times during January and February. Except for twin cress and shepherd's purse, plants were sampled for insects by inspecting damage on individual plants and beating or sweeping 0.5–1.0 m² samples from stands of the plants. A suction device (Vortis), was used to sample six 4 m² areas of twin cress in the first survey period and two in the second period. Irregular areas of shepherd's purse were sampled to accumulate a total sample area of 0.25 m² in each sampling period. The survey was extended to other areas of Auckland and South Auckland to include another nine potential host plants that occurred in habitats not represented in the grounds of the Pukekohe Research Centre. Roadside and fence line surveys of Solanaceae were carried out by searching for mines of PTM on plants of black nightshade, woolly nightshade, poroporo (*Solanum aviculare*) and potato volunteers at two week intervals on seven occasions from December to March. The collected insects were identified and the rate of infestation for each plant species was compared with the infestation of vegetable brassicas or potato crops.

Field trials

The infestation rates of plant species chosen to represent a range of previously recorded host plants that were relevant to the region were compared in two field trials at the Pukekohe Research Centre. In the Brassicaceae trial, plots of six potential alternate host plants were sown in a 6 x 6 Latin square design. Hedge mustard and cabbage seedlings were transplanted on 5 November 1995 and the remaining species were sown as seed on 14 November. Each plot consisted of a 4.0 x 1.5 m bed and the six rows of plots were separated along their length by double guard rows of cabbage. Each species was planted at a typical density for that crop (Table 1). White mustard was sown in a split plot with 2 m rows of each plant, but only one plant species was assessed. The Solanaceae trial used a similar design with six potential alternate host plants transplanted on 28 November between guard rows of potatoes planted on 10 October. A 2 m spacing was provided between all plots.

In the Brassicaceae trial, six plants from each replicate and cabbage were sampled approximately weekly from 21 December to 15 February for DBM larvae using a combination of searching all the leaves on each plant and beating the plant into a tray. In the Solanaceae trial, estimates of PTM were based on counts of the number of mines per plant from six plants per replicate at approximately two week intervals from 18 December to 21 February. Variations in PTM infestations over time were estimated from a weekly score of the percentage of plants showing any infestation.

DBM estimates per plant were transformed for analysis with the square root transformation, and back-transformed bias adjusted means from this analysis were used to estimate the density of DBM for each plant species. Because there were no significant row effects, the Latin square design was analysed as a randomised block design including cabbage plots from the guard rows. Similar analyses were made for the Solanaceae trial.

RESULTS

Brassicaceae

The survey of 2500 ha around the Pukekohe Research Station estimated the area of vegetable brassicas as 42.7 ha (representing 65% of the total area in Brassicaceae, Table 1) and white mustard cover crops as 14.5 ha (21%). Wild radish, the predominant brassica weed, covered 3.3 ha (6% of Brassicaceae) mostly on roadsides, and wild turnip was comparatively rare. In the intensive survey of the less conspicuous weed plants, twin cress was widely dispersed but occurred at sufficient density to approximately equal the area of wild radish. Hedge mustard appeared in rare stands and shepherd's purse occurred occasionally and at a low density (Table 1).

TABLE 1: Areas of brassica crops and weeds as % of total area of Brassicaceae at Pukekohe, and their infestation by diamondback moth (*Plutella xylostella*) in January 1996.

Plant	Scientific name	Survey		Trial		
		Plant area	DBM	̄(DBM	Plants	DBM
		as % of	/m ²	/plant) ^a	/m ²	/m ²
Brassicaceae						
Vegetable brassica	<i>Brassica oleracea</i>	65 ^b	14.3 ^c	1.96	4	14 ^c
White mustard	<i>Sinapis alba</i>	21	62.0	2.41	12	65
Twin cress	<i>Coronopus didymus</i>	8	0.17	- ^d	-	-
Wild radish	<i>Rhaphanus</i> <i>rhaphanistrum</i>	6	4.8	-	-	-
Wild turnip	<i>Brassica rapa</i>	<0.1	4.6	-	-	-
Hedge mustard	<i>Sisymbrium</i> <i>officinale</i>	<0.1	0	2.17	2.7	12
Shepherd's purse	<i>Capsella bursa-</i> <i>pastoris</i>	<0.1	0	-	-	-
Rape	<i>Brassica napus</i> subsp. <i>Oleifera</i>	-	-	1.61	7	15
Radish	<i>Rhaphanus sativus</i>	-	-	0.63	10.3	6
Turnip	<i>Brassica rapa</i>	-	-	1.29	13	17
Indian mustard	<i>Brassica juncea</i>	-	-	2.79	12	89
LSD (P<0.05)				0.463		

^a Square root (mean DBM/plant + 0.5), from 6 plants/plot

^b Refers to all vegetable brassicas

^c Refers to cabbage

^d No observation

White mustard was most heavily infested by DBM, followed by cabbage, wild radish, wild turnip, and twin cress (Table 1). Three white mustard crops were present as cover crops in the survey area. One, an early-sown white mustard crop, was not infested in the November–December survey period, but a summer crop produced the most dense infestations of DBM on any plant surveyed. Wild radish was the most commonly infested weed plant. Although this weed was common from October to March and started to senesce in February, infestations of DBM did not occur until December, about the same time that vegetable brassicas became infested. Minor infestations were found on twin cress and wild turnip was uncommon in the survey but was moderately infested. In the wider survey, winter cress (*Barbarea* sp.), sweet alyssum (*Lobularia maritima*) and water cress (*Nasturtium officinale*) were not infested, although they have previously been reported as hosts (Spiller and Wise 1982). Infestation of small plantings of night-scented stock (*Mathiola bicornis*), wallflower (*Cherianthus cheiri*), candy tuft (*Iberis* sp.), Argentine cress (*Lepidium bonariensis*) and garden cress (*Lepidium sativum*) and shepherd's purse (*Capsella bursa-pastoris*) indicated the potential importance of rarer plants as hosts, although garden flowers are likely to be a very minor and unpredictable source of DBM in vegetable growing regions.

