

**MATING DISRUPTION REDUCES THE RISK OF RESISTANCE
DEVELOPMENT TO TRANSGENIC APPLE ORCHARDS:
SIMULATIONS OF THE LIGHTBROWN APPLE MOTH**

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ABSTRACT

Computer simulations were used to determine the effect of interactions between trees expressing Bt-toxin and mating disruption on resistance evolution in the lightbrown apple moth. In transgenic orchards, we assumed that 22% of the population was in the understorey and not subject to selection. Increasing expression of the Bt-toxin so that mortality increased from 90% to 99.99%, reduced the resistance allele frequency after 1000 d from 82% to 0%. When 11% of the simulated orchards were replaced with wild, nontransgenic habitat (external refuge), the resistance allele frequencies declined compared to simulations without the external refuge. The reduction was greatest when the expression of Bt-toxin was highest. Mating disruption always reduced the resistance allele frequency. When the expression of the Bt-toxin gene killed 90% of susceptible individuals, the resistance allele frequency was 82% after 1000 d, but 61.4% with 10% mating disruption, and 0.154% with 90% mating disruption (the initial resistance allele frequency was 0.1%). Mating disruption decreased the size of the treated population and increased the relative effect of immigration from refugia.

Keywords: leafroller, *Epiphyas postvittana*, apple, transgenic, Bt, resistance, simulation

INTRODUCTION

Current broad spectrum insect control tactics are increasingly recognised as imposing unacceptable costs to environmental and human health, and may present trade barriers in the future (Christie 1993). Transgenic plants will offer significant opportunities for production of high quality fruit with environmentally safe, sustainable and effective pest control practices (Conner *et al.* 1990). Such crops are already under development, and include apples designed to express *Bacillus thuringiensis* (Bt) insecticidal crystal proteins against leafrollers.

Deployment of transgenic crops expressing Bt genes or other environmentally benign insecticidal proteins will undoubtedly have ecological consequences, including selection pressure for pest resistance. Overseas experience has shown several major pest species have already developed resistance to Bt δ -endotoxins in the laboratory and some have evolved widespread resistance to Bt biopesticides in the field (Tabashnik 1994). Commercially-developed transgenic crops expressing Bt-toxins have only just been released and there are, as yet, no records of insect resistance to them.

However, co-evolution of key pests represents a significant threat to this fruit breeding research investment (Wigley *et al.* 1994). High reproductive and dispersal rates, combined with exceptionally wide host ranges and overlapping generations, make leafrollers significant pests of export horticulture. One species of leafroller, the lightbrown apple moth (*Epiphyas postvittana*), has developed resistance to

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organochlorines (Smith 1961), organophosphates and carbamates (Suckling and Khoo 1990). Resistance to organophosphates is also known in one native leafroller, *Planotortrix octo* (Wearing 1995). These pests are likely to be capable of becoming resistant to Bt-toxins if inappropriate management is used. Leafrollers have consequently been chosen as a model for this research, which is designed to provide the basis for managing resistance to Bt δ -endotoxins being incorporated for insect control into transgenic plants and to ensure that their development and deployment has minimal risk of failure.

Evolution of resistance in the field is extremely hard to investigate directly, and models have been used extensively for identifying key parameters relating to resistance development (Tabashnik 1994). Modelling of resistance risk under different management regimes is an essential part of pro-active resistance management for minimising any loss of investment in transgenic plant development. Stochastic simulation, using a mosaic of treated fields and untreated refugia (Caprio 1994) under different regimes has been used to investigate the impact of various deployment scenarios on resistance frequency, including compatible methods for reducing the population in the crop and managing resistance, such as deployment of sex pheromones in mating disruption (Suckling *et al.* 1990a).

MATERIALS AND METHODS

A stochastic, multiple field simulation model was adapted from an earlier model of insecticide resistance (Caprio and Tabashnik 1992; Caprio 1994), to include mating disruption and its effects on resistance evolution. The model simulated resistance development in nine fields (large scale grids). Each field was independent in both population dynamics and genetics, except for gene flow. The life table parameters used in the model are given in Table 1. The generation time for the simulated insect was 68 d, and there are three generations per year, so that 204 simulated days may be considered one physiological year. Fields were further sub-divided into nine separate patches (81 patches in total) which represented either apple or understorey. Adult females would lay eggs at random among all patches within a field (and would mate at random with males in all patches), while larvae were limited by movement to the patch into which they were originally oviposited. *E. postvittana* is highly polyphagous (Danthanarayana 1975), and large numbers can be found in the understorey in orchards (Geier and Briese 1980; Thomas and Burnip 1993). We simulated this understorey by making either 0, 1, or 2 (the default) of the nine patches within a field non-transgenic. This range was chosen to represent conditions which could be achieved through understorey management (such as grazing or mowing), rather than the maximum understorey population recorded. In simulations that included an external refuge, all nine patches in one of the nine large-scale fields were left non-transgenic. This level of immigration was likely to be at the low end of the range, corresponding to larger areas of contiguous orchards. This value was chosen to ensure that resistance arose eventually, permitting a comparison of treatments on a relative, rather than absolute time scale. All treatments were replicated five times.

