Using Mass-Release of Engineered Insects to Manage Insecticide Resistance

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ABSTRACT: Transgenic crops expressing insecticidal toxins derived from *Bacillus thuringiensis* (Bt) are widely used to control insect pests. The benefits of such crops would be lost if resistance to the toxins spread to a significant proportion of the pest population. The main resistance management method, mandatory in the US, is the high-dose/refuge strategy, requiring nearby refuges of toxin-free crops, and the use of toxin doses sufficiently high to kill not only wild type insects but also insects heterozygous for a resistance allele, thereby rendering the resistance functionally recessive. We propose that mass-release of harmless toxin-sensitive insects could substantially delay or even reverse the spread of resistance. Mass-release of such insects is an integral part of RIDL®, a genetics-based method of pest control related to the Sterile Insect Technique. We used a population genetic mathematical model to analyze the effects of releasing male insects homozygous for a female-specific dominant lethal genetic construct, and concluded that this RIDL strategy could form an effective component of a resistance management scheme for insecticidal plants and other toxins.

Key Words: resistance management; *Bacillus thuringiensis*; mathematical modeling; RIDL; high-dose/ refuge strategy

OVERVIEW ARTICLE

Transgenic crops producing insecticidal proteins are now widely used to control insect pests. In 2005, 26 million hectares were planted with crops expressing toxins derived from *Bacillus thuringiensis* (Bt) (James, 2005). These were principally maize (corn) and cotton. Other Bt plants are available, under development or being considered. Such crops can reduce the need for insecticide sprays, particularly broad-spectrum chemicals, and have environmental, health and economic benefits (Shelton et al., 2002).

The benefits of Bt crops would be lost if resistance to the toxins spread to a significant proportion of the insect pest population. Resistance to Bt crops has not yet been documented in the field (Bates et al., 2005). But bearing in mind the history of resistance to chemical insecticides, pathogen resistance to antimicrobial drugs and similar problems, there are concerns that it is a matter of when,

not if, resistance to Bt crops will develop. Indeed, resistance to Bt sprays has been found in the field in *Plutella xylostella* (diamondback moth) (Ferré and Van Rie, 2002) and in the greenhouse in *Trichoplusia ni* (cabbage looper) (Janmaat and Myers, 2003). It is no surprise, therefore, that a lot of effort has been directed towards finding methods to prevent the spread of resistance in wild pest populations, or reverse it if it occurs.

The primary resistance management method currently in use, and mandatory in the US, is the high-dose/refuge strategy. Refuges are areas of non-Bt host plants which are required to be grown near the insecticidal crops. Resistance to Bt is generally thought to be due to a mutant (resistance) allele at one genetic locus. The high-dose part of the strategy requires the Bt crops to express sufficiently high doses of toxin to kill not only wild type insects but also insects heterozygous for a resistance allele, thereby rendering the resistance functionally recessive so that only homozygous resistant insects survive on the Bt crops. In the toxin-free refuges, susceptible insects can survive; resistant insects mate with them, pro-

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ducing heterozygous offspring that cannot survive on Bt crops, which tends to reduce the resistance allele frequency. Mathematical modeling predicts that the high-dose/refuge strategy slows or prevents the spread of resistance (Alstad and Andow, 1995; Carrière and Tabashnik, 2001; Gould, 1998; Tabashnik et al., 2004; Tabashnik et al., 2005; Tabashnik et al., 2003; Vacher et al., 2003).

Refuges are a crucial component of the high-dose/refuge strategy. They are areas where resistant insects are expected to be at a selective disadvantage (due to fitness costs) and they provide a source of susceptible genotypes that will mate with resistant insects from toxin-treated regions. This tends to reduce the frequency of resistance alleles. However, this comes at a cost – insect damage in the refuges and/or additional control costs to mitigate this. Alternative sources of susceptible insects could in theory perform the same function as refuges, but without sacrificing valuable acreage to insect-susceptible crops.

