DYNAMICS OF RESISTANCE TO NOVEL INSECTICIDES IN WHITEFLIES

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Abstract

The successful management of insecticide resistance benefits from a knowledge of the biological characteristics of pests that promote or retard resistance development. For the whitefly *Bemisia tabaci* (= *B. argentifolii*), these factors include its haplo-diploid breeding system, its breeding cycle on a succession of treated or untreated hosts, and its occurrence on and dispersal from high value crops in greenhouses and glasshouses. These factors, in conjunction with often intensive insecticide use, have led to severe and widespread resistance that now affects several novel as well as conventional control agents. Under the ecological conditions prevailing in some cotton-growing areas of Israel, there is evidence that even restricting new insecticides to a single application per year does not suppress resistance indefinitely. The implications of these findings for comparable cotton-growing systems in the southwestern USA are discussed.

Introduction

Managing resistance to insecticides entails finding practical solutions to problems posed by the adaptability of insect pests. The maximum number of insecticide applications that can be accommodated whilst suppressing resistance will depend on several interacting factors including the genetic and ecological attributes of pest species, the number of cross-resisted chemicals available, and the type of management tactics adopted. As a general rule, pests whose biology renders them most vulnerable to developing resistance are those for which restrictions on insecticide exposure should be most stringent (Denholm et. al., 1998a). The basic dilemma is that these are often the species whose biology also renders them the most abundant and damaging, and therefore the primary targets of insecticide treatments. For this reason, they may also be ones in which resistance is already well advanced, diminishing the supply of effective compounds and placing new insecticides under severe threat of overuse and hence resistance from the outset (Cahill and Denholm 1999; Denholm et. al., 1998a).

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The challenges of combating resistance within such constraints are well exemplified by the whitefly *Bemisia tabaci* Gennadius (= *B. argentifolii* Bellows and Perring). In many cropping systems, the capacity of *Bemisia* to evolve resistance has precipitated a classic treadmill of increasing numbers of applications and rapid depletion of effective control agents (e.g. Horowitz et. al., 1994; Denholm et. al., 1996; Dennehy and Williams 1997). In this paper, we explore some of the biological characteristics of *Bemisia* that promote resistance in agricultural and horticultural systems, and consider their implications for resistance management. Particular emphasis is placed on the dynamics of resistance to novel insecticides that currently underpin whitefly control strategies on cotton in Israel (Horowitz and Ishaaya 1994; Horowitz et. al., 1994, 1999), and have recently been introduced for the same purpose on cotton in the southwestern USA (Dennehy and Williams 1997; Dennehy and Denholm 1998).

Key Factors Influencing Resistance in *Bemisia*

Haplo-Diploidy

Like other whiteflies, *Bemisia* has long been assumed to possess a breeding system based on haplo-diploidy, whereby males result from unfertilized, haploid eggs, and females result from fertilized, diploid eggs. Until recently, however, there was no direct evidence for this phenomenon. Blackman and Cahill (1997) have since published karyotypes for several strains of *Bemisia* (covering both *B. tabaci* and *B. argentifolii*), confirming that males possess only half (n=10) the female complement (2n=20) of chromosomes.

From the standpoint of selecting for resistance, the primary consequence of haplo-diploidy is that resistance genes arising by mutation are exposed to selection from the outset in hemizygous males, irrespective of intrinsic dominance or recessiveness. The potential for this to accelerate resistance development has been noted by several authors (e.g. Havron et. al., 1987; Brun et. al., 1995; Denholm et. al., 1998b), and it has undoubtedly contributed to the capacity of *Bemisia* to develop resistance rapidly to newer insecticides, especially in enclosed environments. Although this breeding system need not invariably speed up the appearance of resistance, it does in a sense predispose haplo-diploid species to resistance compared to fully diploid organisms such as bollworms and boll weevils.

