PRELIMINARY DATA OF THE EFFECTS OF COTTON DEFOLIANT CHEMICALS ON *BEMISIA ARGENTIFOLII* (HOMOPTERA: ALEYRODIDAE) MORTALITY AND ITS PARASITOID SURVIVAL

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Abstract

Lethal and sublethal effects of two commonly used defoliants, Def and Dropp, on whitefly, *Bemisia argentifolii*, and its parasitoids, *Eretmocerus eremicus* and *Eretmocerus hyati*, were evaluated in laboratory and greenhouse tests. Whitefly eggs and adults were more susceptible to defoliant treatments than larvae. The reduction in feeding sites differentially affected whitefly nymph mortality depending on instar. Sublethal effects of Def, Dropp or their mixture on whitefly were manifested through reduction of percentage female progeny and the number of eggs deposited per female per day after spraying young nymphs. The timing of application significantly affected parasitoid survival. After defoliant treatments of whitefly nymphs parasitized with early instar *E. eremicus* larvae, the number of parasitoid female progeny was significantly reduced and their longevity was significantly shorter than those of controls.

Introduction

Bemisia argentifolii Bellows & Perring (=sweetpotato whitefly, *Bemisia tabaci* Gennadius, Biotype B) is a polyphagus pest species in the tropics and subtropics on all continents (Brown et al. 1995). The reported host plants of the *B. tabaci* species complex contain more than 500 species representing 74 families (Greathead 1986). Since 1987, annual losses in the United States have exceeded $200 million, with an additional annual loss of 3,000-6,000 jobs (De Barro 1995). Among the agronomic crops which are attacked by whitefly species, cotton is one of the most economically important. Losses in Arizona cotton from whiteflies have averaged $32 per acre (Robinson and Taylor 1995). Beltwide cotton losses due to *B. argentifolii* were estimated at $5.5 million in 1996 and $6.8 million in 1997 (Williams 1997, 1998). Williams (1999) noted that in 1998 about 478,800 acres of cotton in the USA were infested with whiteflies, and total losses from this insect alone were 735,500 kg (=3,453 bales). It causes economic losses through direct feeding damage, excretion of honeydew, and plant virus transmission. Lint quality is also reduced through stickiness and sooty mold development.

Repeated applications of conventional insecticides are ineffective in suppressing whitefly populations on cotton during the season because of problems with coverage and insecticide resistance. Despite numerous insecticide applications from July through September, high densities of sweetpotato whitefly developed on cotton in the Imperial Valley (Horowitz et al. 1988). Application of a chemical defoliant and (or) a growth regulator in July and August is an important component of the mandated short-season production system. Defoliants and growth regulators are useful in causing leaf abscission, earlier boll opening, and the shedding of young fruiting forms, thus denying late season food sources for overwintering pink bollworm and other insect pests (Henneberry 1986). The effects of chemical defoliants on *B. argentifolii* are poorly known. There are only a few reports of reductions in *B. tabaci* populations caused by defoliants (Horowitz et al. 1988; Nuessly et al. 1994; Hernandez-Jasso and Gutierrer-Zamoran, 1996, 1998). The effects of defoliants on whitefly parasitoids have not been investigated. Knowledge of how various defoliants affect whiteflies and their parasitoids is fundamental to reducing the number of insects dispersing to winter crops in the Imperial and Lower Rio Grande Valleys, and to successfully integrating applied biological methods. We report here the results of laboratory and greenhouse tests designed to evaluate the effects of two commonly used defoliants on mortality of different stages of *B. argentifolii* and survival of the whitefly parasitoids, *Eretmocerus eremicus* Rose & Zolnerowich and *Eretmocerus hyati*. We report also on the sex ratio and number of oviposited eggs per female per day among whitefly progeny and the number of females and their longevity among parasitoid progeny.

Material and Methods

Host Plant Culture

Cotton, *Gossypium hirsutum* L., variety DPL-50 was the host plant used in these tests. Cotton leaves were used in the laboratory tests. They were excised and each leaf petiole was placed in a floral aquapic filled with a hydroponic solution (Aqua-Ponics International, Los Angeles, CA). Excised cotton leaves were found to readily root and not deteriorate under fluorescent lighting (20 watt, Vita-Lite ©, Duro-Test Lighting, Elk Grove, IL) within an incubator. Cotton plants were used in the greenhouse tests. They were grown in 30.5-cm diameter plastic pots (5 plants per pot).

Whitefly Culture

The *B. argentifolii* culture originated from individuals collected from cabbage (*Brassica oleracea capitata* L.) in Hidalgo County, Texas in 1994, and has been maintained in

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a greenhouse, primarily on tomato, *Lycopersicon esculentum* Miller. Before starting this experiment, we reared *Bemisia* on cotton leaves for four generations.

