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APHID POPULATIONS IN A FLORIDA CITRUS TRISTEZA VIRUS SUPPRESSION TRIAL

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Abstract. **Aphid populations were monitored weekly during a five-year study conducted in a sweet orange scion (Hamlin, *Citrus sinensis* (L.) Osbeck) on sour orange rootstock young tree planting. Sampling for citrus tristeza virus (CTV) was conducted once a year. Samples for CTV analysis were analyzed using an indirect double-antibody sandwich with enzyme-linked immunosorbent assay (ELISA). The presence of CTV was compared to aphid population increases found during the previous year. The 300 tree block was treated with several chemical aphid control regimes (Temik®, Meta-Systox R® and stylet oil) with little or no effect. Mild and severe isolates of CTV were observed throughout the block. Four double-sided 22.5 cm × 13.5 cm Pherocon® A.M. sticky traps posted on the borders of the block were used to catch and monitor aphids. In 88% of the weeks of observation either *Aphis spiraecola* (Patch), *Aphis gossypii* (Glover) or both species were the predominant aphids collected. These aphids were most abundant in March, April and May with a slight fall presence peaking in November. An increase in the number of *A. spiraecola* in 1993 was found to be correlated with a large increase in the presence of CTV in the block in early 1994. No distinct pattern of aphid movement was evident throughout the block.**

Introduction

Until the November 1995 discovery that the Brown Citrus Aphid (BrCA), *Toxoptera citricida* (Kirkaldy), had arrived in Florida, the state's endemic aphids most commonly found on citrus were: *Aphis spiraecola* (Patch), *Aphis gossypii* (Glover), *Aphis craccivora* (Koch), *Toxoptera aurantii* (Boyer de Fonscolombe), *Macrosiphum euphorbiae* (Thomas) and *Myzus persicae* (Sulzer) (Blackman and Eastop, 1984). The highest rate of transmission of Citrus Tristeza Virus (CTV) in Florida had been attributed to *A. gossypii*, the melon aphid, followed by *A. spiraecola* (=*citricola* van der Goot), the spiraea or green aphid (Norman and Grant, 1954; Yokomi and Garnsey, 1987). *T. aurantii*, the black citrus aphid, rarely transmits the virus (Yokomi and Garnsey, 1987), although it has been reported to do so (Simanton and Knorr, 1969; Norman and Grant, 1956). *M. persicae*, *M. euphorbiae* and *A. craccivora* have not been confirmed as vectors for CTV and are found only occasionally on citrus, preferring to colonize weed hosts (Oldfield and Yokomi, 1990; Yokomi, personal communication).

Since its discovery in Florida in the 1950's, the citrus tristeza virus has spread throughout all of the state's major growing areas (Garnsey, et al, 1980; Grant, 1952). During this time in the Indian River area, tristeza was first diagnosed as a budwood-transmitted disease which naturally spread rapidly during the late 1960's (Brlansky, et al. 1986). In 1963 Florida's budwood certification program still considered CTV-positive trees as registered, thereby causing further spread of the disease unbeknownst to nurseries involved in propagating new trees (Powell and Pelosi, 1993).

The brown citrus aphid is the most efficient known vector of CTV (Costa and Grant, 1951). Since its appearance in the New World, BrCA has quickly become the most abundant and predominant citrus-attacking species in each country it has infested (Yokomi and Tang, 1996). Because the same scenario is expected in Florida, we have summarized pre-*Toxoptera citricida* alate aphid activity (1990-1994) at the Indian River Research and Education Center on Florida's east coast at Fort Pierce.

Materials and Methods

A block of 300 CTV-free Hamlin sweet orange [*Citrus sinensis* (L.) Osbeck] trees on sour orange (*C. aurantium* L.) rootstock was planted May 1989 on double beds 9.15 m apart, 6.1 meters between rows, with between-tree spacing of 4.6 m. The block was established as an aphid/CTV chemical control experiment. The results of the natural field spread of CTV throughout the block have been reported by Powell, *et al*. (1996).

Alate aphid activity in the block was monitored using five yellow water pan traps and four yellow double-sided sticky traps. The pan traps were round, 25 centimeters in diameter, 7.5 cm deep and painted lemon yellow. Four pan traps were located between trees on the outside north and south rows of the block, three trees from

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each end of the row. These were labeled NE, NW, SE and SW. The fifth trap was located near the center of the block. All five traps were placed on platforms 1.5 m above the ground.