In principle, alleles conferring sensitivity to Bt toxins could be provided by mass-rearing susceptible insects and releasing them into the environment. Deliberate release of large numbers of the pest insect is unlikely to be viewed as a good idea. However, with modification, the strategy may have merit.

The release of mass-reared insects is central to the Sterile Insect Technique (SIT) but release only occurs after the insects have been sterilized by irradiation (Dyck et al., 2005; Knipling, 1955; Krafsur, 1998). Because the released insects are sterile, no viable offspring result from matings between the released insects and the wild population, so there is no introgression of sensitivity alleles into the wild population. The toxin-sensitive alleles provided by traditional SIT are not inherited and so can play no part in the evolution of resistance allele frequency in the pest population.

However, we have previously proposed a modification to the SIT in which the released insects are not irradiated, but instead are ho-

mozygous for one or more dominant lethal genes (Release of Insects carrying a Dominant Lethal, RIDL) (Alphey, 2002; Alphey and Andreasen, 2002; Gong et al., 2005; Thomas et al., 2000). The lethal genes are repressible, using a dietary additive, so that mass-rearing can occur. One version of this system posits female-specific dominant lethal constructs and the use of male insects homozygous for one or more of those ("RIDL males"). When RIDL males mate with wild females all of their F1 progeny would inherit a dominant female-specific lethal, so the female offspring would die but the males would live. Like the SIT, the lack of viable female offspring would reduce the reproductive potential of the wild population. The male offspring would inherit genes from their fathers, including the lethal gene and the allele conferring susceptibility to Bt toxins. The nature of repressible female-specific lethal genes means that a male-only population for release can be achieved by withdrawing the repressor for the generation intended for release (male-only release is generally desirable for SIT (Marec et al., 2005; Rendon et al., 2004)). Release of such RIDL males would therefore provide a potential source of sensitivity alleles, combined with a pest control mechanism, that could be appropriate for managing pest resistance to Bt crops.

We explored the question of whether mass-release of toxin-sensitive RIDL male insects could substantially delay or reverse the spread of resistance to Bt crops using mathematical modeling of population genetics. We found that release of RIDL males slows, and can reverse, the spread of resistance. A significant effect was observed at release ratios considerably lower than those typical for SIT programs that provide effective suppression of the target population. There is an interaction between refuge size and RIDL release ratio (the ratio of released RIDL males to males in the wild); resistance spreads faster with a smaller refuge, RIDL release slows or reverses this. A higher initial resistance

allele frequency requires more RIDL males or a larger refuge. For a range of parameter values, we determined the minimum refuge size for control of resistance, i.e. the critical refuge size at which the resistance allele frequency neither increases nor decreases. We showed that RIDL release reduces the minimum refuge size needed to stop the spread of resistance. The proportional reductions in minimum refuge sizes are not very sensitive to differing relative fitness estimates, which is reassuring as empirical methods for estimating these in field populations are problematic and estimated values vary considerably (Bourguet et al., 2000; Tabashnik et al., 2004; Tabashnik et al., 2005).

This system of release of RIDL males would need a smaller refuge to maintain equivalent levels of resistance management, thus allowing for more efficient use of valuable land. For realistic values of initial resistance allele frequency, of the order of 0.001 based on limited field data (Gould et al., 1997; Stodola et al., 2006), a release ratio as low as 1:4 (one RIDL male per four males in the wild) could rationally justify a reduction in refuge size to as low as 2-3% of the value required without RIDL release. In many cases, such a low refuge size of perhaps 0.2-0.3% of the crop area would automatically be provided by alternative host plants or less than universal planting of the insecticidal variety. Therefore, the use of Bt crops in combination with the release of RIDL males, even at a low level, may provide the key resistance management benefits of the high-dose/refuge strategy without the need deliberately to plant valuable land with crops that are vulnerable to insect damage.

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