Leaves were infested by confining about 100 adult whiteflies to the underside of leaves within a 4.5-cm-diam cylindrical clip cage for 24 h. Adult whiteflies were transferred to test leaves after chilling for several minutes in a plastic vial placed in a refrigerator. Two infested clip cages per leaf provided two cohorts of immature whiteflies. Each rooted leaf with eggs was placed in a 120 x 25 mm polystyrene tissue culture dish (Corning Inc., Corning, NY) covered with polyester organdy for ventilation. Hydroponic solution was added to the floral aquapic as required. Dishes were kept in an environmental chamber at 26±2˚C, 55±5% RH, a photoperiod of 16:8 (L:D) h, at 1400-1725 lux. Whitefly nymphs were allowed to develop to the desired instar.

The cotton plants were infested in the blooming stage by placing them for 10 days among plants infested with high densities of *Bemisia* in the greenhouse. Then they were moved to an insect-free greenhouse for an additional 10 days to allow whitefly development.

Parasitoid Culture

Eretmocerus eremicus originated from individuals received from Dan Cahn (Novartis BCM North America, Oxnard, CA, USA) where they were reared on *B. argentifolii*. We maintained the parasitoid cultures on *B. argentifolii* reared on sweet potato, *Ipomoea botatas* (L.) Lam. *Eretmocerus hyati* used in this study was originally collected from Pakistan. Parasitoids were provided by USDA, APHIS, Mission Plant Protection Center, Mission, Texas (culture No. M95012), where they were maintained on *B. argentifolii* reared on *Hibiscus rosa-sinensis* L. Before using either parasitoid species in the laboratory experiments, they were reared for four generations on *B. argentifolii* on cotton. Mated female parasitoids (< 2 d old) were confined with second instar *B. argentifolii* nymphs (one female per ten second instars) in a clip cage for 24 h. The parasitoids were removed, and the assembly of rooted leaves with parasitized nymphs was returned to the environmental chamber for development.

Two formulated defoliants were tested. Def (Bayer, Kansas City, MO)- *S,S,S*-tributylphosphorotrithioate, emulsifiable; and Dropp (AgrEvo, Wilmington, DE) - 490 g/kg thidiazuron, wettable powder. The defoliants were applied at the following rates: Def - 2.3 l/ha (0.94 l/ac), Dropp - 242.8 g/ha (98.1 g/ac), and mixed Def (0.582 l/ha or 0. 235 l/ac) + Dropp (130.4 g/ha or 52.7 g/ac).

Design of Experiments

In laboratory tests to study the effects of defoliants on whitefly mortality, we sprayed cotton leaves infested with different stages of *B. argentifolii* - eggs (one day old), young nymphs (first instar), and old nymphs (fouth instars) with Def, Dropp, and Def + Dropp. As a control, leaves were sprayed with water. Each treatment contained about 220-250 eggs and 250-300 nymphs. There were 5 replicates per treatment. To estimate the effects of defoliants on mortality of newly emerged adults, we aspirated 25 adults into a Petri dish containing cotton leaves sprayed with formulated defoliants. There were ten dishes (replicates) per treatment. We used a laboratory spray chamber (De Vries Mfg., Hollandale, MN), calibrated to deliver 56 liters per hectare using one TXVS-4 nozzle at 1.7 kg/cm^2, and 4.8 km/h, to apply defoliants to leaves. To test the effects of defoliants on parasitoid survival, cotton leaves with parasitized nymphs were sprayed on the 3rd and 14th days after the parasitoid females had been exposed to the host nymphs (n=5, 230 nymphs per treatment). In greenhouse tests, cotton plants were sprayed with the same defoliants as in the laboratory tests on the 10th day after the plants were moved from the greenhouse, where they were infested, to the insect-free greenhouse. This allowed us to attain different-aged *Bemisia* on the leaves on the day of spraying. Plants were sprayed with a trigger-actuated hand sprayer (Sprayco, Detroit, MI 48238) (n=5; 1,250 per treatment).

Experimental Indices and Their Assessment

The following parameters were estimated in the laboratory tests: continuation of development of whitefly eggs and young nymphs; mortality of eggs, young and old insect nymphs, and adults; number of female progeny ; and number of eggs oviposited per female per day. The continuation of development and mortality of different whitefly stages were monitored daily with a dissecting microscope. In the case of eggs, they were observed until hatched or desiccated; of nymphs, until adult eclosion or the nymphs desiccated; and of adults, until 48 h after spraying, when they either moved or failed to move when prodded by a probe. The number of female progeny was examined by sexing 50-100 individuals from each treatment. The number of eggs deposited per female per day was determined on ten 2-d old females. They were confined individually to the undersides of cotton leaves using clip cages. Leaves were visually inspected at 2 d intervals over a period of 10 d. All eggs during the 48 h interval were counted and removed. In the greenhouse tests, the mean proportion of each whitefly stage present per leaf and total mortality were evaluated.