Ecology in Field Crops

For highly polyphagous pests such as *Bemisia*, interactions between ecology and resistance can be complex, reflecting the seasonality and relative abundance of treated and untreated hosts, and patterns of migration between hosts at different times of the year. There are still few agricultural systems for which these interactions have been resolved sufficiently to understand their influence on resistance development. However, the best understood ones involve cotton, especially those in the southwestern deserts of the

USA, where long-term research into whitefly ecology is complemented by extensive monitoring of changes in resistance levels (e.g. Dennehy and Williams 1997).

The key feature of these systems is the continuous availability of whitefly hosts, enabling *Bemisia* populations to develop actively throughout the year. However, variation between regions evidently has profound implications for the speed at which resistance is selected and for the effectiveness of resistance management recommendations. In the Imperial Valley of California, spring melons remain an important crop but the acreage of cotton has declined dramatically. Monitoring of whitefly resistance since 1993 has shown that resistance has generally increased each season as a consequence of insecticide use on spring melons. However, it has tended to decline subsequently on cotton, due partly to the extensive immigration of susceptible whiteflies into cotton from adjacent, unsprayed hosts. As a consequence, there has been no overall increase in the severity of resistance problems over successive seasons (Castle et. al., 1996).

In Arizona, prior to the introduction of a resistance management strategy in 1996, resistance to insecticides used most frequently on cotton was consistently higher in the centre of the State, where cotton constitutes approximately two-thirds of the acreage of cultivated whitefly hosts, than in southwestern Arizona, where it accounts for less than one quarter of the total acreage (Dennehy et. al., 1996; Williams et. al., 1997). As a result, the risk of resistance to newer insecticides (e.g. insect growth regulators) used on cotton appears greater in the centre than in the southwest of the State. Conversely, risks of resistance to compounds (e.g. imidacloprid) still used primarily on vegetables and melons appear greater in the southwest, where these crops are proportionally much more abundant than elsewhere in Arizona (Williams et. al., 1997).

Ecology in Protected Crops

Enclosed environments such as greenhouses and glasshouses, which restrict immigration and escape from insecticide exposure under climatic conditions favouring rapid and uninterrupted population growth, provide near-ideal conditions for selecting resistance genes (e.g. Denholm et. al., 1998a). Selection for resistance in such localities not only generates control problems *in situ*, but can have wider implications for the spread of resistance genes. On a local scale, active migration of insects from protected environments to adjacent field crops has the potential to 'seed' outdoor populations with resistance and thereby accelerate its selection to damaging frequencies. To our knowledge this has not been demonstrated directly for *Bemisia*, but it constitutes a significant threat where outdoor and protected hosts are grown in close proximity. On a broader scale, the risk of inadvertent movement of resistant insects between regions or even countries via trade in plant produce has become clearly apparent from the high levels of resistance found in individuals of *Bemisia* newly imported into northern European glasshouses (Denholm et. al., 1996).

Implications for Resistance

Throughout the world, the above factors combined with often intensive insecticide use have led to strong resistance encompassing the great majority of insecticide classes. The extent and nature of resistance to older compounds, including pyrethroids and organophosphates, have been reviewed extensively (e.g. Dittrich et. al., 1990; Cahill et. al., 1995; Denholm et. al., 1996). A particularly worrying development has been the increase in reports of resistance to newer molecules, on which the future of whitefly control now depends in many countries, including the USA. Many of these products offer the considerable advantage of being unaffected by older resistance mechanisms, and many also exhibit more favourable environmental profiles than broad spectrum agents employed in the past.

The chemicals probably attracting most attention in this respect are the chloronicotinyls or neonicotinoids, whose forerunner, imidacloprid ('Admire') is now in widespread use against whiteflies and other pests including aphids and Colorado beetles. Other members of this class are currently being introduced or in late stages of development. Their efficacy both as highly persistent systemic treatments and as shorter-lived foliar sprays offers outstanding versatility, but also renders them very susceptible to overuse. Their structural similarity, coupled with a likely common target site, also raises the possibility of cross-resistance affecting the group as a whole (Elbert et. al., 1996; Cahill and Denholm 1999). The risk of resistance is therefore considerable, and reinforced by the speed with which resistance to imidacloprid has developed in *Bemisia* under sustained selection pressure in the laboratory (Prabhakar et. al., 1997) and in greenhouses in southern Europe (Cahill et. al., 1996a), and the rate at which it appears to be increasing on field crops in Arizona (Dennehy and Denholm 1998; T.J. Dennehy unpublished data). Given the current scale of reliance on imidacloprid and the impending release of other chloronicotinyls, it is essential to identify conditions under which this important new class of insecticide chemistry can be used sustainably.