The four sticky traps, (model Pherocon® A. M., manufactured by Trécé Inc., Salinas, Ca.) were placed at the midpoint along each side of the block. Traps were folded so that aphids could be collected from outside or inside the plot and aphid counts were tabulated with respect to the direction each side faced. Traps were posted 2.2 m above the ground and held firmly by a clip mounted on the end of a PVC pipe. On a weekly schedule, alate aphids were collected from water traps, the traps were refilled and new sticky traps were posted.

Sticky traps with aphids were returned to the lab, counted and identified under 7X to 30X magnification. Only aphids known to colonize citrus were counted. Non-citrus feeding aphids and specimens which were decomposed beyond identification were categorized as "Other." *A Field Key to the Citrus Aphids in Florida* (Entomology Circular No. 10, Florida Department of Agricultural and Consumer Services, Division of Plant Industry. Denmark, H. A.) and *Aphids on the World's Crops: An Identification Guide* (Blackman and Eastop, 1984) were used to identify aphid species. A sampling of aphids was sent to the Division of Plant Industry's H. A. Denmark for species identification confirmation.

Results

Aphids captured and identified throughout the course of the test included *Aphis spiraecola*, *Aphis gossypii*, *Aphis craccivora*, *Toxoptera aurantii*, *Macrosiphum euphorbiae* and *Myzus persicae*, listed in order of abundance. *A. spiraecola* represented 49.3% of all aphids collected and *A. gossypii* was second most prevalent at 14.1%. Also encountered were *A. craccivora* (3.4%), *T. aurantii* (2.9%), *M. euphorbiae* (1.4%) and *M. persicae* (1.3%).

Water traps were not an effective means of sampling aphid populations in this study. With each growing season, expanding flush cycles grew the tree canopies closer to the five water traps. It is believed that aphids easily differentiated between the water traps and the nearby trees and often selected flush in the canopies, resulting in a very low capture rate in the traps. In 37% of the weeks, no aphids were captured in the water traps. Aphid colonies were often observed on surrounding trees while water traps remained empty. During the test's peak month of spirea aphid activity (April of 1993), the five water traps captured a combined total of 12 aphids while the four sticky traps surrounding the plot captured 1,647. Therefore, only data collected from sticky traps will be reported.

Aphis spiraecola was the most abundant species captured every month except January 1990 when *Aphis gossypii* was the predominant species captured. Either *A. spiraecola, A. gossypii* or both species were present in the test's monitoring traps in 97% of the survey weeks, but no aphids of any species were captured in June or July of 1989, the first two months following the planting of the test trees. The spirea aphid population increased to high levels between March and May each year. In four of the five years, the month of April accounted for 47% of each year's total number of captured aphids.

The fall peak occurred consistently in November with 11% of each year's total caught. The highest populations of *Aphis gossypii* were captured in November 22.5%, April 21.8% and March 21.6%. Of the experiment's total number of collected aphids, only .07% occurred in June, .06% in July and 1.3% in August.

A. gossypii is reported to be a very effective vector of CTV (Yokomi and Garnsey, 1987; Yokomi, et al, 1987). This test demonstrated that *A. spiraecola* is capable of similar efficacy.

Comparison of direction of aphid travel for *A. spiraecola* and *A. gossypii* was analyzed using several different methods. First, species were compared with respect to traps in which they were caught. The number of aphids collected in traps and with respect to a trap's position in the block were examined using analysis of variance, and no significant differences were found.

The number of aphids collected on the side of a trap facing the outside of the block was added to the number of aphids collected from the trap on the opposite side of the block facing the same cardinal direction. For each species, its "direction of movement" was analyzed with respect to cardinal direction for the six-month periods January-June and July-December. No trends were found.

The number of aphids collected on the sides of all four traps facing the inside of the block was compared with the number of aphids collected on the sides of all four traps facing the outside of the block. For each species, the two conditions were compared using yearly totals in a t-test comparison. No significant difference between the two conditions was found.

For the years 1990-1994, a monthly comparison of aphid occurrence for each species was investigated using analysis of variance. *A. spiraecola* population increased during the spring months: April (average total of 486 aphids per month), May (155.5 apm) and March (89.25 apm). November was the third most populous month (119.5 apm).

For *A. gossypii*, monthly totals for April (51.75 apm), February (51.5 apm) and November (51.5 apm) were all significantly higher ($P <= 0.05$) than monthly totals for September (6.25), August (3.25), June (1.75) and July (1.5) (Fig. 1).

