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Host plant pubescence: Effect on silverleaf whitefly, *Bemisia argentifolii*, fourth instar and pharate adult dimensions and ecdysteroid titer fluctuations

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Abstract

The ability to generate physiologically synchronous groups of insects is vital to the performance of investigations designed to test insect responses to intrinsic and extrinsic stimuli. During a given instar, the silverleaf whitefly, *Bemisia argentifolii*, increase in depth but not in length or width. A staging system to identify physiologically synchronous 4th instar and pharate adult silverleaf whiteflies based on increasing body depth and the development of the adult eye has been described previously. This study determined the effect of host plant identity on ecdysteroid fluctuations during the 4th instar and pharate adult stages, and on the depth, length and width dimensions of 4th instar/pharate adult whiteflies. When grown on the pubescent-leafed green bean, tomato and poinsettia plants, these stages were significantly shorter and narrower, but attained greater depth than when grown on the glabrous-leafed cotton, collard and sweet potato plants. Thus, leaf pubescence is associated with reduced length and width dimensions, but increased depth dimensions in 4th instars and pharate adults. For all host plants, nymphal ecdysteroid titers peaked just prior to the initiation of adult development. However, when reared on pubescent-leafed plants, the initiation of adult development typically occurred in nymphs that had attained a depth of 0.2 to 0.25 mm (Stage 3 - 4). When reared on glabrous-leafed plants, the initiation of adult development typically occurred earlier, in nymphs that had attained a depth of only 0.15-0.18 mm (Stage 2 Old - early 3). Therefore, based on ecdysteroid concentration, it appears that Stage-2, -3 and -4/5 nymphs reared on pubescent-leafed plants are physiologically equivalent to Stage-1, -2 Young and -2 Old/3, respectively, nymphs reared on glabrous-leafed plants. The host plant affected the width but not the height of the nymphal-adult premolt ecdysteroid peak. However, leaf pubescence was not the determining factor. Thus, host plant identity affects physiological events as well as structural characteristics during whitefly nymphal and adult development.

Keywords: whitefly-plant interactions, 20-hydroxyecdysone

Abbreviation:
EIA enzyme immunoassay

Introduction

The silverleaf whitefly, *Bemisia argentifolii*, is polyphagous, attacking more than 500 different species of plants including food, fiber and ornamental species. This homopteran causes hundreds of millions of dollars of damage in crop losses each year by feeding on plant phloem, transmitting plant pathogenic viruses and producing honeydew that causes stickiness in cotton and other produce and supports the growth of sooty mold (Heinz, 1996; Henneberry et al., 1997). Recently, precise staging systems for identifying silverleaf whitefly instars and for tracking developmental progress of 3rd instar and of 4th instar/pharate adult whiteflies grown on green bean plants has been described (Gelman et al., 2002b). During any given instar, length and width measurements remain the same while depth increases (Hargreaves, 1915; Gelman et al., 2002a,b). Thus, the staging systems for 3rd instars and for 4th instar/pharate adults, respectively, were based wholly or partly on increasing body depth. The development of a precise staging system for silverleaf whitefly 3rd and 4th instars and pharate adults made it possible to monitor ecdysteroid (the molting hormone, its precursors and metabolites) titer fluctuations during these stages and to determine premolt peak chronology and size. The external appearance of the silverleaf whitefly as well as its developmental time have been reported to vary depending upon the identity of the host plant (Mound, 1963; Bethke et al., 1991; Rosell et al., 1996; Neal and Bentz, 1999; Guershon and Gerling, 2001). Thus, it became important to determine if the nature of the host plant affects developmental progress and ecdysteroid titer fluctuations in 4th instar and pharate adult silverleaf whiteflies. Here we report that host plant identity influences not only mean length

and width of silverleaf whitefly nymphs and pharate adults, but also depth attained. We also describe the effect of the host plant on the timing, magnitude and shape of the ecdysteroid peak in last instar and pharate adult silverleaf whiteflies.

Materials and Methods

Chemicals

20-hydroxyecdysone was purchased from Sigma (www.sigmaaldrich.com). T. Kingan (University of California at Riverside, USA) provided the ecdysone antiserum and the peroxidase-labeled ecdysone conjugate used in the EIA. The antiserum has a high affinity for ecdysone, 20-hydroxyecdysone, makiserone A, 20,26-dihydroxyecdysone, 26-hydroxyecdysone and 3-dehydroecdysone (Kingan, 1989; Kingan, personal communication). The enzyme substrate, 3,3',5,5'-tetramethylbenzidine, and the goat antirabbit IGG were obtained from American Qualex (Miracle-Gro Products, Inc., http://2001.scotts.com) and from Jackson Immuno Research Laboratories (www.jacksonimmuno.com), respectively.

