Chemical control and resistance

Session VI

Insecticide resistance in *Bemisia* : a global perspective

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In several countries, the capacity for *Bemisia tabaci* to develop resistance to insecticides has had severe repercussions for sustained control of this polyphagous pest. Factors likely to promote the build-up and spread of resistance in this species include: (i) a haplodiploid breeding system enabling the rapid selection and fixation of resistance genes (irrespective of their dominance characteristics); (ii) its breeding cycle on a succession of treated host plants; and (iii) its occurrence on high value crops that are widely traded internationally. Reports of resistance continue to proliferate, both in new regions of the world and to more novel insecticides including growth regulators and neonicotinoids. Although there has been substantial progress with resolving the mechanistic basis of many forms of resistance, others remain poorly characterised other than at a phenotypic level. This may change through exploitation of genomics-based techniques for localising and identifying resistance genes, aided by the publication of full genome sequences for an expanding number of insect species. This paper will review current knowledge of the incidence and mechanisms of resistance in *B. tabaci*, and consider how attempts to combat resistance can benefit from improved insights of whitefly genetics and ecology. One exciting area for further investigation is the distribution and homology of resistance genes across potentially reproductively-isolated biotypes, and the possible role of resistance in influencing changes in biotype composition. The time is ripe to incorporate resistance work into expansive and co-ordinated studies of population genetics and micro-evolutionary processes. *Bemisia* is an ideal model organism to exploit in this respect.

Perspectives on insecticide resistance in *Bemisia tabaci*

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Insecticides have played a significant role in managing agriculture insect pests. However, if insecticides are relied upon overwhelmingly, resistance can be expected to express itself in the target pest due to a plethora mechanisms in the insect. To avoid or delay resistance development in the whitefly, *Bemisia tabaci*, a major pest on agricultural and horticultural crops in the USA, it is important to understand nature of resistance. In the USA the rate of resistance development to insecticides varied in *B. tabaci*. This variation in resistance development was based on a number of factors which included the whiteflies life cycle, generations per year, fecundity, capacity for high dispersal and frequency of insecticide applications. Also, differences in the measurement of resistance could vary depending on the type of bioassay method used in the study. An insecticide resistance monitoring program was conducted in California to determine the relative susceptibilities of *B. tabaci* (B type) to various classes of insecticides. Results of five years of resistance monitoring data did not indicate that grower management practices were selecting for whiteflies tolerant to insecticides. Whiteflies appeared to be more susceptible to insecticides over time in spite of continuous chemical use. An analysis of chemical control for the last decade indicated the importance of research and development of 'resistance management programs' for continuing use of insecticides.

Stability of resistance to pyriproxyfen in *Bemisia tabaci*

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Pyriproxyfen, a juvenile hormone mimic, has been used on cotton in Israel since 1991. It is a very potent insect growth regulator (IGR) with persistent activity against several insects, including the whitefly, *Bemisia tabaci* (Gennadius). On the other hand, it has been reported that pyriproxyfen is prone to development of resistance in the whitefly. Consequently, high resistance to pyriproxyfen evolved in greenhouses in Israel one year after its introduction, after three successive applications. In cotton fields, after ten years of pyriproxyfen use, with only one application per season, a high level of resistance was observed in some areas in Israel, but its rate of development differed among localities. The stability of resistance to pyriproxyfen in *B. tabaci* has been studied under field and laboratory conditions. *Bemisia tabaci* field-collections were monitored annually for resistance to pyriproxyfen. In addition, several field populations were maintained under standard laboratory conditions and checked in subsequent generations for their resistance. Due to the absence of applications of pyriproxyfen in some cotton fields, resistance levels tended to decline between 1998 to 2002, but this decline has been rather slow. On the other hand, a significant reduction in resistance levels was observed in a pyriproxyfen-resistant field strain that was maintained under standard laboratory conditions for 15 generations. In the 26th generation, the susceptibility to pyriproxyfen was restored completely reaching the level of the susceptible strain. The experiments support the hypothesis that this decline in resistance reflects, in part, fitness costs associated with pyriproxyfen resistance. An assumption that *B. tabaci* biotypes are also involved in this phenomenon is discussed.