In laboratory tests, we estimated the effects of defoliants on survival of young and old *E. eremicus* and *E. hyati* stages, as well as on the number of female progeny produced and their longevity. In each treatment, we recorded the number of parasitoids that emerged successfully from the parasitized nymphs. Sex of the adult progeny was determined by examining the antennae which are sexually dimorphic. To examine longevity, the female progeny were held as honey-

fed individuals in 1 cm x 3 cm glass vials. Mortality was checked daily at 1100 h.

Statistical Analyses were conducted using analysis of variance (ANOVA), and means were separated using Tukey's studentized range test (Wilkinson et al. 1992). Percentage data were transformed using the arcsine square root method before statistical analysis, but results are presented as nontransformed means (Sokal and Rohlf 1981).

Results and Discussion

Effects of Defoliants on Whitefly Mortality and Other Biological Parameters (Laboratory Tests)

The effects of chemical defoliants on mortality of different stages of *B. argentifolii* are presented in Table 1. The treatments with defoliants and their mixture significantly increased the number of desiccated whitefly eggs (85.5 - 89.0 %) compared with water (control) treatment (4.5 %). After being sprayed with Def, Dropp, or Def + Dropp, young whitefly nymphs (1st or 2nd instars) continued developing similar to controls (89.7%, 92.7%, 91.4%, and 91.1%, respectively). However the percentage emergence of *B. argentifolii* adults after treatment with defoliants (29.8 - 32.2 %) was significantly less than that of the control (95.1%). The effect of Def and Def + Dropp on mortality of old whitefly nymphs (4th instars) was also significant, but of much less magnitude than on the young nymphs. The mortality of old nymphs after application of Dropp was not significantly different from the control. The chemical defoliants were significantly toxic to the whitefly adults. Mortality in the defoliant treatments ranged from 94.4 to 96.8%, while in the control it was only 2.4%.

The percentage of females among progeny of *Bemisia* that had been sprayed as young and old nymphs did not significantly differ between defoliant and control treatments. But those in the set of the young nymphs was much less (37.3 - 43.4 %) in defoliant applications than in the control (52.0%) (Fig. 1). The average number of oviposited eggs per female per day was 1.7-fold less among progeny of whiteflies treated with defoliants as young instars than among progeny of controls. However, this effect was not observed in the case of old nymphs (Fig. 2).

Effects of Defoliants on Whitefly Mortality in Greenhouse Tests

At the time of defoliant applications the following average proportions of different stages of *Bemisia* were present on infested plants: eggs - 3.5%, young nymphs - 34.2%, old nymphs - 51.3%, and adults - 11.0%. We observed the same relative effects of defoliants on whitefly mortality as in the laboratory tests, but overall mortality was less in the greenhouse test than laboratory (Table 2). The reduction in whitefly densities in the greenhouse after defoliant treatments compared with the control were: Def - 6.4 fold, Dropp - 5.5 fold, Def + Dropp - 6.8 fold.

Effects of Defoliants on Whitefly Parasitoid Survival

The survival of *E. eremicus* in young nymphs in the control treatment was 2.8-3.6 times higher than in nymphs sprayed with defoliants. However, *E. eremicus* and *E. hyati* developing in old nymphs were not significantly affected (Fig. 3).

The percentage of female progeny produced by females parasitizing young nymphs in the defoliant treatments was significantly lower (40.0 - 42.2%) than in the water treatment (58.0%). We did not observe this difference when old nymphs were parasitized (Table 3).

Longevity of *E. eremicus* that emerged from nymphs treated when young with Def (4.4 d), Dropp (4.6 d), or Def + Dropp (4.5 d) was significantly shorter than those reared from water-treated nymphs (6.8 d). Mean longevity of *E. eremicus* reared from nymphs treated when old did not differ among treatments.

The reduction in feeding sites differentially affected whitefly nymph mortality depending on instar. The cotton leaves desiccated on the 7th day after treatment, while total developmental time through adulthood required about 20 days. Thus, the main reason for the observed defoliant-induced mortality was a reduction in feeding sites for whiteflies. We did not observe a direct affect of defoliant on young instars. It is possible that the protective waxy covering secreted by the nymphs stemmed penetration of the defoliants. The eggs and adults of whiteflies were more susceptible to defoliant treatments, maybe due to toxicity of some of the components, and to the lesser exterior protection of these stages. Sublethal effects of chemical defoliants on whiteflies were manifested through reduction of percentage female progeny and the number of eggs deposited per female per day after spraying young nymphs. Possibly this occured because the nutritional needs of the insect were not adequately met by the host plant.