Insect rearing

Silverleaf whiteflies used in these studies were obtained from a laboratory colony maintained at the Insect Biocontrol Laboratory, Beltsville, MD, USA. The whitefly colony was housed in a walk-in, climate-controlled, insect growth chamber (26 ± 2° C, L:D 16:8 and relative humidity of 60-80%). Whiteflies were reared on a variety of plants, including green bean cv. Roma II (Burpee, Warminster, PA, USA), cotton cv. Stoneville ST 474 (Stoneville Pedigreed Seed Co., Maricopa, AZ, USA), sweet potato, collard cv. Champion (Meyer Seed Co., Baltimore, MD, USA) tomato cv. Bush Big Boy (Burpee,Warminster, PA, USA), poinsettia cv. Freedom Red (Paul Ecke Ranch, Encinitas, CA, USA) and eggplant cv. Millionaire Hybrid (Burpee, Warminster, PA, USA). All plants were grown from seed except for sweet potato and poinsettia. Sweet potato was propagated vegetatively from sweet potato tubers purchased at the local supermarket, and poinsettia was grown from cuttings supplied by Paul Ecke Ranch (Encinitas, CA, USA). Plants were fertilized weekly using a 1% solution of Mira-gro (15% N, 30% P_2O_5, 15% K_2O) (Miracle-Gro Products, Inc., http://2001.scotts.com). Leaves of experimental host plants [green bean, tomato and poinsettia (pubescent-leafed) and cotton, collard and sweet potato (glabrous-leafed)] were infested by placing greenhouse-grown plants into the growth chamber containing the whitefly colony for approximately 6 h. Adult whiteflies were then removed from the experimental plants, and the plants were transferred to a second growth chamber, or to an incubator box, and maintained under the same rearing conditions as described previously for the colony.

Insect staging

Silverleaf whitefly 4th instar/pharate adults were staged as described elsewhere (Gelman et al., 2002b). In order to measure body depth, an optical micrometer (0.14 mm x 0.14 mm with each subunit being 0.1 mm square) mounted on a stereoscopic microscope was used. Briefly, Stages 1, 2 and 3 were characterized by body depths of 0.1, 0.15 and 0.2 ± 0.02 mm, respectively; stage 4 had a body depth of 0.23–0.26 mm. Nymphs with a body depth about 0.27mm were assigned to Stage 5. For nymphs reared on cotton, collard and sweet potato, Stage 2 was subdivided into Stage 2 Young (0.125–0.145 mm in depth) and Stage 2 Old (0.15-0.17 mm in depth). Stages 6 through 9 were identified based on the appearance of the developing adult eye. Nymphs entered Stage 6 when the small intense red dot characteristic of the eye of Stages 1 through 5 began to diffuse. A light red, medium red bipartite, and dark red or red-black bipartite adult eye characterized stages 7, 8 and 9, respectively. Stage 6 was further subdivided into 6A (diffusion limited to the anterior-medial portion of the eye), 6B (diffused pigment had begun to radiate in all directions) and 6C (diffused pigment was in the form of a circle whose diameter was approximately 0.05 mm). Approximate duration of each stage has been described previously (Gelman et al., 2002c)

Determination of silverleaf whitefly 4th instar/pharate adult dimensions and weight

An optical micrometer mounted on a stereoscopic microscope was used to measure length and width as well as depth. Determinations were made along the line of maximum dimension. Individual whiteflies were removed from leaves and weighed using a Cahn Model C-34 microbalance (ATI Orion, www.orionres.com). When measuring body dimensions and determining body weight, silverleaf whiteflies from at least three and two separate cohorts, respectively, were sampled.

Ecdysteroid determination

For each determination, two to ten (depending on stage) 4th instars or pharate adults were removed from the leaf and extracted in 75% aqueous methanol. Ecdysteroid was determined by EIA (Gelman et al., 2002a). Briefly, whiteflies were homogenized, homogenates were sonicated and centrifuged, and supernatants plus washes were placed in 6 x 50 mm borosilicate glass tubes. Tubes were dried and ecdysteroid was determined using an EIA developed by T. Kingan (Kingan and Adams, 2000; Gelman et al., 2002a). The range of the EIA is 500 to 40,000 fg. Thus the EIA is 50 to 100 times more sensitive than radioimmunoassays that have been used to determine ecdysteroid concentration in more concentrated samples (Borst and O'Connor, 1972; Gelman et al., 1997). The EIA is performed in a 96-well microtiter plate and is based upon the competition between ecdysteroid (in standard or sample) and a known amount of peroxidase-labeled conjugated ecdysone for the ecdysteroid antiserum that has been bound to the IGG-coated wells. After several washes, the addition of substrate followed by phosphoric acid (1M) produced a yellow color. Absorbance was measured at 450 µm. Ecdysteroid, in femtograms, present in each sample was determined from a standard curve (semi-log plot with fg ecdysteroid plotted on the log scale). In order to eliminate the contribution of ecdysteroid present in the whitefly gut, ecdysteroid content of filter chamber/gut complexes was also measured for selected stages of nymphs reared on green bean, sweet potato, cotton and collard. For each determination, 10 gut complexes (sometimes with hindgut attached) were collected, homogenized, extracted and subjected to EIA (Gelman et al., 2002a).