We simulated mating disruption (Suckling and Shaw 1992, 1995) by reducing the number of eggs laid by the female population. Thus, with 90% mating disruption, females only laid 10% of their normal reproductive capacity. To simplify the model, all females in a field were assumed to have the same mating status. This had the disadvantage that mated females immigrating into a field with mating disruption were treated as if they had to remate, which was unrealistic. The bias introduced by this assumption was likely to be small, however, as such females were apt to be susceptible and produce susceptible offspring, most of which would die due to the Bt-toxin. This relationship held until resistant adults began to form a significant proportion of the immigrants, by which time we may assume that the refuge had been contaminated by resistance alleles and resistance was developing rapidly.

Suckling *et al.* (1990b, 1994) found adult *E. postvittana* did not disperse long distances (80% of released males were recaptured within 100 m of the release point over their 2-3 week lifespan). We simulated this adult dispersal with a multi-step

random walk with a 90% probability of terminating the walk after each iteration. This resulted in fewer than 5% of the moths dispersing a distance of more than 1 field (not all movements are away from the original field).

We assumed that the Bt-toxin produced in the trees generated mortality only in larvae; adults, pupae and eggs were unaffected by the toxin. The increase in the mortality rate due to the presence of Bt-toxin for neonate susceptible larvae was varied from 90% to 99%, 99.9% and 99.99%. This value will ultimately depend upon the level of expression achieved in the transgenic trees. While the expression of Bt-toxin in cotton plants has been shown to vary with crop phenology, we made the simplifying assumption that toxin expression (and mortality) was constant. We also assumed that the resistance ratio of the LC_{50} of the resistant larvae compared to the susceptible larvae was 100 fold. The dominance of the resistance gene (h) was either 0.1 (a recessive gene) or 0.5 (an additive or co-dominant gene). We chose to concentrate on co-dominant resistance alleles as these pose greater risk for the development of resistance. Most cases where resistance has been reported to Bt-toxin have been due to recessive alleles, though in one case of field resistance the resistance was dominant (Gould *et al.* 1992). Recessive counterparts to most of the co-dominant simulations were run, but provided little additional information and are not reported here. We examined the development of resistance under three experimental conditions.

Experiment 1

This experiment was designed to determine the importance of the internal and external refugia, with varying expression of the Bt toxin. We assumed that there was no foliage available for *E. postvittana* outside the orchards (no external refuge), similar to very large areas of contiguous orchards. The dominance of the resistance gene was varied from recessive ($h=0.1$) to co-dominant ($h=0.5$). The size of the internal refuge in the understorey was then varied from 0 to 2 patches (0 to 22% of the available habitat in the orchard). We also varied Bt-toxin expression in the transgenic trees by changing mortality of susceptible neonates due to the toxin from 90% to 99%, 99.9% and 99.99%. In a further run, we left one out of nine fields as untransformed habitat (an external refuge, beyond the understorey in each orchard that constituted the internal refuge). This was based on the dense areas of orchards in some fruitgrowing regions.

Experiment 2

To determine the interaction of variable mating disruption and Bt-toxin expression, we had one field as an external refuge and two patches within each field as an internal refuge, and varied the amount of mating disruption from 10% to 30, 50, 70 and 90%. The mortality of susceptible neonates was again varied from 90% to 99, 99.9, and 99.99%.

TABLE 1: Life table parameters used for the *E. postvittana* model.

