WHITEFLY CONTROL IN ARIZONA: DEVELOPMENT OF A RESISTANCE MANAGEMENT PROGRAM FOR IMIDACLOPRID

Livy Williams, III[1], Timothy J. Dennehy[1] & John C. Palumbo[2]
The University of Arizona,
Department of Entomology[1,]
Extension Arthropod Resistance Management Laboratory
Tucson, AZ
Yuma Agricultural Center
Yuma, AZ[2]

Abstract

In 1995 we initiated a resistance management program aimed at sustaining the efficacy of imidacloprid. This paper delineates the groundwork for the program, and describes methodological and conceptual advances toward our goal. Bioassay methods developed for adult whitefly consisted of a 1 day hydroponic uptake procedure using cotton seedlings. A reliable mortality criterion was also established. Results from a statewide survey suggested slight geographic variation in whitefly susceptibility to imidacloprid. Long-term studies will 1) evaluate the risk of resistance to whitefly populations in commercial greenhouses, and relate this to field populations, and 2) characterize the development of resistance in relation to cropping systems and spatial dynamics of whitefly. The overall objective of these investigations is to determine if a sustainable use strategy can be identified for imidacloprid.

Introduction

Imidacloprid is a new insecticide of the chloronicotinyl group that exhibits both systemic and contact activity, primarily against sucking insects (Mullins 1993). It has a novel mode of action, binding to the nicotinergic acetylcholine receptor in the post-synaptic region of the insect nerve. Imidacloprid plays an important role in whitefly management in a broad range of crops (Mullins & Christie 1995; Palumbo 1994a,b; Palumbo et al. 1994; Palumbo 1995). Additionally, it has relatively low mammalian toxicity (rat dermal LD50 >5000 mg/kg). Trade names in the United States include Gaucho®, Admire®, Provado®, Marathon®, and Merit®.

Imidacloprid was first introduced to Arizona agriculture in 1993 as Admire®, under a Section 18 registration. This was necessary because pest managers were unable to control *Bemisia* in lettuce, cole crops, and melons in southern Arizona. Since then, imidacloprid has been granted full registration by the EPA and continues to provide critical control of whitefly on vegetables and melons. It is essential that we sustain the effectiveness of this compound as long as possible. Studies in California have shown that resistance to imidacloprid can be selected relatively rapidly in whiteflies (Prabhaker et al. 1995).

In response to this threat, we initiated a resistance management program for imidacloprid in 1995, the ultimate goal of which was to sustain the efficacy of this insecticide against *Bemisia*. In this paper we present the following building blocks of the program.

1. Development of Bioassay Methods
2. Statewide Survey of Baseline Susceptibility
3. Isolation and Characterization of Homozygous Resistant Strains
4. Ecosystem Study of Resistance Dynamics
5. Monitoring Susceptibility in Commercial Greenhouses

Development of Bioassay Methods

Efficient and reliable bioassay methods are a prerequisite for an effective resistance management program. Considering the systemic action of imidacloprid, we developed a bioassay that exposed whitefly to the chemical through voluntary feeding on leaf tissue. In a sequential manner, we evaluated the effect of 1) host type, 2) interval of hydroponic uptake, and 3) mortality criterion on whitefly susceptibility to imidacloprid.

Cotton, *Gossypium barbadense* L. (var. Pima S-7), and bean, *Phaseolus vulgaris* (var. Landmark), seedlings were subjected to imidacloprid in a hydroponic procedure adapted from Prabhaker et al. (1995). Seedlings were grown in the greenhouse in plastic flats with potting soil, and were transported to the Extension Arthropod Resistance Management Laboratory (EARML) at the 2 true leaf stage. The potting soil was thoroughly washed off the roots and the seedlings were placed into 400 ml beakers containing 200 ml of the appropriate imidacloprid concentration. The seedlings were then held in an environmental chamber for the desired interval of hydroponic uptake (1, 2, or 5 days) at 27(C ((1(C), 50-60% RH, and a 16 hour photophase. Dilution series were made using formulated insecticide (Admire® 2F) and distilled water, on the basis of μg active ingredient per ml of solution. Solutions were prepared immediately before use. Controls consisted of distilled water treatments.