Insecticide resistance and resistance management in cotton populations of B-biotype *Bemisia tabaci* in Australia

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B-biotype *Bemisia tabaci* (silverleaf or poinsettia whitefly), were first detected in Australia in 1994. This exotic whitefly invaded Australia, as part of a world-wide trade in Poinsettia cuttiings. The silverleaf whitefly has since spread to most Australian states and has become a significant pest of horticultural crops and ornamentals in Queensland, the Darwin area of the Northern Territory and northern New South Wales. Surveys from 1995 onward, indicated that the silverleaf whitefly was present, albeit at low numbers, in cotton crops in Queensland and New South Wales. During the summer of 2001/2, B-biotype *B. tabaci* populations exploded on cotton at Emerald, and other locations in central Queensland. There were also significant populations of silverleaf whitefly in cotton from other districts of Queensland and New South Wales. In an attempt to protect cotton lint from honeydew, insecticidal control in central Queensland was largely directed at adult whiteflies. Insecticides used were: pyrethroids, diafenthiuron, amitraz, and some organophosphates. Some insect growth regulators (buprofezin and pyriproxyfen), were used against immature silverleaf whitefly. There was an extreme and constantly evolving insecticide resistance situation and spray failures had to be re-sprayed. High levels of resistance to pyrethroids, diafenthiuron, amitraz and the insect growth regulators were rapidly selected by insecticide use on cotton. Insecticides applied as mixes rapidly built up multiple resistance. Insecticide resistance management is imperative to control the silverleaf whitefly. In Australia, however, a whitefly resistance management strategy needs to be integrated with existing *Helicoverpa armigera* and *Aphis gossypii* resistance management straetgies on cotton. The management of resistance in multiple pests on cotton presents considerable difficulties for effective silverleaf whitefly management in Australia.

Biochemical investigations of neonicotinoid cross-resistance in Q-and B-type *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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The sweet-potato whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) is a serious pest in numerous cropping systems worldwide and this species occurs in different, sometimes also geographically separated biotypes such as the Q- and B-type. The most common biotype, designated type B and sometimes described as *Bemisia argentifolii* Bellows & Perring, is by far the most devastating one. Biotype determinations of Spanish populations of *B. tabaci* indicated at least two different biotypes, the B-type and a so-called non B-type (also referred to as Q-type). Resistance to insecticides such as organophosphates, carbamates, pyrethroids and insect growth regulators such as buprofezin is well described for several strains collected from different geographic areas. One of the latest group of insecticides introduced to the market for whitefly control were the neonicotinoids (chloronicotinyls) which act agonistically on insect nicotinic acetylcholine receptors. Resistance to neonicotinoid insecticides has recently been shown to occur in *B. tabaci* Q-type in some places in Almeria, Spain, whereas control of B-type *B. tabaci* in many other intense cropping systems worldwide remained on high levels. In addition to the Spanish strains we also detected strong neonicotinoid cross-resistance in one B-type strain from the Mediterranian bassin. Studies to investigate the biochemical mechanisms of resistance were carried out on both biotypes. Metabolic enzymes such as esterases, glutathione S-transferases and cytochrome P450-dependent monooxygenases were studied and a fluorometric microplate assay with 7-ethoxycoumarin as substrate revealed a clear correlation between neonicotinoid resistance and enhanced microsomal 7-ethoxycoumarin-O-deethylase activity. Metabolism of [14C]imidacloprid in vivo was investigated in several *B. tabaci* strains, too. Two metabolites were detected, 5-hydroxy-imidacloprid and the olefine compound. The olefine showed the same insecticidal activity and binding affinity to nAChR than imidacloprid itself, whereas 5-hydroxy-imidacloprid was more than 10 times less active. Furthermore we demonstrated that neonicotinoid-resistance is not due to an altered [3H] imidacloprid binding site of nicotinic acetylcholine receptors in Q- and B-type strains.

Control of the tobacco whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) in covered vegetables in southern Spain: latest findings and revised Insecticide Resistance Management guidelines

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The recent and current status of resistance to neonicotinoid insecticides in the tobacco whitefly *Bemisia tabaci* in southern Spain is reviewed with the cross-resistance relationships between neo-nicotinoids and resistances to other insecticide groups being highlighted. Recent studies comparing insecticide efficacy against whitefly in Almeria and Murcia have examined changes in neonicotinoid resistance in response to intense neonicotinoid selection and compared these changes to those resulting from the use of realistic management strategies involving multiple modes of action. The effects of selection of *B. tabaci* with thiamethoxam or pymetrozine have been studied and the resultant changes in resistance are demonstrated. Evidence for the existence of specific resistance mechanisms in *B. tabaci* is reviewed and implications for cross-resistance are discussed. Based on these findings, a revised, recommended insecticide resistance management strategy for the control of *B. tabaci* in covered vegetables crops in southern Spain is presented.