Statistical analysis

A one-way ANOVA was used to analyze all data sets. To analyze for significant differences among the experimental groups

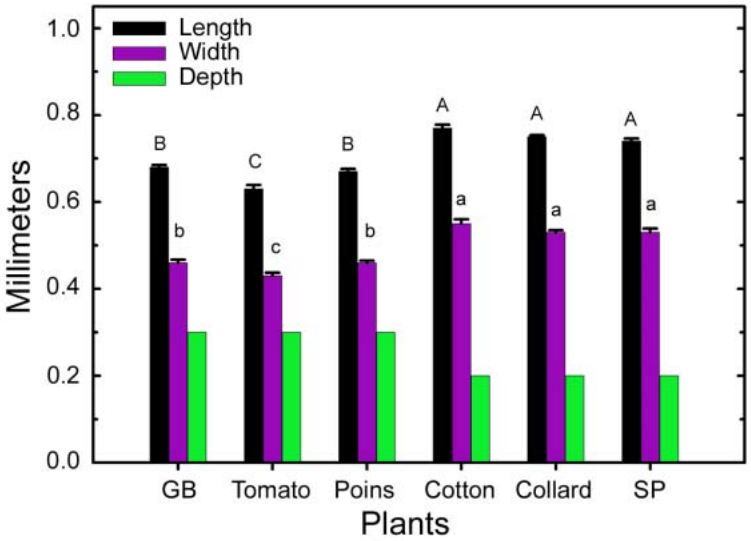

Figure 1. Mean length, width and maximum depth dimensions of 4th instars reared on six host plants. Plants were infested with silverleaf whiteflies that had been maintained on a variety of plants as described in Materials and Methods. Length, width and depth were measured using an optical micrometer. For each stage, length and width dimensions of at least 20 individual whiteflies were determined. Each value represents the mean ± S. E of these stage means. Approximately equal numbers of all stages of 4th instars and pharate adults were included. The depth dimension is the maximum observed prior to the initiation of adult development. Means having the same upper case or lower case letters were not significantly different. GB = greenbean; Poins = poinsettia; SP = sweet potato. Greenbean, tomato and poinsettia plants have pubescent leaves; cotton, collard and sweet potato plants have glabrous leaves.

when F-tests were significant (P = 0.05), the Fisher's Least Significant Difference (LSD) (Figure 3) or the Tukey's HSD (all other figures and tables) Comparison of Means Test was used (α = 0.05).

Results

Effect of host plant on s 4th instar and pharate adult dimensions

The host plant species significantly affected the mean length (F=64.92; df=5.39; P d" 0.0001) and width (F=36.59; df=5.39; P d" 0.0001) of 4th instar/pharate adult whiteflies (Fig. 1). Whiteflies reared on cotton, collard and sweet potato (glabrous-leaved plants) were significantly longer and wider than whiteflies reared on green bean, tomato and poinsettia (pubescent-leaved plants). Mean lengths of silverleaf whiteflies reared on green bean, tomato, poinsettia, cotton, collard and sweet potato were 0.68, 0.63, 0.67, 0.77, 0.75 and 0.74, respectively. Mean widths were 0.46, 0.43, 0.46, 0.55, 0.53 and 0.53, respectively. In contrast, maximum depth observed for 4th instars was greater when nymphs were reared on green bean, tomato and poinsettia (0.30 mm) than when they were reared on cotton, collard and sweet potato (0.21 mm) (Fig. 1). Newly ecdysed nymphs reared on the glabrous-leafed plants (cotton, collard and sweet potato) were less thick than those reared on the pubescent-leafed plants (green bean, tomato and poinsettia). Because of their thinness, it was difficult to accurately measure the depth of these whiteflies. We estimate, their depth to be approximately 0.05 mm as compared to the 0.08 to 0.1 mm of nymphs reared on the pubescent-leafed plants.