Others have observed the effects of defoliants on whiteflies. *Bemisia tabaci* densities can be substantially reduced in the fall by incorporation of an insecticide (94.7-99.2%) with regular Def application (81.1%) on cotton (Horowitz et al. 1988). Thidiazuron in combination with S,S,S-tributyl phosphorotrithioate was the most effective defoliant against *B. tabaci* in Iran. Twenty-one days after treatment no individuals of *Bemisia* were recorded (Moghaddam et al. 1993). Nuessly et al. (1994) noted that significant reductions in *B. tabaci* adults and immatures did not occur until after the cotton plots were treated with defoliants. Hernandez-Jasso and Gutierrez-Zamorano (1996, 1998) demonstrated that the defoliants Ginstar and Dropp did not affect lint or seed cotton

yield, but early defoliation has been proposed to decrease *B. argentifolii* populations by reducing feeding sites.

The timing of defoliant application significantly affected *E. eremicus* and *E. hyati* survival. The defoliants significantly reduced emergence of parasitoids when applied at early nymphal stages, but treatment of old nymphal stages had no effect on emergence. Mortality during the early nymphal stages was most likely due to direct host death. *Eretmocerus eremicus* needed 22 days at 26ºC for full development, while leaves with parasitized nymphs desiccated on the 7th day after spraying them with defoliants. There is no published information concerning the secondary effects of chemical defoliants on survival of whitefly parasitoids, but there are several reports on the effects of insecticide on the survival of parasitoids at different stages. Jones et al. (1998) showed that survival of *Eretmocerus tejanus* Rose and Zolnerowich when sprayed as pupae with the insect growth regulator buprofezin was three times greater that when sprayed as young larvae. Gerling and Sinai (1994) found that buprofezin significantly reduced emergence of *Eretmocerus californicus* Howard (=*E. eremicus*) when applied just after oviposition. Uygun et al. (1994) also demonstrated that timing of pesticides had a differential effect on survival of *Eretmocerus debachi* Rose and Rosen, depending on parasitoid developmental stage within its host, *Parabemisia myricae* (Kuwana).

After defoliant treatments of whitefly nymphs parasitized with young *E. eremicus* larvae, the number of parasitoid female progeny and their longevity were significantly shorter than in the control treatment. Possibly, this is due to differences in nutritional quantity as a consequence of host mortality arising from reduced feeding sites, resulting in exhaustion of food for developing parasitoids. It is also possible, that males can attain maturity on less food than females. Flanders (1968), Waage and Lane (1984), Greenberg et al. (1995) demonstrated this in some species studied. We did not observe sublethal effects of defoliants on *E. eremicus* and *E. hyati* when applied at the old nymph stages.

These studies clearly indicate that defoliants can be used to reduce *B. argentifolii* populations in cotton, and therefore to reduce the number of whiteflies dispersing to winter crops, such as in the Lower Rio Grande Valley. Timing of sprays is important for whitefly mortality and parasitoid survival. Under field conditions, application methods and abiotic influences could be expected to mediate the affects of chemical defoliants.

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Table 1. Effect of chemical defoliants on *B. argentifolii* mortality (laboratory test).[1]

	Mortality, %			
Treatments	Eggs	Young nymphs	Old nymphs	Adults
Control	4.5±1.9b	4.9±1.9b	10.1±2.8b	2.4±1.7b
Def	89.0±4.9a	70.2±4.4a	28.8±14.5a	96.4±1.7a
Dropp	86.5±13.5a	67.8±7.5a	21.8±5.5b	94.4±6.1a
Def+Dropp	85.5±5.4a	68.5±6.9a	30.0±5.8a	96.8±2.3a

[1]Means (±SD) in each column followed by different letters are significantly different at the 5% level, as determined by Tukey's studentized range test.

Table 2. Effect of chemical defoliants on *B. argentifolii* mortality (greenhouse test).[1]

	Mortality, %			
Treatments	Eggs	Young nymphs	Old nymphs	Adults
Control	10.4±7.0b	5.6±1.9b	14.5±2.0b	3.6±3.8b
Def	57.7±19.6a	81.6±4.1a	18.7±3.0bc	60.7±8.3a
Dropp	52.7±11.9a	76.7±10a	15.4±2.8bc	55.1±6.0a
Def+Dropp	69.0±2.4a	78.8±4.6a	21.4±1.3ac	65.2±3.0a

[1]Means (±SD) in each column followed by different letters are significantly different at the 5% level, as determined by Tukey's studentized range test.