Stage	Development time (d)	Daily mortality rate (%)	Eggs /female /day	Dispersal (% of individuals)
Egg	12 ^a	19 ^{b,c}	0	0
Larvae	39 ^{a,c}	6 ^b	0	0
Pupae	12 ^{a,c}	0.5 ^b	0	0
Young adults	10 ^b	0.7 ^a	40 ^a	18 ^f
Old adults	7 ^{a,d}	15 ^a	15 ^a	13 ^f

^a Geier and Briese (1981)

^b Geier and Briese (1980)

^c Mortality estimated, up to stage of established larvae

^d Older adults that contribute little to future generations were not simulated

^e Tomkins *et al.* (1989)

^f Suckling *et al.* (1994)

Experiment 3

Finally, in order to investigate the influence of mating disruption and immigration on resistance evolution, we held mortality of susceptible neonates at 90%, and maintained one field as an external refuge, but varied the amount of the understorey from 0% to 22%, while varying the amount of mating disruption from 10% to 30, 50, 70 and 90%.

RESULTS AND DISCUSSION**Experiment 1**

Increasing Bt-toxin gene expression in orchards, with an internal refuge of non-transgenic plants (and no immigration from non-transgenic fields) decreased the mean resistance allele frequency after 1000 d (Fig. 1A). This is an example of a "high dose" strategy (Tabashnik and Croft 1982; Roush 1989) combined with an internal refuge. Removing the internal refuge resulted in an increase in the resistance allele frequency with higher levels of Bt-toxin expression unless the population went extinct. The

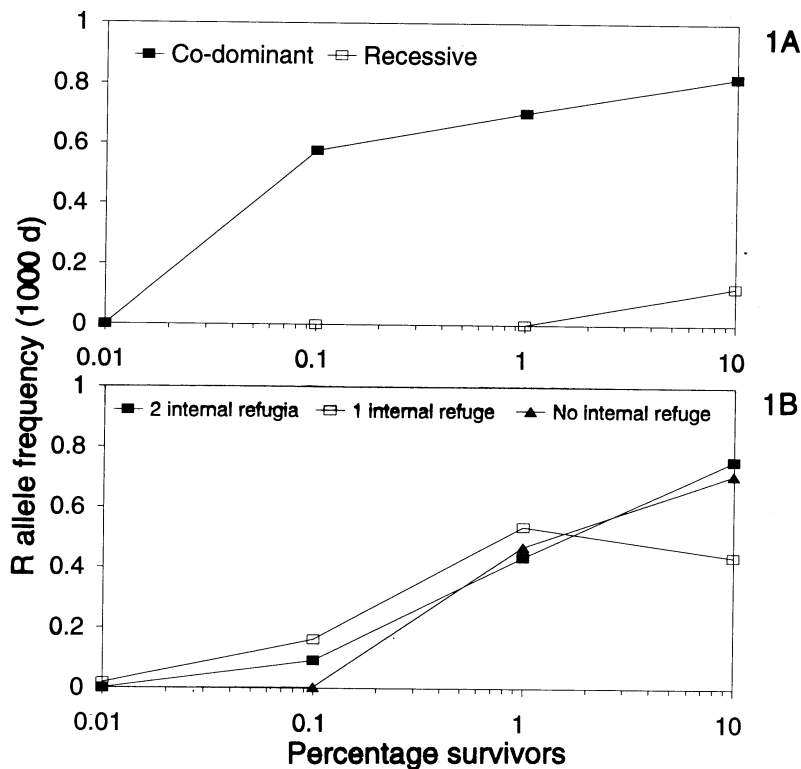


FIGURE 1: Resistance allele frequency after 1000 days in simulations of four levels of Bt-toxin gene expression with: (1A) nine transgenic orchards, with no external refuge or mating disruption, to compare co-dominant and recessive alleles, or with (1B) eight of nine fields treated as transgenic orchards and one of nine fields as an external refuge (11% of fields), to compare three levels of internal refuge (0, 11 or 22% of each transgenic field).

probability of extinction decreases with increasing population size (Caprio 1994), which makes the high dose strategy without a refuge a high risk strategy. When one out of nine fields was non-transgenic and added to the systems as an external refuge, it was more effective at delaying resistance evolution than the internal refuge (black squares in Figs. 1A and 1B). The external refuge may be more effective for several reasons. Females emerging in the internal refuge lay most of their eggs on transgenic tissue, few of which survive. This effectively reduces the reproductive capacity of females in transgenic fields. The external refuge also takes longer to become contaminated with resistance alleles because of limited gene flow out of transgenic orchards, and it retains its effectiveness longer. In many real life situations, the external refugia would be much larger.

When these simulations were run with a recessive resistance allele, the *E. postvittana* population persisted only when there was low expression of Bt-toxin in transgenic trees (90% mortality). At higher levels of expression, the moth population went extinct (Fig. 1A).