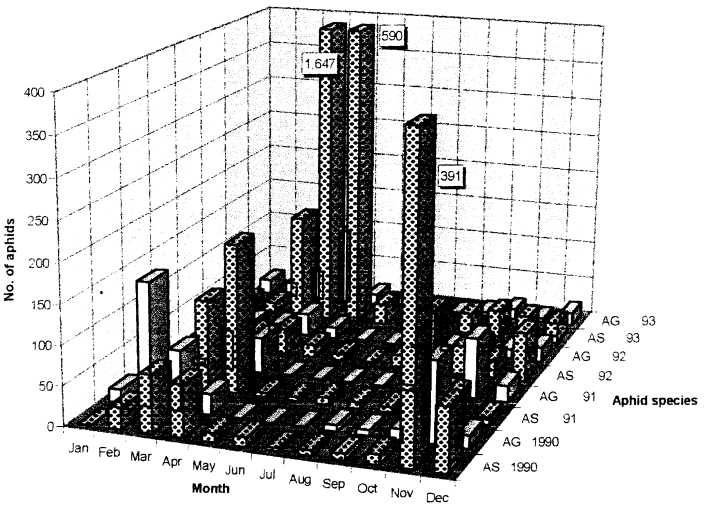

Figure 1. Monthly captures of *Aphis spiraecola* (AS) and *Aphis gossypii* (AG) at IRREC from 1990-1993.

As suggested by Powell, et al., (1996), the increased incidence of CTV in 1994 correlated with the heavy flights of *Aphis gossypii* and *Aphis spiraecola* in April and May of 1993.

Table 1. Total number of *A. spiraecola* and *A. gossypii* captured on yellow sticky traps and percentage of trees diagnosed with CTV in 300 tree block of young Hamlin oranges 1990-94.

Collection year	No. of *A. gossypii*	No. of *A. spiraecola*	CTV sample year	CTV % in block	Increase in % of CTV
1989*	72	48	1990	0.0%	N/A
1990	434	689	1991	1.2%	1.2%
1991	225	424	1992	11.8%	10.6%
1992	141	320	1993	13.8%	2.0%
1993	244	2594	1994	38.2%	24.4%

*aphids collected for only seven months (June-December)

Three aspects of the data collected were tested for correlation: yearly population of *A. gossypii* compared to yearly percentage of trees newly affected by CTV; yearly population of *A. spiraecola* compared to yearly percentage of trees newly affected by CTV; and the combination of both species' yearly populations compared to yearly percentage of trees newly affected by CTV. Using data published by Powell (1996) reporting the spread of CTV each year between 1990 and 1994 within this block, this correlation can be made. Powell sampled leaves annually in the experimental plot each winter. Samples were analyzed for mild and severe isolates of CTV by indirect double-antibody sandwich with enzyme-linked immunosorbent assay (ELISA) (Bar-Joseph et al., 1980) (Powell et al., 1992) except the monoclonal antibody (Hooker et al., 1993) was used instead of 3DF1. The percentage of trees infected with CTV after growing seasons 1, 2, 3, 4 and 5 was 0.0, 1.2, 11.8, 13.8 and 38.2, respectively.

The degree of association between aphid population and the spread of CTV may be measured mathematically using the correlation coefficient. The correlation coefficient (r) may be determined by (LeClerg, et al., 1939):

$$r = \frac{\Sigma[(x-\bar{y})(y-\bar{y})]}{\sqrt{[\Sigma(x-\bar{x})^2][\Sigma(y-\bar{y})^2]}}$$

Significance of *r* was tested using a *t*-test for P<= 0.05 or 0.01

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

The number of *A. gossypii* collected (Table 1) was not correlated with the percentage of CTV incidence within the plot. For *A. gossypii*, r = 0.0078 and t = 0.0135 which was not significantly different than zero at P<=0.05. However, for *A. spiraecola* where r = 0.900 and t = 8.21, there was a significant correlation (P<=0.01). The combination of both species also correlated where r = 0.855 and t = 5.508, (P<=0.05).

Discussion

In past spatial studies on the spread of CTV epidemics throughout a block of trees, Chellemi et al. (1991) concluded that the presence of a tree with CTV appeared to have little or no effect on the condition of its immediate neighbors. Furthermore, spread within and between groves appears to take place in CTV epidemics resulting in a more explosive spread of the virus.

Although *A. gossypii* may have been primarily responsible for the spread of CTV throughout the test plot, the magnitude of the spirea population may have compensated for the specie's lower vectoring ability.

