Studies on Stubborn Disease and its Vector in Arizona Citrus Groves and Nurseries[1]

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Abstract

Seasonal flight of beet leafhopper vectors of stubborn disease was monitored at wholesale nurseries in Yuma County, and at young citrus groves in Maricopa County using yellow sticky traps exposed at successive two-week intervals. Trapped leafhoppers were removed and assayed for presence of the citrus stubborn agent by PCR. Leafhoppers were collected live from weed plants in groves and nurseries using an insect vacuum and both leafhoppers and tissue from weed plants were assayed by PCR for the stubborn agent. Selected trees in groves in Yuma and Maricopa Counties were visually inspected for stubborn symptoms.

Introduction

Stubborn disease of citrus causes severe losses in areas of the Near East, north Africa and southwestern USA where the beet leafhopper occurs. Annual plants, many of which originated in the Old World, constitute sources from which the beet leafhopper acquires the stubborn agent. In the absence of suitable annual plants upon which they reproduce, beet leafhoppers fly to other plants and feed long enough to transmit the stubborn agent. California and Arizona citrus may be visited by leafhoppers carrying the stubborn pathogen during most months of the year. Although stubborn has been observed in Arizona citrus for several decades, little is known concerning when the leafhopper is most likely to visit citrus or when during the year, and to what extent, populations may carry the disease agent. Additionally, little information is available concerning specific infection rates in Arizona citrus. Herein we report the results of a two-year study of the seasonal occurrence of 1) the vector in Arizona citrus areas, 2) the stubborn agent in the vector and in weed plants, and we report our visual observations on stubborn incidence in groves in the two citrus producing areas.

Materials and Methods

Yellow cards manufactured by Seabright Laboratories, "Sticky Aphid Whitefly Trap", suspended about a foot above ground level on steel stakes, and placed at two wholesale nurseries in Yuma County and in young citrus at several locations in Maricopa county were used to trap flying leafhoppers. Traps were exposed at the two nurseries throughout 1995-96, and at the Maricopa County locations in 1996. Replaced every two weeks, traps were

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examined for beet leafhoppers at the Department of Plant Pathology, University of California, Riverside. Leafhoppers were removed from traps, washed in kerosene, then stored in kerosene until analysis for the stubborn pathogen by PCR. Leafhoppers collected live with a DeVac insect vacuum and those removed from traps were examined under a dissecting microscope to assure that they were the beet leafhopper, and to determine if they were males or females. Leafhoppers collected alive were frozen immediately after identification pending PCR assay for the stubborn agent. Weed plants suspected of harboring the stubborn agent were collected in plastic bags and stored at about 40 F until they were assayed by PCR.

Several rows of trees at one grapefruit grove in each of the two counties were visually inspected for symptoms of stubborn during April 1996. Each tree was examined for the presence of small lop-sided fruit, shortened twig internodes, small upturned leaves, and a shrubby appearance of all or part of the canopy compared to other trees in the grove.

Results and Discussion

Preliminary trapping from late 1993 through 1994 in Yuma County detected the presence of flying adult beet leafhoppers only during late March/early April and early June of 1994. During 1995 a total of 12 traps were exposed (six per nursery) at two nurseries in Yuma County. Total numbers of beet leafhoppers trapped on the various traps varied widely from trap to trap. At three trap locations within the two nurseries, 50 or more leafhoppers were trapped during the year; at the other nine locations seventeen or fewer adults were trapped throughout the year, indicating that flight may vary widely within a nursery. At one location where high numbers were trapped, annual hosts of the vector were particularly abundant near the trap. PCR assay of nearby wild turnip and London Rocket host plants that exhibited yellows symptoms, and from which we collected large numbers of beet leafhoppers during the spring, indicated that these hosts were infected with the stubborn agent.

By late March 1995, when trapping started at the two Yuma nurseries, biweekly totals of trapped leafhoppers numbered 20; this increased to 88 during early April. Although numbers remained substantial for the next month (late April, 43 trapped; early May, 49 trapped), numbers declined through early June (15 and 23 for the succeeding two 2-week periods) and during latter June and July no leafhoppers were trapped. Eleven leafhoppers were trapped in each of the next two periods, then a total of four or fewer (that is substantially less than one leafhopper per trap) were trapped during each two-week period for the rest of the year. In all over 90% of all leafhoppers were trapped between April and latter June.

Trapping at the Yuma nurseries continued through 1996, with the number of traps reduced to 4/nursery as trapping in Maricopa County commenced. As the date of receipt of ACRC funds had not allowed increased trapping before March 1995 at the Yuma County nurseries, 1996 trapping revealed for the first time a substantial flight activity during February that was not detected the previous year.