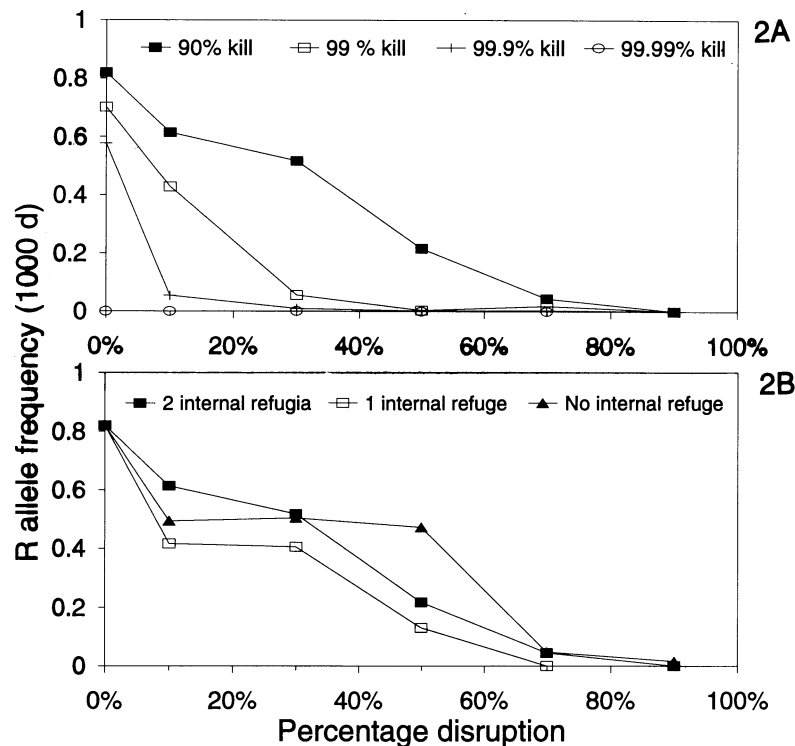


FIGURE 2: The impact of variable levels of mating disruption on resistance allele frequency after 1000 days in simulations with (2A) eight of nine fields treated as transgenic orchards, with one external refuge, and two internal refuge patches (22% of each transgenic field) at four levels of Bt-toxin gene expression, or with (2B) one external refuge (11% of fields), and three levels of internal refuge patches (0, 11 and 22% of each transgenic field).

Experiment 2

Simulating six levels of mating disruption combined with four levels of Bt-toxin expression showed that mating disruption decreased the resistance allele frequency after 1000 d (Fig. 2A) compared to simulations without disruption. With low Bt-toxin expression, mating disruption decreased the resistance allele frequency from 82% to 0.15%. Mating disruption was more effective when Bt expression was higher. These results suggest that mating disruption can synergize the maintenance of susceptibility to Bt-toxins. Mating disruption acted as the equivalent of an additional mortality factor in the transgenic orchards, reducing the population size in the orchards. Populations in orchards without mating disruption (or in wild habitat) remained large and continued to produce the same number of emigrants. Because of the reduced population size in transgenic orchards, these emigrants made up a larger proportion of the population, and were more effective at delaying resistance development.

Experiment 3

When we varied the amount of internal refuge in the transgenic orchards with mating disruption, there was little effect on the overall development of resistance (Fig. 2B). This result again suggests that the external refuge are more effective than the internal refuge at delaying resistance.

CONCLUSIONS

These simulations suggest that mating disruption, in combination with refugia, can be effective for maintaining susceptibility in *E. postvittana* populations to Bt-toxins expressed in transgenic orchards. The results also suggest that maintaining wild-type habitats (or non-transgenic orchards) is important in delaying resistance development. These external refugia appear more effective at reducing the risk of resistance development than internal refugia, such as the understorey in a transgenic orchard. Other mortality factors are likely to produce similar effects in delaying resistance development. For example, increasing the level of biological control in a transgenic orchard may also delay resistance development. It is possible that pyramiding multiple toxins into transgenic trees will also reduce the risk of resistance development, though here the additional mortality factor is also a selective agent, and the benefits may not be as great. Further simulations are needed to address this important question.