Table 1. Effect of host plant identity on the mean depths (mm x 10) of SLWFs during pharate adult development

	PHARATE ADULT STAGE				
PLANT	**6A**	**6C**	**7**	**8**	**9**
Greenbean	2.26 ± 0.04^{Aa}	2.26 ± 0.04^{Aa}	2.33 ± 0.03^{ABa}	2.36 ± 0.02^{ABa}	2.43 ± 0.03^{Ba}
Tomato	2.19 ± 0.03^{Aa}	2.18 ± 0.04^{Aab}	2.22 ± 0.04^{Aa}	2.20 ± 0.03^{Ab}	2.32 ± 0.03^{Bb}
Poinsettia	2.20 ± 0.03^{ABa}	2.11 ± 0.04^{Ab}	2.23 ± 0.03^{ABCa}	2.28 ± 0.03^{BCab}	2.35 ± 0.03^{Cab}
Cotton	1.67 ± 0.03^{Ab}	1.70 ± 0.03^{ABc}	1.79 ± 0.02^{BCb}	1.81 ± 0.02^{Cc}	1.93 ± 0.02^{Dc}
Collard	1.59 ± 0.02^{Ab}	1.74 ± 0.04^{Bc}	1.81 ± 0.02^{Bb}	1.79 ± 0.03^{Bc}	1.91 ± 0.02^{Cc}
Sweetpotato	1.66 ± 0.03^{Ab}	1.66 ± 0.02^{Ac}	1.78 ± 0.03^{Bb}	1.80 ± 0.02^{Bc}	1.89 ± 0.02^{Cc}

Depths were determined for SLWFs from 3-5 different cohorts. Each value represents the mean of at least 40 separate determinations ± S. E. Across horizontal rows, means with the same upper case superscript are not significantly different. Within vertical columns, means with the same lower case superscript are not significantly different.

Prior to the initiation of adult development (Stage 6), some 4th instars reared on green bean, tomato and poinsettia were observed to reach Stage 5, while the maximum stage reached by those reared on cotton, sweet potato and collard was Stage 3. Typically, nymphs grown on green bean and tomato achieved Stage 4, while those grown on the other three plants achieved Stage 2 Old, prior to entering Stage 6. Since measuring depth involved removing and killing the whitefly nymph, depth measurements could not be tracked daily for a given nymph. Rather, our conclusions regarding maximum depth attained prior to the initiation of adult development were based on the large numbers of nymphs observed that had depths of 0.15-0.17 mm when reared on sweet potato, cotton or collard compared with depths of 0.23-0.26 when reared on green bean, tomato and poinsettia, before having undergone adult development (entering Stage 6).

Analysis of the depth measurements of Stage 6A, the stage when adult development had just been initiated, provided information regarding mean depth achieved just prior to the initiation of adult development for nymphs reared on the glabrous-leafed sweet potato, cotton and collard plants (Table 1). The mean depth for these Stage-6 whiteflies was equivalent to the depth of a 4th instar Stage-2 Old whitefly nymph. However, the relatively low number or absence of Stage 6A whiteflies reared on green bean, tomato and poinsettia, respectively, with depths of between 0.27 and 0.3 mm

Table 2. Effect of host plant identity on the percent[1] of pharate adult SLWFs with depths equivalent to the depths of Stage-2 Young, -2 Old, -3, -4, and -5 nymphs

	PHARATE ADULT STAGE												
	6A					7				9			
Nymphal→ stage	2Yg	2Old	3	4	5	2Old	3	4	5	2Old	3	4	5
PLANT ↓													
Greenbean			42	53	5		30	60	10		22	63	15
Tomato			62	35	3	2	47	44	7		38	46	16
Poinsettia			62	38		1	51	42	6		33	53	14
Cotton	6	66	28			35	65			9	89	2	
Collard	8	78	14			20	80			10	88	2	
Sweetpotato	13	53	34			40	60			19	81		

[1] Each percentage was calculated using at least 40 separate determinations. Depths were determined for SLWFs from three to five different cohorts.

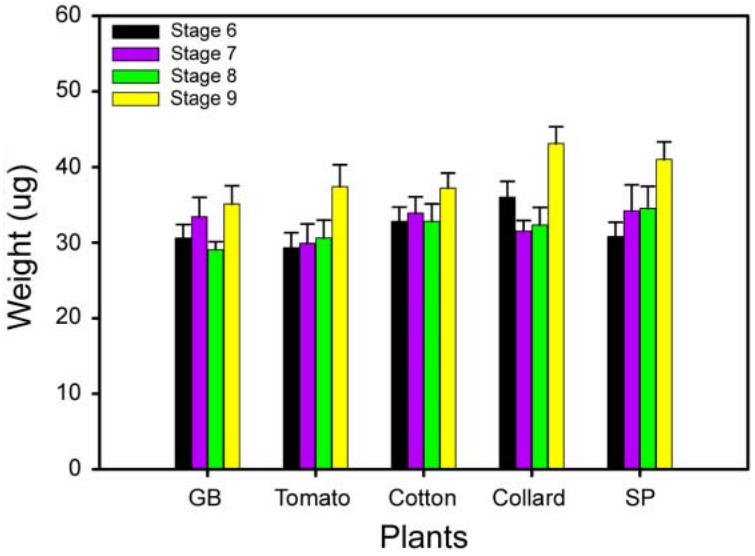

Figure 2. Mean weights of Stage-7, -8 and –9 silverleaf whiteflies reared on five host plants. Plants were infested with whiteflies that had been maintained on a variety of plants, and weights of individuals were determined as described in Materials and Methods. Each value represents the mean ± S. E. of at least 10 separate determinations. GB = greenbean; SP = sweet potato. Greenbean and tomato plants have pubescent leaves; cotton, collard and sweet potato plants have glabrous leaves.