Biochemical and toxicological studies of insecticide resistance in *Bemisia*

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Toxicological bioassays are essential for the identification of insecticide resistance in insect pests. Biochemical techniques have played an important role in identifying many of the mechanisms involved. By combining both toxicological and biochemical studies, the contributions of specific mechanisms to resistance can be very accurately quantified. To illustrate this, biochemical and toxicological data will be presented showing the relative contributions of esterase metabolism and acetylcholinesterase insensitivity to pyrethroid and organophosphate resistance in *Bemisia*.

Impact of insecticide resistance on chemical control of *Bemisia tabaci*

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Insecticides have proven to be highly effective at protecting cotton and vegetable crops under extreme pressure from *Bemisia tabaci*. Unfortunately, indiscriminate and widespread use of insecticides jeopardizes the longevity of insecticides due to development of resistance. Insecticide resistance has been implicated as a major factor for whitefly outbreaks. In the past resistance in *B. tabaci* to organophosphates and pyrethroids was reported during the 1980s, mostly in Sudan and California and was accompanied by economic disasters. In general, resistance has been trans-global in this pest to all classes of insecticides and more recently to the newest class of chemistry, the neonicotinoids. Comparative bioassay results with imidacloprid on whiteflies from Guatemala indicate some resistance to this compound. Similarly whiteflies from Almeria, Spain, appear to be resistant to imidacloprid in addition to other insecticides. Even though imidacloprid has been used heavily in many regions, progression towards high levels of resistance has not been equal because resistance evolves at different rates in different pests and even in the same species. The current status of insecticide resistance in *B. tabaci* to various insecticides with special reference to neonicotinoids will be examined and the various systems that may have contributed to the differences will be compared. A number of factors that may have influenced the resistance patterns to insecticides as evident by the differences in responses of whiteflies from various regions are compared and discussed with emphasis on agro-ecosystem, crop rotation, insecticide-use patterns and refugia concept.

Host plant influence on susceptibility of *Bemisia tabaci* to insecticides

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Populations of *Bemisia tabaci* from different host crops can vary considerably in their susceptibility to insecticides. Extensive resistance monitoring data collected in California over 3 consecutive years showed a consistent pattern of higher LC50s when *B. tabaci* was collected on broccoli or other cruciferous crops. Lower LC50s were obtained when *B. tabaci* was collected on cotton, with intermediate LC50s on melon. Differences in mean LC50s among crops was greatest for bifenthrin (pyrethroid) and least for chlorpyrifos (organophosphate). Because broccoli, melon and cotton are grown during different seasons, there was some question as to whether observed differences in LC50s were due to host plant effects or to other seasonal factors. A reciprocal transplant experiment was performed by collecting *B. tabaci* from melon and kale crops grown adjacent to one another and establishing greenhouse cultures on melon and broccoli for each field strain. Insecticide bioassays using bifenthrin, endosulfan and chlorpyrifos were performed on test subjects at the time of the field collection, one week after establishing the greenhouse cultures, and on the F1 generation. As expected, whiteflies that originated on kale yielded a higher LC50 than those originating on melons. After one week in greenhouse culture, the LC50 for kale-originating whiteflies cultured on broccoli was significantly higher (non-overlap of 95% C.I.s) than the LC50 for those cultured on melon. Similarly, LC50s increased on broccoli for melon-originating whiteflies while decreasing on melon. Bioassay results for the F1 generation remained similar to the results at the one-week interval. As with the resistance monitoring data, greatest differences in the reciprocal transplant study were observed with bifenthrin. Host plant-induced changes in the susceptibility of *B. tabaci* populations to certain insecticides may contribute to the relative success or failure of chemical control.