Whitefly susceptibility to imidacloprid was assessed using a completely randomized design with ten replicates per treatment. Leaf disks measuring 2.5 cm in diameter were excised from the true leaves and were each placed into 20 ml glass scintillation vials containing 1.5 ml agar (1.3% by

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:752-755 (1996)
National Cotton Council, Memphis TN

weight). Agar prevented the leaf disks from wilting during the bioassay. The top surface of the leaf disks was placed on the agar, leaving the lower surface exposed to whiteflies. Twenty-five adult whiteflies were aspirated into each scintillation vial. Vials were then capped with a piece of dialysis membrane, inverted, and placed in an environmental chamber maintained at 27° C ±1° C), 50-60% RH, and a 16 hour photophase. After a 2 day exposure period, mortality was assessed by observing individuals in each vial with a Wild M3Z dissecting scope. Two mortality criteria were compared. In the first, individuals not exhibiting repetitive (non-reflex) movement were scored as 'dead'. For the second, individuals not able to move one body length in a normal manner (walking or flying) were scored as 'dead'. After several trials, 'dead' individuals were placed on clean petri dishes in the presence of cotton plants for 2 days after which recovery (ability to colonize the cotton) was determined. Mortality data were arc sine transformed prior to two-way ANOVA.

Results indicated that host plant had no influence on whitefly susceptibility at any of the concentrations tested ($P>0.05$) (Fig. 1a). Therefore, cotton was used in subsequent trials, since it is the more preferred of the two hosts. Interval of imidacloprid uptake did not significantly affect whitefly mortality ($P>0.05$) (Fig. 1b). Though not significant, control mortality increased with duration of the uptake interval. This trend was probably due to a reduction of host quality as the interval of uptake increased. Also, phytotoxic effects were observed at the 2 and 5 day intervals for the highest imidacloprid concentration tested (1000 μg/ml). These results indicate that a 1 day interval of hydroponic uptake is appropriate for cotton. Results from trials comparing mortality criteria were similar at low concentrations, but at high concentrations the criterion requiring normal locomotion led to greater mortality (Fig. 1c). Recovery of 'dead' individuals was most common with one-body-length criterion. Therefore, in subsequent trials, repetitive movement was used as the mortality criterion.

Our results indicate that a reliable bioassay procedure for assessing the susceptibility of adult whitefly to imidacloprid was a 1 day hydroponic uptake using cotton seedlings and the above-mentioned mortality criterion. We are currently evaluating bioassay methods for immature whitefly and plan to correlate adult and immature susceptibility in the coming season.

Statewide Survey of Baseline Susceptibility

Statewide assessment of whitefly susceptibilities to imidacloprid will allow us to detect resistance once it appears in Arizona, and will provide a foundation for managing resistance once it appears. Whiteflies were collected from eight locations throughout the cotton-growing regions of Arizona during the 1995 field season. At each location, approximately 8000 whiteflies were collected in plastic vials (13 dram) by vacuuming the foliage with a Makita Cordless Vacuum((Model 4071D). Collection vials were chilled and transported to the EARML within 8 hours of collection. Whiteflies were then released into cages containing several cotton, *G. barbadense* L. (var. Pima S-7), plants at the 5-7 true leaf stage. Bioassays using the hydroponic uptake method described above were conducted approximately 36 h after field collection.

Results illustrated a high degree of similarity in susceptibilty of Arizona populations. to imidacloprid (Fig. 2). Whitefly populations from four of eight locations sustained 100% mortality at 1000 μg/ml. However, at concentrations of 10 and 100 μg/ml, only the population from the Yuma Valley Agricultural Center sustained 100% mortality. Differences were greatest at 1μg/ml. For example, Casa Grande exhibited 40% mortality and Yuma >95% mortality at this concentration. These results parallel those from a related survey which indicate that whitefly from western Arizona, e.g. Yuma Valley Agricultural Center, were more susceptible to pyrethroid-organophospate mixtures than populations elsewhere in the State (Dennehy et al., this volume). We hypothesize that this may be attributable to a higher proportion of unsprayed hosts, e.g. alfalfa, in western Arizona than in other parts of the state. These unsprayed hosts may act as a buffer to the development of resistance.