suggests that for nymphs reared on pubescent-leafed plants, there may be a decrease in depth upon entrance into Stage 6. At each stage of pharate adult development [6A, 6B (results not shown), 6C, 7, 8 and 9], mean depth was significantly greater for whiteflies reared on green bean, tomato and poinsettia than for those reared on cotton, collard or sweet potato (Table 1). Only 10% of pharate adult whiteflies (Stages 6-9) reared on green bean, tomato and poinsettia had depths less than 0.20 mm, while only 2% of pharate adults grown on cotton, collard and sweet potato had depths greater than 0.20 mm. During pharate adult development, an increase in depth was observed, with most Stage-9 whiteflies reared on the glabrous-leafed plants having a depth equivalent to that of a 4th instar Stage-3 nymph, and most reared on the pubescent-leafed plants having a depth equivalent to a 4th instar Stage-4/5 nymph (Table 2).

Effect of host plant on the weight of pharate adult silverleaf whiteflies

Unlike length, width and depth dimensions, mean weights of pharate adults in Stages 6, 7, 8 and 9 were not significantly affected by the identity of the host plant on which the whitefly was reared (Stage 6: F=1.2; df=4,57; *P*= 0.32; Stage 7: F=0.54; df=4,53; *P*= 0.71; Stage 8: F=1.23; df=4,62; *P*= 0.31; Stage 9: F=1.49; df=4,45; *P*= 0.22 (Fig. 2). However, in general, maximal values were exhibited by Stage 9 whiteflies no matter which plant served as the host plant (Fig. 2). When whiteflies were reared on collard, the mean weight of Stage-9 whiteflies was significantly greater than the mean weights of Stage 6, 7 and 8 whiteflies (F=5.57; df=3,43; *P*= 0.0025).

Effect of host plant on ecdysteroid titer fluctuations during the 4th instar/pharate adult

The ecdysteroid content of filter chamber-gut complexes removed from whiteflies reared on green bean, cotton, collard and sweet potato ranged between 52 and 94 fg/complex and there was no significant difference in mean ecdysteroid levels of gut complexes (F=1.23; df=7,16; *P*= 0.34) (fig. 3). The ecdysteroid content of the gut complex of silverleaf whiteflies reared on tomato was not determined. The mean value for all the determinations, 71.8, was selected as the value to be subtracted from each whole body ecdysteroid determination.

Ecdysteroid fluctuations during the 4th instar/pharate adult were determined for whiteflies reared on each of five of the experimental plants used in these studies (Fig. 4a-e). Whether expressed as fg/whitefly or fg/10 µg wet weight, the patterns of ecdysteroid fluctuation were similar, although titers peaked between 700 and 1,100 fg and between 250 and 400 fg, respectively, when expressed as fg/whitefly and fg/10 µg wet weight. In green bean-reared nymphs, the premolt ecdysteroid peak (expressed as fg/10 µg wet weight) occurred between Stages 4 and 6A, in tomato-reared whiteflies, between Stages 4 and 5, in cotton- and collard-reared whiteflies at Stage 2 Old, and in sweet potato-reared whiteflies, between Stages 2 Old and 6B. In green bean- and tomato-reared nymphs, ecdysteroid levels were not significantly different for extracts prepared from Stage-4 and Stage-5 whiteflies; in extracts prepared from cotton-, collard- and sweet potato-reared whiteflies, ecdysteroid levels were not significantly different for Stage-2 Old and –3 whitefly extracts (results not shown). When reared on cotton, whitefly ecdysteroid levels increased again in Stage 7, but the increase was not statistically significant. The breadth or plateau of the ecdysteroid peak was greater when whiteflies were reared on green bean and sweet potato (peaked between Stages 4-5 and 2 Old- 6B, respectively) than when they were reared on the other