Monitoring susceptiblity of *Bemisia argentifolii* to imidacloprid in Florida

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Imidacloprid is applied at transplanting or within two to three weeks after transplanting to nearly 100% of the tomato acreage in Florida for control of the silverleaf whitefly (SLWF), *Bemisia argentifolii* Bellows & Perring, and the geminiviruses it transmits, primarily tomato yellow leaf curl virus (TYLCV). A cut leaf petiole (CLP) method using cotton seedlings was used to establish the baseline susceptibility of whitefly adults from a laboratory colony to imidacloprid. The CLP method was used to estimate the susceptibility of whitefly populations from three imidacloprid-treated tomato fields in the spring of 2000, nine in the spring of 2001, two in the fall of 2001, and 13 in the spring of 2002 using adults reared from field-collected nymphs. Standard probit analyses were used to calculate the slopes and intercepts of the linear equations describing the log dose response and to estimate the LC50 values for the laboratory colony and for each field population. LC50 values of field populations ranged from about 2 to 35 times that of the highly susceptible laboratory colony. Values on the high side of the range were found at three sites in 2001 and three sites in 2002. Two of the sites that had high values in 2001 did not have high values in 2002. The LC50 values of one population from 2001 and one from 2002 declined to acceptable levels after rearing in the laboratory for an estimated 4-6 generations. In addition, SLWF populations were not reported by growers to be out of control at any of the sites, and in-field evaluations of imidacloprid efficacy in 2002 indicated expected levels of control of SLWF nymphs, even at the site that had an LC50 value 35 times the laboratory colony.

Monitoring insecticide resistance of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Crete

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The past two years (2000 - 2001) an outbreak of tomato yellow leaf curl virus (TYLC), noticed on both greenhouse and field tomatoes in Crete and in other regions of Greece, was associated with *Bemisia tabaci* (Hemiptera: Aleyrodidae). In addition growers report difficulties to control *B. tabaci* using registered insecticides at recommended dosage. This project aims to monitor the level and the extent of insecticide resistance throughout Greece. Here we present results from populations collected from different host species in the island of Crete. Responses to insecticides were studied by toxicological bioassays. The insecticides tested were pirimiphos methyl, alpha-cypermethrin, and bifethrin commonly used against *B. tabaci* adults in greenhouse crops. In the case of the insecticides pirimiphos methyl and alpha-cypermethrin the observed LC50's were highly variable among the populations. On the other hand, in the presence of bifethrin a different response pattern was recorded; no significant differences in the LC50's were observed between the geographically close populations. Toxicological tests with other groups of insecticides as well as with populations from additional regions of Greece are in progress.

Evidence of *Bemisia tabaci* resistance to some commonly used insecticides in greenhouse tomatoes in Morocco

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Until 1997 whiteflies were not considered a major pest of tomatoes in Morocco and subsequently never required pesticide applications for their control. *Trialeurodes vaporariorum* was the predominant whitefly species in greenhouse tomato and *Bemisia tabaci* was only occasionally recorded in greenhouse tomatoes, both species populations were always maintained at low level by the naturally occurring predatory bug *Nesidiocoris tenuis*. Since 1998 and with the introduction of TYLCV to Morocco we have observed a dramatic change in the whitefly populations. *Trialeurodes vaporariorum* populations were quickly displaced by that of *B. tabaci* which has become the major pest of greenhouse tomato in Morocco requiring over 30 insecticide applications per crop cycle. In the mean time, farmers were faced with a short list of insecticides registered for use against whiteflies in tomatoes. This has led to frequent use of certain active ingredient that were highly effective in the beginning but unfortunately became less effective with time. In this paper we report on *Bemisia* resistance to 6 insecticides that are commonly used by farmers to control whiteflies in Morocco. The technique used for evaluation of *Bemisia* resistance is the leaf-dip bioassay. Adult whiteflies used in the bioassays were collected in farms where there was failure in controlling *B. tabaci* populations. The results of the bioassays indicated various levels of resistance to the insecticide tested. The highest resistance factors was obtained for Imidacloprid and Cypermethrin followed by Deltamethrin, Thiamethoxam and Methomyl. Endosulfan continue to be effective against adult *B. tabaci*. The pattern of resistance reflects the frequency of use, Imidacloprid and Cypermethrin are the most widely used insecticides in greenhouse tomatoes in the Souss Valley of Morocco.