In the Brassicaceae trial, DBM populations for all plant species peaked in January. Three plant species supported population densities similar to cabbage (Table 1) and two species, the taller more densely planted species Indian mustard (*Brassica juncea*) and white mustard, supported several times the populations found on cabbage. To provide an index of DBM populations on the main hosts in the survey area we multiplied the area of each host plant by the density of DBM/m² (Table 1). This index ranked white mustard (899 x 10⁴ DBM) as a slightly more common host than vegetable brassicas (610 x 10⁴ DBM), with populations on wild radish an order of magnitude less (15.8 x 10⁴). Twin cress was a minor host with a population of 0.5 x 10⁴ DBM in the survey area.

Solanaceae

In roadside surveys, black nightshade (*Solanum nigrum*) formed 50% of the solanaceous plant population, followed by woolly nightshade (*Solanum mauritanium*) which formed 37%, and volunteer potatoes (13%). None of these plants was infested with PTM, although 70% of volunteer potatoes in cropping fields were infested. Thorn apple (*Datura stramonium*) was not encountered in the survey but was infested in neighbouring areas. By contrast with roadside surveys, several species of Solanaceae were infested in the field trial (Table 2). The first infestations occurred in potatoes, with half the potato plots showing evidence of infestation in late December and peak infestations occurring from mid January to late February. Poroporo and pepino (*Solanum muricatum*) were infested over a similar period, and eggplant 2-3 weeks later. Potato was the most heavily infested plant with a peak of 8.6 mines per plant compared with 2.8 mines on poroporo. The number of mines per plant and estimates of the percentage of plants infested showed a similar pattern of infestation (Table 2).

TABLE 2: Infestation by potato tuber moth (*Phthorimaea operculella*) of Solanaceae at Pukekohe in January-February 1996.

Plant	Scientific name	Survey % infested plants	Trial ^a % infested plants	š(mines /plant) ^b	Mines /plant ^c
Potato crop	<i>Solanum tuberosum</i>	100	100	3.02	8.6
Potato volunteer		70	-	-	-
Potato roadside		0	-	-	-
Black nightshade	<i>Solanum nigrum</i>	0	0	-	0
Woolly nightshade	<i>Solanum mauritanium</i>	0	-	-	-
Eggplant	<i>Solanum melongena</i>	-	50	1.24	1.1
Pepino	<i>Solanum muricatum</i>	-	68	1.53	1.8
Poroporo	<i>Solanum aviculare</i>	0	48	1.80	2.8
Tomato	<i>Lycopersicon</i> <i>lycopersicon</i>	-	0	-	0
Capsicum	<i>Capsicum annuum</i>	-	0	-	0
LSD (P<0.05)			11.0	0.183	

^a Species with 0 or 100% infestation in the trial were omitted

^b Square root (mines/plant + 0.5) from 6 plants/plot

^c Back transformed bias adjusted means

DISCUSSION

The three host plants supporting the majority of the DBM population in the Pukekohe survey area were cover crops of white mustard, vegetable brassica crops and wild radish in marginal areas. Twin cress was a host to less than 1% of the population. The main factor determining the proportion of the DBM population supported by a host plant species was its abundance in the area surveyed. Many of the additional garden plants surveyed were infested but would not usually be present in vegetable growing areas. The study also demonstrated that the trial results were not always representative of infestations in natural locations. For example, hedge mustard was infested in the trial

but not in the survey. This may have been caused by the proximity of infested cabbage plants or differences in growing conditions. However, both the survey and trial results indicated that summer plantings of white mustard supported DBM populations that were of a similar magnitude to the total population levels found on vegetable brassicas. The persistence of wild radish on roadsides over the whole growing season appeared to provide a small but stable host plant source for DBM at Pukekohe. By contrast, the sowing of white mustard crops is more variable in time and space. The contribution of each of these hosts to the seasonal development of DBM populations is unclear. Our monitoring to date suggests that populations build up synchronously on the different host plants.

PTM populations were almost totally confined to potatoes. Despite their ability to develop on poroporo, eggplant and pepino in the field trial, PTM was not found on naturally occurring poroporo, the only one of these species to occur as a weed. This distribution and the occurrence of PTM on volunteer potatoes in fields but not on roadsides suggested that the adults are not very mobile and would be unlikely to find isolated weed hosts. The dispersal of PTM and DBM, and their ability to transfer between plant species is the subject of further research.

These results suggest that transformation of the main vegetable brassicas - cabbage, cauliflower and broccoli - would expose about half of the DBM in the survey area to *B. thuringiensis*. By contrast, transformation of potatoes would expose almost all of the PTM population to *B. thuringiensis*, either in crop potatoes or in volunteers. This information suggests that the use of transformed potatoes should be limited in space or in time to minimise possible selection for resistant PTM. As DBM have significant refugia, it may be possible to utilise transformed vegetable brassicas more widely than potatoes without significant risk of the development of resistance.

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