Despite their key role in current resistance management strategies for whiteflies on cotton in Israel and Arizona, the insect growth regulators (IGRs) buprofezin ('Applaud') and pyriproxyfen ('Tiger' or 'Knack') are also proving vulnerable to resistance by *Bemisia*. Although both chemicals act primarily against immature stages of whiteflies, they possess distinct modes of action and are therefore very unlikely to be affected by the same resistance mechanism. These differences, coupled with a high degree of species selectivity, make them ideally suited as rotation partners in control programs that place strong emphasis on the preservation of natural enemies of *Bemisia* and co-existing pest species (Horowitz et. al., 1994).

As with imidacloprid, resistance to both IGRs first became apparent under the intensive selection operating in protected environments. Resistance to buprofezin was first detected in glasshouses in the Netherlands, but has since been demonstrated elsewhere in northern Europe and in greenhouses in Israel and Spain (Horowitz and Ishaaya 1994; Cahill et. al., 1996b). Buprofezin is still less widely used in the open field, where resistance has been slower to develop. However, monitoring of the susceptibility of *Bemisia* on cotton in Israel, where this chemical has been restricted to a single application per year since its introduction in 1989, has shown small increases in LC_{50} values consistent with the presence of resistant insects at some localities (Horowitz et. al., 1994).

The history of pyriproxyfen use against *Bemisia* in Israel provides a striking example of how genetic and ecological factors can combine to promote resistance, despite conscious efforts to prevent this occurring. Within one year of its introduction in Israel in 1991, high resistance was demonstrated in some greenhouses housing ornamental plants after successive applications (Horowitz and Ishaaya 1994; Horowitz et. al., 1994). The maximum resistance recorded was 550-fold at LC_{50}, from a rose greenhouse that had previously been sprayed only three times with this chemical. The linearity of dose-response data obtained from bioassays at this stage implied a high degree of homozygosity for one or more pyriproxyfen-resistance genes (Denholm et. al., 1998b; Horowitz et. al., 1999).

As with buprofezin, pyriproxyfen was released for use on Israeli cotton with a restriction to one application per season, in order to prevent or delay the development of resistance. This restriction was facilitated by the outstanding effectiveness of pyriproxyfen, which in many cases proved sufficient to control *Bemisia* during the whole cotton season. Despite excellent compliance with this recommendation, resistance was first detected on cotton as early as 1992 at some localities, where it has since undergone a gradual increase in frequency within and between seasons. Initial observations of an increase within seasons did not cause alarm, due to the tendency for populations to revert to susceptibility by the start of the following season. The reason(s) for this reversion are still unclear, but could relate to differences in the inherent fitness of pyriproxyfen-susceptible and resistant phenotypes, and/or gene flow between treated and untreated hosts.

Factors that initially moderated the selection of resistance on cotton have nonetheless been unable to prevent a systematic increase in resistance, albeit at different rates in different regions. The most dramatic changes were recorded in the vicinity of Nachshon in the Ayalon Valley in central Israel, where egg survival at a discriminating concentration of 0.16 ppm showed a stepwise increase between 1992 and 1997 (Figure 1a). Until 1994, however, levels of resistance early in the season were still relatively low, enabling effective control of whiteflies with pyriproxyfen. From 1995 onwards, susceptibility of *Bemisia* in this region decreased substantially and was no longer restored between seasons. At Kefar Aza in the western Negev, early-season susceptibility to pyriproxyfen did not change significantly until 1997 (Figure 1b). However, following the use of pyriproxyfen in 1996 and 1997, resistance increased to levels comparable to those observed in the Ayalon Valley during the same seasons.