Table 3. Sublethal effects of chemical defoliants on *E. eremicus* female progeny and their longevity.[1]

	Female progeny, %		Female progeny longevity, d	
Treatments	On young whitefly nymphs	On old whitefly nymphs	On young whitefly nymphs	On old whitefly nymphs
Control	58.0±7.0a	58.0±5.7a	6.8±2.5a	7.2±2.7a
Def	40.0±10.0b	55.0±7.9a	4.4±2.0b	6.8±2.6a
Dropp	42.2±3.8b	56.0±6.5a	4.6±2.7b	7.1±2.5a
Def+Dropp	40.0±1.6b	54.0±8.2a	4.6±2.2b	6.9±2.1a

[1]Means (±SD) in each column followed by different letters are significantly different at 5% level, as determined by Tukey's studentized range test.

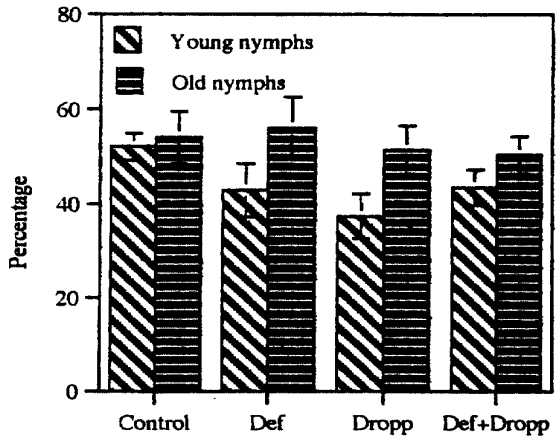

Figure 1. Effects of defoliants on *Bemisia* female progeny.

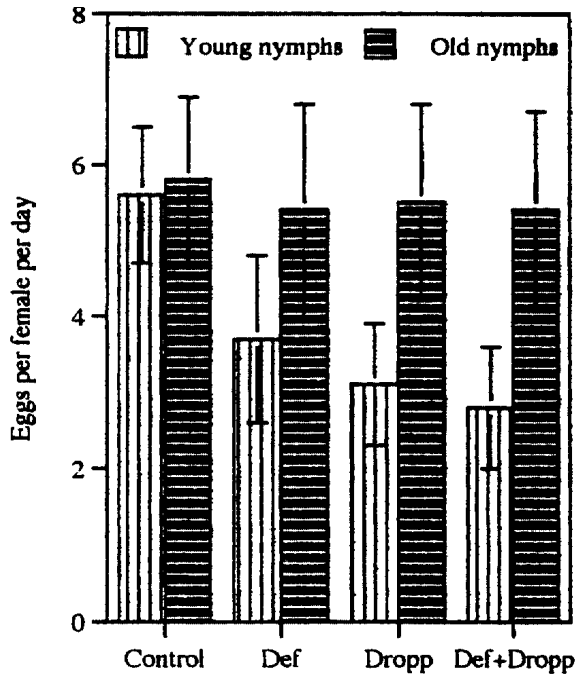

Figure 2. Effects of defoliants on *Bemisia* oviposition per female per day.

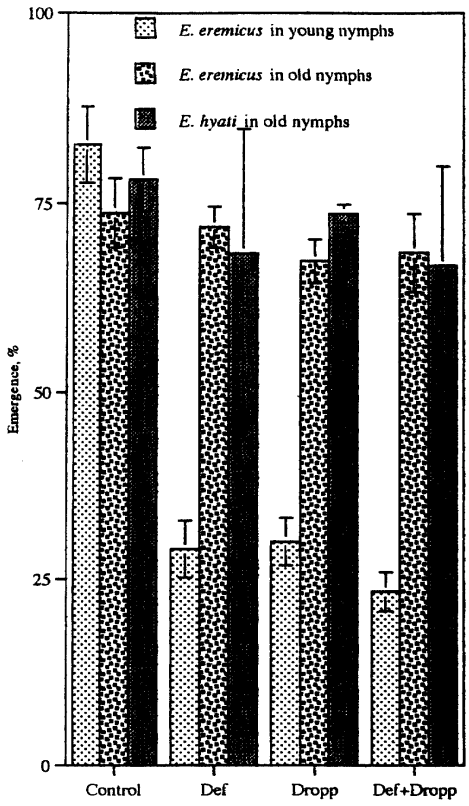

Figure 3. Defoliants affect on parasitoids survival.