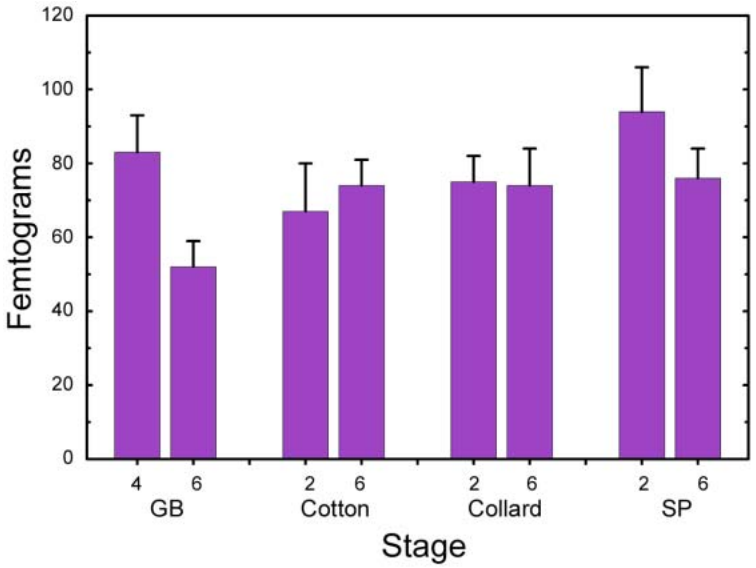

Figure 3. Mean ecdysteroid titers of filter chamber/gut complexes of silverleaf whiteflies reared on greenbean (GB), cotton, collard and sweet potato (SP) plants. Whiteflies were staged and collected, extracts prepared and ecdysteroid concentrations determined by EIA as described in Materials and Methods. Each sample contained 10 complexes. Each value represents the mean ± S. E. of at least three separate determinations and is expressed in femtograms 20-hydroxyecdysone equivalents/filter chamber gut complex.