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REFERENCES

- Caprio, M.A., 1994. *Bacillus thuringiensis* gene deployment and resistance management in single and multi-tactic environments. *Biocontrol Science and Technology* 4: 487-497.
- Caprio, M.A. and Tabashnik, B.E., 1992. Gene flow accelerates local adaptation among finite populations: simulating the evolution of insecticide resistance. *J. Econ. Entomol.* 85: 611-620.
- Christie, R., 1993. Plant protection and international trade. Pp 1-7 *In: Plant Protection: Costs, Benefits and Trade Implications*. Suckling, D.M. and Popay, A. (Eds). N.Z. Plant Protection Society, Christchurch. 161p.
- Conner, A.J., Bezar, H.J. and Ashby, J.W., 1990. Genetic engineering of plants for weed, disease and pest control: science versus politics. *Proc. 43rd N.Z. Weed and Pest Control Conf.*: 200-208.
- Danthanarayana, W., 1975. The bionomics, distribution and host range of the light brown apple moth, *Epiphyas postvittana* (Walk.) (Tortricidae). *Aust. J. Zool.* 23: 419-437.
- Geier, P.W. and Briese, D.T., 1980. The light-brown apple moth, *Epiphyas postvittana* (Walker): 5. Variability of demographic characteristics in field populations of southeastern Australia. *Aust. J. Ecol.* 5: 135-142.

- Geier, P.W. and Briese, D.T., 1981. *Epiphyas postvittana* (Walker): a native leafroller fostered by European settlement. Pp 131-149 In: The Ecology of Pests. Some Australian Case Histories. Kitching, R. L. and Jones, R.E. (Eds.) CSIRO, Melbourne.
- Gould, F., Martinez-Ramirez, A., Anderson, A., Ferre, J., Silva, F.J. and Moar, W.J. 1992. Broad-spectrum resistance to *Bacillus thuringiensis* toxins in *Heliothis virescens*. *Proc. Nat. Acad. Sci. USA*. 89: 7986-7990.
- Roush, R. T. 1989. Designing resistance management programs: how can you choose? *Pestic. Sci.* 26: 423-441.
- Smith, A.G., 1961. Control of apple leafrollers in Nelson. *Orchardist of N.Z.* 34: 315-317
- Suckling, D.M. and Khoo, J.G.I., 1990. Cross-resistance in the lightbrown apple moth *Epiphyas postvittana* (Lepidoptera: Tortricidae). *N.Z. J. Crop and Hort. Sci.* 18: 173-180.
- Suckling, D.M., Khoo, J.G.I and Rogers, D.J., 1990a. Resistance management of *Epiphyas postvittana* (Lepidoptera: Tortricidae) using mating disruption. *N.Z. J. Crop and Hort. Sci.* 18: 89-98.
- Suckling, D.M., Khoo, J.G.I and Rogers, D.J., 1990b. Disruption of lightbrown apple moth *Epiphyas postvittana* (Lepidoptera: Tortricidae) trapping in Nelson, New Zealand. *Aust J. Zool.* 38: 363-373.
- Suckling, D.M. and Shaw, P.W., 1992. Conditions that favor mating of disruption of *Epiphyas postvittana* (Lepidoptera: Tortricidae). *Environ. Entomol.* 21: 949-956.
- Suckling, D.M. and Shaw, P.W., 1995. Large scale trials of mating disruption of lightbrown apple moth in Nelson, New Zealand. *N.Z. J. Crop and Hort. Sci.* 23: 1-11.
- Suckling, D.M., Burnip G.M, Brunner, J.F. and Walker, J.T.S., 1994. Dispersal of *Epiphyas postvittana* (Walker) and *Planotortrix octo* Dugdale (Lepidoptera: Tortricidae) at a Canterbury, New Zealand orchard. *N.Z. J. Crop and Hort. Sci.* 22: 225-234.
- Tabashnik, B.E., 1994. Evolution of resistance to *Bacillus thuringiensis*. *Ann. Rev. Entomol.* 39: 47-79.
- Tabashnik, B. E. and Croft, B. A., 1982. Managing pesticide resistance in crop-arthropod complexes: interactions between biological and operational factors. *Environ. Entomol.* 11: 1137-1144.
- Thomas, W.P. and Burnip, G.M., 1993. Lepidopterous insect pests and beneficials - the influence of the understorey. *HortResearch Technical Report No. 93/3*: 13-19.
- Tomkins, A.R. Penman, D.R. and Chapman, R.B., 1989. Effect of temperature and host plant on development of three species of leafroller (Lepidoptera: Tortricidae). *New Zealand Entomologist* 12: 438-53.
- Wearing, C.H., 1995. Management of organophosphate insecticide resistance in *Planotortrix octo* using mating disruption. *Proc. 48th N.Z. Weed and Pest Control Conf.*: this volume
- Wigley, P.J., Chilcott, C.N. and Broadwell, A.H., 1994. Conservation of *Bt* efficacy in New Zealand through the planned deployment of *Bt* genes in transgenic crops. *Biocontrol Science and Technology* 4: 527-534.