The statewide survey provided growers with real-time information on the imidacloprid resistance status of specific whitefly populations. In one such case in 1995, a pest manager contacted the EARML to report a whitefly control failure in a fall melon crop treated with imidacloprid. Up until this event, imidacloprid had provided the manager with good control of whitefly, even in fields surrounded by cotton, as the field in question was. Intensive late summer flights of whitefly from cotton to melons can occur as cotton plants deteriorate and become less suitable whitefly hosts (Byrne et al. 1990). Therefore, it is possible that the 'control failure' was due to whitefly movement from cotton into melons and not resistance to imidacloprid.

Subsequent bioassays showed no difference in susceptibility between the field-collected population (purportedly resistant) and a population never exposed to imidacloprid (pristine population) (Fig. 3). Therefore, we attribute the 'control failure' to movement of whitefly from adjacent cotton.

This 'pseudo-resistance episode' illustrates the interdependence between vegetable/melon and cotton production in Arizona. Cotton growers depend on vegetable/melon growers for timely plowdown in order to limit the whitefly dispersing their crop. Similarly, fall vegetable/melon growers rely on cotton growers to limit whitefly movement from cotton by making necessary treatments and timely defoliation and plowdown of the crop. This inter-relatedness of the well-being of cotton and vegetable/melon production in Arizona points to the benefits for production and resistance management to be gained from cross-commodity cooperation.

Isolation and Characterization of Homozygous Resistant Strains

Selection of resistant strains is an essential prerequisite for studies of resistance dynamics, development and validation of resistance detection methods, and characterization of cross-resistance and resistance mechanisms. We are currently selecting for imidacloprid resistance in Arizona whitefly populations. However, we have already determined the baseline susceptibility of many Arizona field populations, including some never exposed to imidacloprid, to be intriguing (Fig. 2). We have consistently observed a 'plateau' in the response of Arizona populations, yielding approximately 10% survival at relatively high concentrations of imidacloprid (Fig. 4). These data strongly suggest the presence in Arizona of a polymorphism conferring reduced susceptibility to imidacloprid. The selection experiments underway should confirm or reject this hypothesis. If resistance is selected in the laboratory, future work will focus on the stability of imidacloprid resistance, and characterization of cross-resistance.

Ecosystem Study of Resistance Dynamics

Interactions between insecticide resistance and the spatial dynamics of whiteflies, hosts, and cropping systems represent an ecological approach to resistance management. Such studies will allow the concept of 'sustainable efficacy' to be evaluated on a regional scale and to interface with the area-wide pest programs underway in Arizona.

We know that resistance of whiteflies is less severe in Yuma, Arizona and the Imperial Valley, California, than in central Arizona. We hypothesize that this phenomenon is predicated on the prevalence of untreated hosts, such as alfalfa and weeds, upon which there is little or no selection for resistance. Such hosts act as reservoirs of susceptibility in whiteflies.

In 1995 we initiated a long-term study exploring these interactions in southwestern Arizona. Whitefly susceptibility to imidacloprid will be monitored at five sites throughout the year, each site being characterized by production of lettuce, cole crops, alfalfa, cotton, and melons. By contrasting the development of resistance in treated crops and its impact on untreated crops nearby we hope to elucidate use patterns and refuge conditions that sustain efficacy of imidacloprid.

Monitoring Susceptibility in Commercial Greenhouses

Imidacloprid (Marathon®) was registered for use in commercial greenhouses in Arizona in August 1995. We expect many greenhouse populations of *Bemisia* to be subjected to intensive selection pressure by imidacloprid because 1) this chemical is very stable in the soil, and 2) low tolerances of cosmetic damage to ornamental plants promote frequent application in greenhouses.

Monitoring greenhouse populations will help us anticipate the time-course of resistance development in field populations. We hypothesize that the use of imidacloprid in Arizona greenhouses will substantially increase the rate at which resistance will occur in field populations. This is because, once selected in greenhouses, resistant whiteflies are transported throughout Arizona (and elsewhere) on deliveries of ornamental plants to garden shops. Consumers further disseminate the whiteflies into urban areas, many of which are adjacent to agricultural fields. Thus, we expect that use of imidacloprid in commercial greenhouses will serve as a very effective mechanism for selecting and distributing imidacloprid-resistant whitefly throughout Arizona. Documenting resistance to imidacloprid in greenhouses will help us assess the validity of this hypothesis.