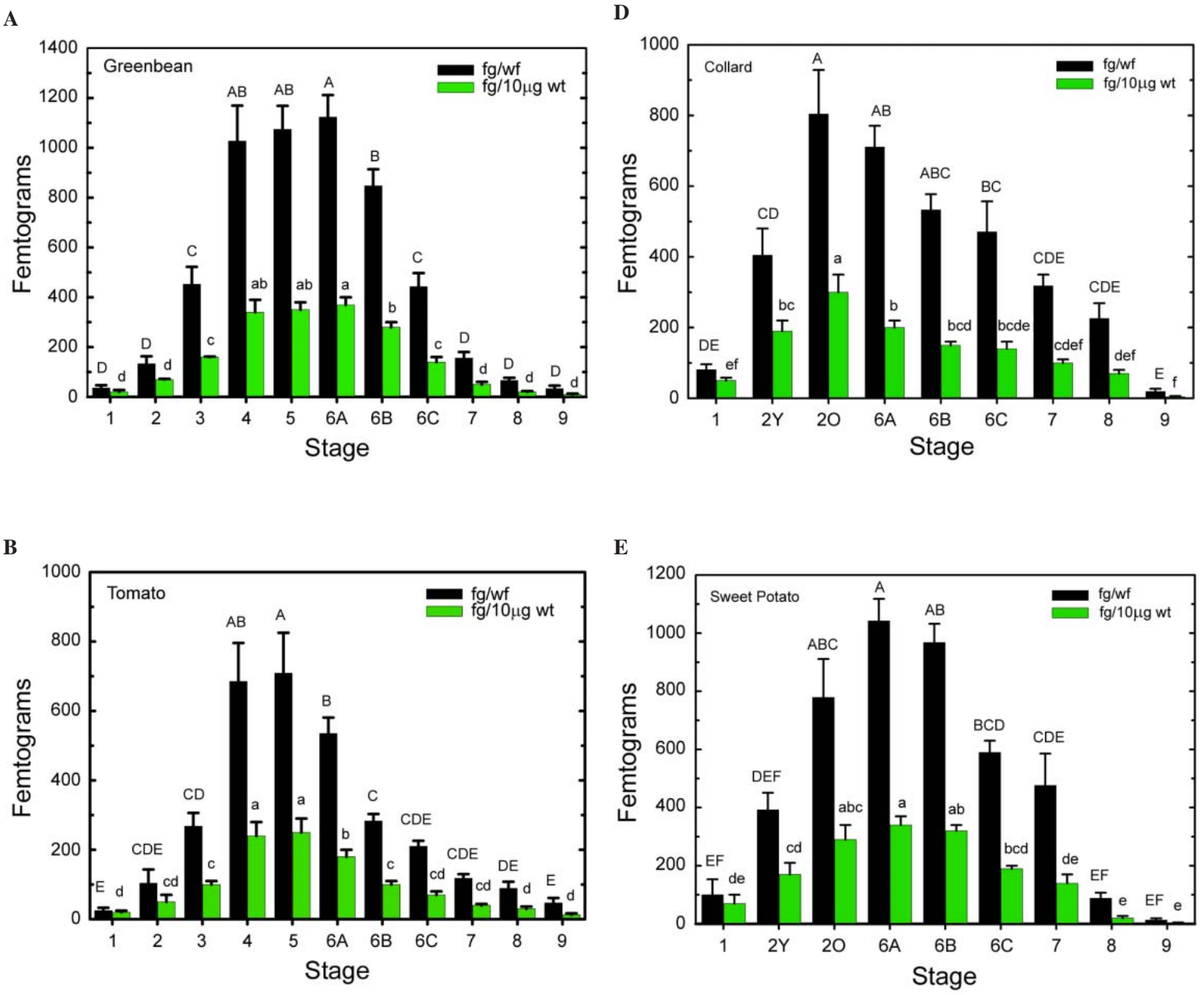

Figure 4. Ecdysteroid fluctuations during the 4th instar and during pharate adult development for silverleaf whiteflies reared on five host plants. Whiteflies were staged and collected, extracts prepared and ecdysteroid concentrations determined by EIA as described in Materials and Methods. Each value represents the mean ± S. E. of at least five separate determinations and is expressed in femtograms 20-hydroxyecdysone equivalents/whitefly or /10 µg wet weight. Means having the same letters were not significantly different. Greenbean and tomato plants have pubescent leaves; cotton, collard and sweet potato plants have glabrous leaves.

three experimental plants (peaked at Stage 4-5 or 2 Old) (Fig. 5). Interestingly, the developmental time from the 3rd-4th instar ecdysis to the onset of adult eye formation was approximately the same for whiteflies reared on the five experimental plants (results not shown).

Discussion

Results of this study show that 4th instar silverleaf whiteflies

reared on the pubescent-leafed green bean, tomato and poinsettia plants attained a significantly greater depth than those reared on the glabrous-leafed cotton, collard and sweet potato plants. However, length and width dimensions of 4th instar whiteflies were significantly greater when the whiteflies were reared on the glabrous-leafed plants. Since whiteflies feed throughout the pharate adult stage (Lie et al., 1996; Costa et al., 1999; Gelman, unpublished results), it is not surprising that they were observed to increase in depth during the pharate adult stage. Most of the Stage-8 whiteflies reared on the glabrous-leafed plants had a greater depth dimension than the 0.15-0.17 mm typically observed as the greatest depth attained for 4th instars. However, the depth of whiteflies reared on green bean, tomato and poinsettia did not increase as much during pharate adult development (between Stages 6A and 9) as did the depth of those reared on the glabrous-leafed cotton, collard and sweet potato plants. Thus, only 14-34% of new pharate adults (Stage 6A) reared on the glabrous-leafed plants had depths equivalent to a Stage-3 4th instar, while just prior to emergence (Stage 9), 81-88% exhibited depths equivalent to a Stage-3 4th instar. In contrast, the percent of whiteflies that had a depth equivalent to a Stage-4/5 4th instar nymph, only increased from 58% (Stage 6A) to 78% (Stage 9) for whiteflies reared on green bean, from 38% to 62% for whiteflies reared on tomato, and from 38% to 67% for whiteflies reared on poinsettia.

Plant identity had no effect on the weights of Stages 6-9, which were the same for a given stage of the pharate adult. In addition, whitefly weights reached their highest values in Stage 9, the stage in which mean depth was also the greatest. Also, although the typical nymphal stage at which maximum depth was reached differed for whiteflies reared on pubescent-leafed (Stage 4) and glabrous-leafed (Stage 2 Old) plants, the developmental time between the 3-4th instar ecdysis and the onset of adult development (Stage 6) was similar for whiteflies reared on the five experimental plants.

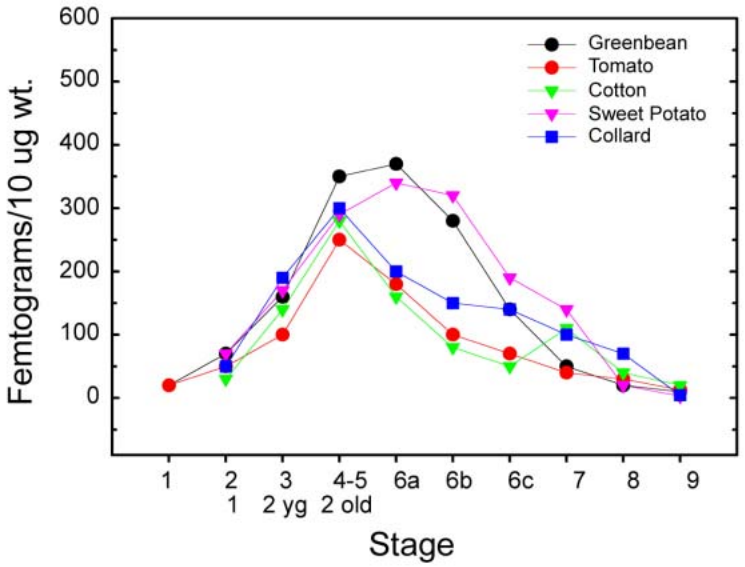

Figure 5. Comparison of the height and breadth of the ecdysteroid peak in silverleaf whiteflies 4th instars and pharate adults reared on five host plants. Whiteflies were staged and collected, extracts prepared and ecdysteroid concentrations determined by EIA as described in Materials and Methods. Each value represents the mean of at least five separate determinations and is expressed in femtograms 20-hydroxyecdysone equivalents/10 µg wet weight. To avoid confusion, S.E. bars have not been drawn. Stage designations in white are for nymphs reared on greenbean and tomato plants (pubescent-leafed); stage designations in yellow are for nymphs reared on cotton, sweet potato and collard plants (glabrous-leafed).