Field test with a new neonicotinoid insecticide to control *Bemisia tabaci* on vegetable protected crops in Sicily

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In order to obtain a safer and high quality production, on vegetable protected crops control of *Bemisia tabaci* should be mainly based on biological, cultural or physical methods. Nevertheless, severity of this whitefly as virus vector imposes on some crops, such as tomato, to maintain its populations at very low levels, and chemical applications may be therefore required also within IPM programs. In these cases, insecticides must be carefully evaluated and selected, in order to reduce risks of insecticide resistance (easily acquired by *B. tabaci*) as well as undesirable effects on beneficial organisms (natural enemies or pollinators) and the environment. Such considerations are becoming a starting point also to industry, which is required to direct the production of new insecticide compounds towards molecules answering to these needs. Whiteflies are among declared target species of a new neonicotinoid insecticide: thiacloprid. Field tests already realised in south-eastern Sicily have shown a satisfactory selectivity of this insecticide on *Bombus terrestris*, in protected tomato crop grown in cold greenhouses. Further investigation is carrying out in order to verify the insecticide activity on *B. tabaci* under local environmental and cultural conditions. A test has been realised in an unheated plastic house, located in the territory of Santa Croce Camerina (province of Ragusa), on an autumn-winter cycle egg-plant crop. In the experiment here reported, thiacloprid has been compared to other insecticides largely used at present by growers. Mortality has been evaluated on both adults and young stages of the whitefly and all results have been statistically analysed.

Evaluating the mode of action for a novel, non-toxic whitefly pesticide

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A new, non-toxic pesticide for managing whiteflies, AGRI-777E (Cal-Agri Products, Los Angeles California), was examined using laboratory bioassays to determine efficacy against the pupal stages of *Bemisia tabaci* and *Trialeurodes vaporariorum* and to ascertain the underlying mode of action. The first bioassay compared AGRI-777E (333ml/hl) with Thiamethoxam (20g/hl) and Buprofezine (100ml/hl) and a water-only control (Distilled water). Each treatment was replicated 10 times. Foliar discs were dipped in the treatment solution and left to dry for about one hour before placing them in separate petri-dishes prepared with Agar (1,5%) gel. Petri dishes were then covered and left at ambient temperature and examined at 2 hours, 4, 8 and 12 days after treatment. A second bioassay was conducted using *B. tabaci* pupa with 4 treatments as follows: A low AGRI-777E treatment (250ml/hl), a high AGRI-777E treatment (500ml/hl), an AGRI-777E wash treatment (250ml/hl), and a water-only control. Each treatment was replicated 4 times. The "wash" treatment subjected pupa to a 6 second washing with water for 5 minutes after application of AGRI-777E. The first bioassay using *B. tabaci* revealed that 0.82% of the Agri-777E treated pupae successfully emerged as adults whereas the Thiamethoxam and Buprofezine showed 45.7 and 32.9 percent adult emergence, respectively. Water only treated pupae showed 57.83 percent emergence. For the *T. vaporariorum* bioassay, no adult emergence was observed for either the AGRI-777E or the Thiamethoxam treated pupae, while 2.5 percent emerged for the Buprofezine treatments compared to 65.22 percent for the water treated pupae. For the third bioassay, similar reductions in adult emergence for the low and high AGRI-777E treated pupae showing no adult emergence for either of the treatments. However, the washing treatment revealed that much of the effect of AGRI-777E was reversed when subjected to washing, adult emergence was observed to increase from zero to 47.9 percent compared to the water-only control of 99.4%. The first bioassay demonstrated that AGRI-777E was able to kill *B. tabaci* pupae and eliminate adult emergence giving AGRI-777E an advantage in performance over many conventional pesticides that have difficulty with this life stage. Interestingly, there was no difference in performance between the high and low rates of Agri-777E indicating that adequate performance was achieved at lower rates in the field. *Trialeurodes vaporariorum* pupa, on the other hand, was very susceptible to each of the three pesticide treatments. The results of the third bioassay also showed that a significant reduction in AGRI-777E efficacy resulted by washing, indicating that AGRI-777E operates external to the insect cuticle as opposed to conventional pesticides that effect insect metabolism. This observation is consistent with a physical mode of action and provides evidence that AGRI-777E kills insects by this mode of action. The reason washing did not remove all the effect of Agri-777E is not known, but may indicate insufficient washing or additional factors may be involved.