Acknowledgements

We are grateful to Xiaohua Li, Monika Wigert, Amanda Simmons, and June Russell for technical assistance. Ian Denholm and Matthew Cahill (Rothamsted Experimental Station) supported multiple aspects of this project. The cooperation of Tracy Everingham and Mark Carson (University of Arizona, Campus Agricultural Center), Curt Engle (Bayer Corp.), Steve Castle (USDA-ARS, Western Cotton Research Laboratory), Larry Antilla (Arizona Cotton Research & Protection Council), Nilima Prabhaker (The University of California), Todd Hannon (Barkley Co. of Arizona), Robert Nichols (Cotton, Inc.) and Scott Tollefson (Del Monte Fresh Fruit, Inc.) is also appreciated.

References

Byrne D. N, T. S. Bellows, Jr., and M. P. Parrella. 1990. Whiteflies in agricultural systems, pp. 227-261. In: D. Gerling (ed.), Whiteflies: their Bionomics, Pest Status and Management. Intercept, Andover, U.K.

Mullins, J. W. 1993. Imidacloprid: a new nitroguanidine insecticide. Am. Chem. Soc. Sym. Series No. 524: 183-198.

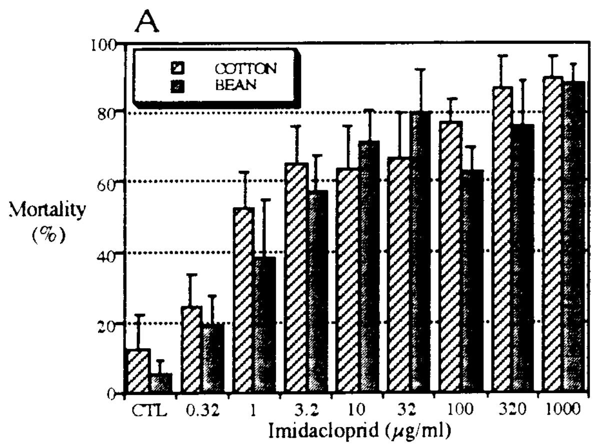
Mullins, J. W. and D. Christie. 1995. Management of aphids, whiteflies and plant bugs with foliarly applied imidacloprid. In: Proceedings of the Beltwide Cotton Production Conference: 868-870.

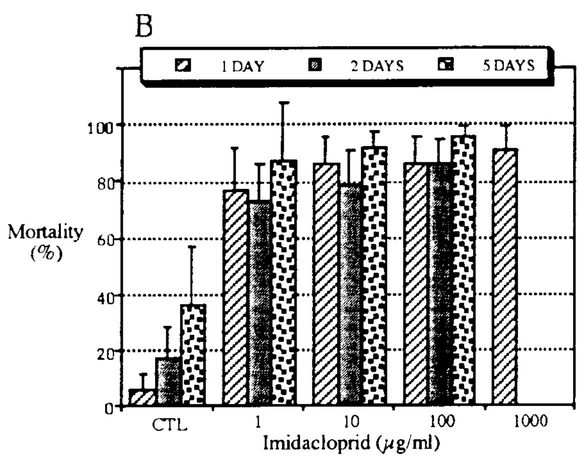
Palumbo, J. C. 1994a. Insecticidal control of sweetpotato whitefly on spring melons, p. 106. In: T. J. Henneberry and N. C. Toscano (eds.), Silverleaf Whitefly (formerly Sweetpotato Whitefly) 1994 Supplement to the Five-Year National Research and Action Plan, USDA-ARS No. 125.

Palumbo, J. C. 1994b. Evaluation of Admire® for control of sweetpotato whitefly in commercial head lettuce in Arizona, p. 73. In: T. J. Henneberry & N. C. Toscano (eds.), Silverleaf Whitefly (formerly Sweetpotato Whitefly) 1994 Supplement to the Five-Year National Research and Action Plan, USDA-ARS No. 125.

Palumbo, J. C., C. H. Mullis and F. J. Reyes. 1994. Control of sweetpotato whitefly in cantaloupe with various pesticides. Arthropod Manag. Tests 19: 80-81.Palumbo, J. C. 1995. Evaluation of Admire® for management of sweetpotato whitefly and green peach aphid populations on lettuce. Final Report for Arizona Iceberg Lettuce Research Council, 15 pp.