Russell (1948) reported that the nature of the host plant affected the appearance of *Trialeuodes vaporariorum* (the greenhouse whitefly). Pharate adult (referred to as pupae by Russell) *T. vaporariorum* had larger papillae and longer setae when reared on pubescent as opposed to glabrous-leafed plants. Neal and Bentz (1999) and Guershon and Gerling (2001) reported similar findings for *Bemisia tabaci* (sweet potato whitefly). Mound (1963) found that the pharate adult of *B. tabaci* had more irregular margins when reared on plants with a rough leaf cuticle. Regarding pharate adult dimensions, Bethke et al. (1991) reported that *B. tabaci* reared on cotton were significantly longer and wider than those reared on poinsettia (pubescent leaves). Our data supported these findings using *B. argentifolii.* We observed that the maximum depth reached on poinsettia was 0.3 mm (Stage 5) although since we detected relatively few Stage-5 whitefly nymphs, it appears that most tended to enter Stage 6 from Stage 3 or 4 (unpublished results). When *B. tabaci* were bred for several generations on the same host plant as was used as the test plant (i.e., reared on cotton and tested on cotton, or reared on poinsettia and tested on poinsettia), differences in length and width measurements among individuals were even greater than when whiteflies were reared for several generations on poinsettia and tested on cotton or vice versa (Bethke et al., 1991). When Tsai and Wang (1996) compared the 2nd, 3rd, and 4th instar lengths of *B. argentifolii* on eggplant (pubescent), tomato (pubescent), sweet potato (glabrous), cucumber (pubescent) and garden bean (pubescent), for both male and female 4th instars, there were significant differences in mean length with whiteflies grown on the glabrous-leafed sweet potato being the longest. Yet, for the 2nd and 3rd instars, the identity of the host plant did not influence the mean whitefly length.

A comparison of the patterns of ecdysteroid fluctuation during the 4th instar/pharate adult for whiteflies reared on the five test plants revealed that titers peaked in the stage that precedes Stage 6 (Stage 2-old/3 and 4-5 for whiteflies reared on glabrous- and pubescent-leafed plants, respectively). Based on ecdysteroid concentration, it appears that Stage-2, -3 and -4/5 nymphs reared on pubescent-leafed plants are physiologically equivalent to Stage-1, -2 Young and -2 Old/3 nymphs reared on glabrous-leafed plants. It is probable that the mean ecdysteroid titers of nymphs with depths <0.08 mm reared on the glabrous-leafed plants would be equivalent to the mean ecdysteroid titers of Stage-1 nymphs reared on green bean and tomato. However, titers for these nymphs were not determined because the magnitude of the difference between Stage-1 and Stage-2 mean ecdysteroid titers for whiteflies reared on the pubescent-leafed plants was very small.

It is likely that there is some variance in regard to the last stage attained during the 4th instar before adult development is initiated in Stage 6. Few nymphs reared on glabrous leaves attained Stage 3 and on pubescent leaves attained Stage 5 indicating that nymphs do not attain the maximum possible 4th instar depth before undergoing adult development. Furthermore, although many

nymphs achieved Stage 2 Old on glabrous plant hosts and many achieved Stage 4 on pubescent plant hosts, it is probable that a few nymphs reared on glabrous-leafed plants proceeded directly from Stage 2 Young to 6A, and a few reared on pubescent-leafed plants proceeded from Stage 3 to 6A. Mean ecdysteroid titers began to increase in Stage 2 Young and 3 when glabrous- and pubescent-leafed plants, respectively, served as hosts.

For whiteflies reared on tomato, cotton and collard, ecdysteroid titers began to decline upon entrance into Stage 6, while for those reared on green bean and sweet potato they remained high for a longer period of time. Thus, the identity of the host plant influences the stage in which ecdysteroid titers begin to decline, but leaf pubescence does not appear to be the determining factor.

In summary, when selecting 4th instar/pharate adult silverleaf whiteflies that are developmentally synchronous, it is important to take into consideration the identity of the plant. Fourth instar/pharate adults reared on glabrous-leafed plants were significantly longer and wider than those reared on pubescent-leafed plants. However, when reared on pubescent-leafed plants they tended to achieve a greater depth prior to the initiation of the premolt ecdysteroid peak and the initiation of adult development as well as at the completion of adult development than did those reared on glabrous-leafed plants. Once a physiological event is associated with a particular stage, stage can be used as a criterion for collecting large numbers of physiologically synchronous whiteflies. Our results also demonstrate that while the breadth of the premolt ecdysteroid peak is affected by the nature of the host plant, the height of the peak is not.

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