During December, 1995 no flight was detected; numbers increased during January 1996, and peaked at more than 7 leafhoppers/trap/two-week period during mid-February, declining gradually to nearly zero in late March then increasing to a second peak of nearly 6/trap during late April. Numbers decreased to less than one per trap by early May and remained low, never rising above 2/trap/2-weeks throughout the rest of the year. Thus, during both of the years in which flight of the vector was monitored at the two nurseries, most adults were trapped during late winter and spring when annual hosts known to harbor the stubborn pathogen germinate. Later, these hosts become infected with stubborn then are infested with reproducing vectors, and provide inoculative vectors that leave to feed on citrus when the hosts plants dry up and die in late spring.

In the nurseries, some of the traps were located where many annual hosts of the vector and pathogen were found during both years. Young seedlings and grafted trees in such nurseries would be expected to be particularly susceptible to infection as annual hosts become stressed and leafhoppers (already having fed for several weeks, acquired the stubborn agent and become inoculative) leave to find another plant to feed upon. From observations

during these two years, nursery trees would be expected to be exposed to highest numbers of vectors at approximately the same time that many of them become inoculative, that is two or more weeks after they first feed on infected annual plants in late winter. By contrast, with the virtual disappearance of many annual hosts of the vector and pathogen after late spring, the likelihood of transmissions to citrus for the rest of the year would be very much reduced.
In the Maricopa County area, results of trapping vectors at different grove sites indicated a similarity in seasonal flight activity to that detected in Yuma County. Traps were exposed at four sites starting in early March 1996. Numbers were already substantial by mid March, with 40, 74, 65, and 58 leafhoppers trapped during successive 2week intervals ending in early May. A location along the Gila River showed flight activity that was several times greater and lasted later in the spring than at the other locations south and west of Phoenix. This activity ended abruptly at all locations by early May and virtually no leafhoppers were trapped again until the end of October. During November, numbers increased to levels approximating those detected in the spring, then declined abruptly to practically zero by late December.

Although unfunded, trapping continued at a reduced rate in 1997. In January 1997, a predominance of female beet leafhoppers was detected on traps at the two Yuma County nurseries. This is the first time mostly females have been trapped at any time of the year in Arizona or in California. Information from extensive studies done by this project's personnel during the 1980s in the San Joaquin Valley of California indicated that most flight to traps during the year is done by male leafhoppers, presumably after they have mated with females. Particularly in the fall of the year, females in the San Joaquin Valley, inseminated by males, leave summer hosts in the valley floor and fly to surrounding foothills. There, leafhoppers feed on perennial plants until the annual host plants germinate in the winter, then they move to those annuals to reproduce the generation that returns to the valley in the spring. The predominance of females on traps in early winter in Yuma County indicates that they probably persist *in* nurseries and lay there eggs on host annual weeds *within* the nurseries provided weeds are allowed to germinate and mature there.

Beet leafhoppers trapped at each location throughout 1996 and those collected live periodically from annual weed hosts are still being analyzed by PCR for the stubborn agent. Leafhoppers collected from wild turnip at a Yuma nursery in early March 1995 transmitted stubborn when fed on healthy radish in the laboratory. PCR assay of wild turnip and London Rocket plants collected in late April from the University of Arizona Citrus Station and from a nursery in Yuma revealed that two of five wild turnip plants from the Citrus Station and one London Rocket from the nursery were infected with the stubborn agent. Several other wild turnips from the nursery were not tested but exhibited symptoms of asymmetrical small yellow leaves in a rosette that is characteristic of stubborn in this species. At Somerton, in Yuma County, all the grapefruit trees in seven rows of 17 trees were inspected for foliar and fruit symptoms characteristic of stubborn. Five of the 117 grapefruit trees showed classic advanced symptoms of stubborn; 32 % of the trees exhibited small lop-sided fruit, a symptom usually associated specifically with stubborn, and many other trees showed a few lop-sided fruit. A random inspection of a few trees in other rows revealed five other trees with classic symptoms of stubborn. Several varieties of citrus at a property north of Phoenix were examined for stubborn symptoms. A tree by tree inspection of one row of grapefruit trees revealed strong stubborn symptoms in about 60 % of the trees; in one row of an adjoining navel orange block about 35% of the 86 trees exhibited symptoms of stubborn but navels were generally less severely affected.

Although many leafhoppers collected from sticky traps await PCR analysis at the time of this report, we have for the first time been able to detect the stubborn spiroplasma in leafhoppers trapped on sticky cards, treated with kerosene, and held in kerosene for one year. We recently detected he stubborn spiroplasma in the bodies of such leafhoppers collected in March 1996 at a grove near the Gila River in Maricopa County. This first demonstration of the ability of PCR to detect in leafhoppers treated and stored this way, and our successful PCR detection of stubborn spiroplasmas in live-collected/frozen leafhoppers allows us to proceed more efficiently with studies of the seasonal occurrence of the disease agent in leafhoppers and plants. Although unfunded, we are continuing to trap leafhoppers at the two nurseries in Yuma County and at the Citrus Station. We will obtain further information on seasonal phenology of the vector and will test them by PCR as we are presently doing with leafhoppers trapped in 1996.