Yokomi and Garnsey (1987) and Yokomi, et al. (1987) have reported that *A. gossypii* is up to three times more efficient as a CTV vector than *A. spiraecola.* Our findings however, confirm previous observations that the spirea aphid occurs in greater abundance than *A. gossypii* (Yokomi and Garnsey, 1987). For example, in 1993 we collected 2,594 spirea aphids against 244 melon aphids, or 10.6 times as many spirea aphids. For the entire length of the experiment, 4,402 spirea aphids were captured against 1,200 melon aphids or 3.67 times as many.

More work on the population dynamics of domestic citrus aphids could provide more complete models for seasonal movement, vectoring efficiency and tree damage potential. With the recent arrival of *Toxoptera citricida* in Florida, models of movement of indigenous aphids may enable extension agencies to more accurately predict and prepare for the brown aphid's impact on each citrus growing region in the state.

Additional information on aphid population dynamics in the field must be obtained in order to help forecast the brown aphid's impact on domestic aphids. Competition for food supply and other environmental factors may possibly result in the displacement of a specie such as *Toxoptera aurantii*.

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PREPLANT LEAF NITROGEN EFFECTS ON GROWTH AND FERTILIZER REQUIREMENT OF YOUNG 'HAMLIN' ORANGE TREES

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Abstract. **Fertilization rate and growth response of young trees vary both in citrus nurseries and in new plantings. Experiments were conducted with 'Hamlin' orange trees [*Citrus sinensis* (L.) Osb.] on 'Swingle' citrumelo rootstock [*C. paradisi* Macf. × *Poncirus trifoliata* (L.) Raf.] rootstock to determine the optimum N rate for greenhouse nursery trees and the effect of N nutrition of greenhouse nursery trees on growth and fertilization response of trees in the field. Greenhouse nursery trees received 12, 50, 100 or 200 ppm N weekly via drip irrigation for one year. Optimum tree growth occurred at the two highest rates, while trees that received 12 or 50 ppm N were stunted and chlorotic. In another experiment from 0 - 0.75 lb N/tree/year were applied for 2 years to young field trees with initial leaf N levels of 1.4 - 4.1% when planted. Preplant leaf N level had no effect on trunk diameter, height, shoot growth and number or dry weight of trees for year one and two in the field. Fertilizer rate in the field did not affect tree growth in year one but did in year two, with maximum growth occurring at 0.37 lb N/tree/ year.**

Nutrition of citrus is one of the most important aspects of nursery tree production. Fertilization programs for young trees are highly variable in Florida. Different fertilizer formulations (dry or liquid), frequencies, and rates are used for seedlings and budded, container-grown, and field-grown trees. High N rates are commonly used in Florida greenhouse and field citrus nurseries, ranging from 1,058 to 2,903 lbs/acre. Despite use of such high rates, only 5 to 20% of the N applied could be found in the leaves of 'Valencia' orange trees grown in container nurseries (Castle and Rouse, 1990).

Nitrogen nutrition for young trees is highly variable, as reflected by the optimum N fertilization rates reported for the first year in the field which range from 2 (Rasmussen and Smith, 1961) to 2.4 (Marler et al., 1987) to 3.8 (Obreza and Rouse, 1993) to 8 oz/tree/year (Willis et al., 1990). Moreover, some studies suggest that fertilization rate has no effect on tree growth for the first (Rasmussen and Smith, 1961; Obreza and Rouse, 1993) and second years in the field (Calvert, 1969 and Rasmussen and Smith, 1961). In other studies, Obreza and Rouse (1993) found that tree growth increased with increasing N rate.

Factors that may contribute to different fertilization responses by young citrus trees include soil type (Obreza and Rouse 1993), tree age and size (Calvert, 1969), rootstock (Wustcher, 1989), amount of stored reserves (Legaz et al., 1995) or type of nursery trees (bareroot or container grown) (Davies unpublished).

The objectives of these studies were to determine the N rate that produced the greatest tree growth in the greenhouse using 'Hamlin' orange on 'Swingle' citrumelo rootstock and to determine if there is a residual effect of greenhouse nutrition on subsequent growth and fertilization response of young citrus trees planted in the field.

Materials and Methods

Nursery studies. Experiment 1: Four hundred bare-root 'Swingle' citrumelo liners were purchased from a commercial nursery and planted on 25 Feb. 1992 in citripots containing a commercial medium composed of 1 perlite:1 peatmoss (v/v), 14.6 lbs of limestone and 0.13 lb of superphosphate/yard^3. Liners were budded with Hamlin orange on 3 Mar., 7 and 11 Apr., and 3 and 6 May

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