Prabhaker, N., N. Toscano, S. Castle and T. Henneberry. 1995. Assessment of a hydroponic bioassay for evaluation of imidacloprid against whiteflies. 1995 Proc. Beltwide Cotton Conf. (addendum). pp. 72-73.





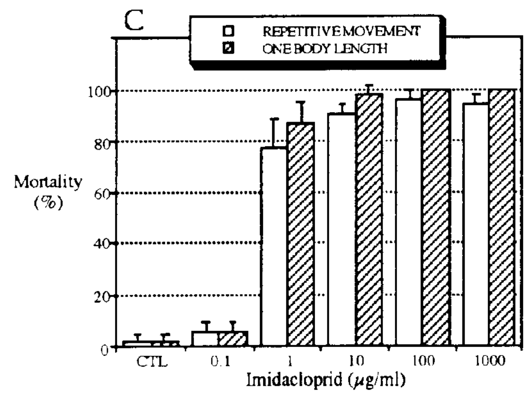


Figure 1. Comparison of host plant (a), interval of uptake (b), and mortality criterion (c) for development of bioassay methods (mean mortality + SD).

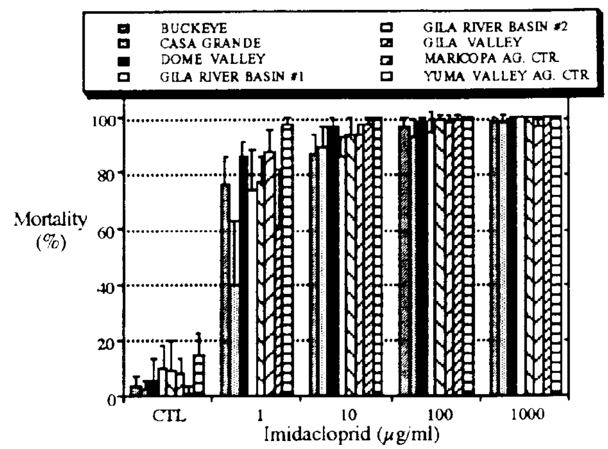


Figure 2. Statewide susceptibility of whitefly to imidacloprid, 1995 (mean mortality + SD).

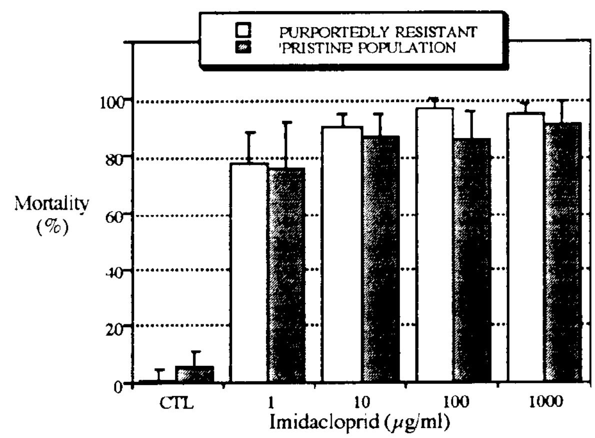


Figure 3. Diagnosis of a pseudo-resistance episode (mean mortality + SD).

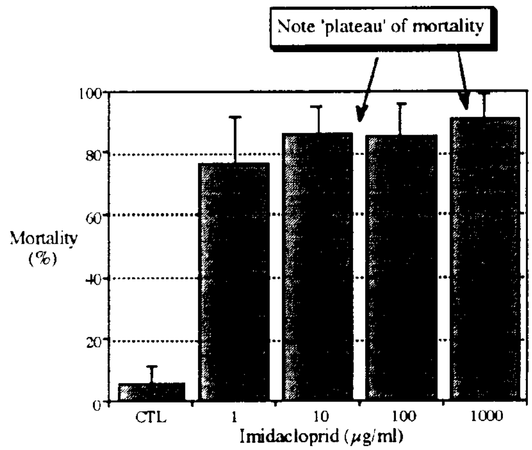


Figure 4. Baseline susceptibility of a whitefly population never exposed to imidacloprid (mean mortality + SD).