Reasons(s) for the differential speed of resistance development in the two areas can only be speculated on at present. Each year in the spring and early summer (April-May), infestations of *Bemisia* occur earlier and develop faster in the Ayalon Valley than in other parts of Israel. Based on resistance monitoring data it appears that they originate from the same source each year, even though very low numbers of whiteflies are considered to overwinter in the vicinity (Gerling 1984). Geographically, the Ayalon Valley is relatively isolated, being surrounded by hills that could prove a potent barrier to the movement of *Bemisia*, thereby mimicking the enclosed environment of a greenhouse. Furthermore, agricultural production is very intensive in the Ayalon Valley, with sunflowers and cotton being the main host plants of *Bemisia*. Although largely unsprayed, sunflowers are planted and harvested much earlier than cotton, and do not therefore constitute an effective refuge when insecticides are applied against whiteflies on cotton. In the western Negev there is a greater diversity of crops including melons, tomatoes, potatoes and greenhouse ornamentals, most of which have not been treated recently with pyriproxyfen. The later build-up of whiteflies and diversity of pyriproxyfen-untreated hosts may therefore explain the slower appearance of resistance in this region. However, these factors have clearly not prevented resistance., which in some areas now threatens to become as frequent as in the Ayalon Valley.

Conclusions

Results obtained in Israel and elsewhere have potentially important implications for managing resistance of *Bemisia* to pyriproxyfen and other novel insecticides. Even a restriction to one application per season has been insufficient to combat the onset of resistance in particular localities in Israel for more than five years. Regions such as the southwestern USA with comparable climates, cropping systems and/or histories of whitefly resistance problems must clearly pay careful attention to anticipating such rapid responses and to modifying insecticide use recommendations accordingly.

The critical question when formulating any resistance management strategy is "how much use of a particular insecticide against a particular pest can a particular regional cropping system sustain?" Assessing the risk of resistance is always a complex task, but at the very least requires regular and systematic monitoring of pest sensitivity in order to detect shifts in tolerance at a sufficiently early stage for restrictions on exposure to specific products on different crops to be refined if necessary. This is the approach currently being adopted at the Extension Arthropod Resistance Management Laboratory (EARML) at the University of Arizona. The most recent results of this work and its implications are reported elsewhere in this volume (Dennehy et. al., 1999).

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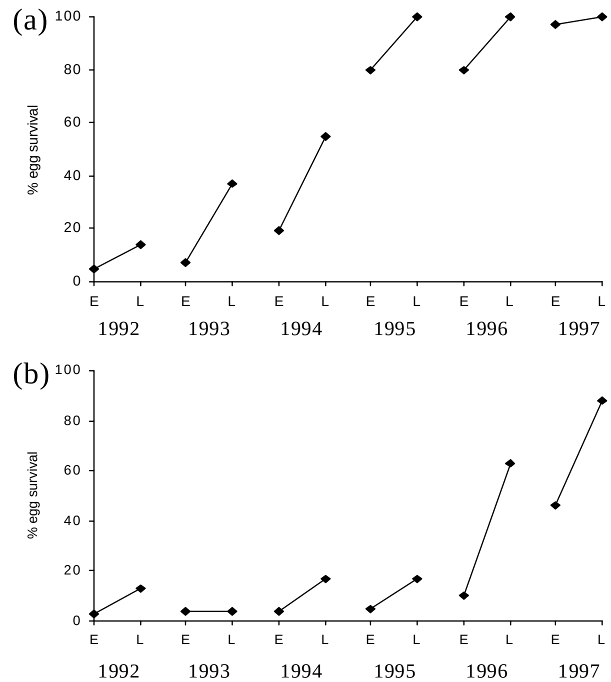

Figure 1. Changes in the response of *Bemisia* from early-season (E) and late-season (L) Israeli cotton at (a) Nachshon in the Ayalon Valley and (b) Kefar Aza in the western Negev to a discriminating concentration of 0.16 ppm pyriproxyfen between 1992 and 1997. Data relate to a leaf-dip bioassay exposing adults to the toxin and measuring subsequent egg-hatch.