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The Role of Leafhoppers in Transmission of *Spiroplasma Citri* with Special Reference to Stubborn Disease of Citrus

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Title

The role of leafhoppers in transmission of Spiroplasma citri with special reference to stubborn disease of citrus

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B. Detailed description of the research activities and results

ABSTRACT

In the present report, we present for the first time, insect transmission of Spiroplasma citri in the Middle East by local variants of the beet leafhopper Circulifer tenellus (Baker). While in California, the identity of the vectors was established several years ago, little was known on natural transmission of the disease either in Israel or in the Mediterranean. A major difficulty was the multitude of variants of of two species in the Circulifer genus, namely, haematoceps and tenellus. Morphological differences, host preference and courting sound signals were used in order to distinguish between certain variants. Being aware of the reports on the possible role of Circulifer haematoceps in transmission of S. citri in Syria, we made attempts to distinguish between variants of the species which might be epidemiologically important to others. Two variants of leafhopper which belong to Circulifer haematoceps complex were characterized in this project. One was found to breed on Salsola kali in the coastal plains of Israel, the other multiplied on Chenopodium album in the Golan Heights. Differences were found between these variants as for their host range in captivity, the one from S. kali breeds on turnip only, while the one from C. album has been found to be polyphagous, reproducing and surviving on most of the plant hosts tested. Morphologically, difference were found between the two variants in male genitalia and in the female's 7th sternite.

We also found two variants of C. tenellus one trapped on Atriplex halimus in the Jordan Valley. Another variant was trapped on Portulaca

oleraceae in Bet Dagan. A partial host range is described herein.

Transmission of a Spiroplasma citri isolates was obtained for the first time in Israel, in 1988, using a variant of the Circulifer tenellus complex which was trapped from Atriplex halimus in the Jordan Valley. Later, in 1989, the variant from P. oleraceae was also found to transmit three isolates of S. citri from Israel. S. citri was isolated from six citrus varieties growing in different regions. Isolation was carried out from seed coats of infected trees.

Infectious leafhoppers were obtained first by injecting pathogen-free insect with S. citri. These leafhoppers were capable to inoculate Matthiola incana. All subsequent transmissions were carried out by pathogen-free leafhoppers which were allowed acquisition access feeding on herbaceous host infected by S. citri. Infectious leafhoppers have served to examine the possibility of various hosts to harbor S. citri. S. citri was leafhopper transmitted to additional cruciferous hosts as well as to oranges (Citrus sinensis). The presence of the pathogen was verified by serology and by cultivation of the organism both from plants showing symptoms and from Insects.

Research efforts in the USA were aimed at the characterization of the beet leafhopper transmitted virescence agent (BLTVA). The transmission characteristics, the host reactions and range are described in the report and in attached manuscripts.

Also, and the various isolates of Spiroplasma citri collected from various states in the USA and from infectious insects. Characteristics of the isolates included determination of the host range, leafhopper transmissibility and serological reaction. Unfortunately, the USDA laboratory in Riverside was closed in late 1987, not allowing to conclude this part of the research in the USA.

OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL

Identify the arthropods responsible for transmission of Spiroplasma citri in Israel and identify non-citrus natural hosts that may play a role in the spread of stubborn.

Establish the roles of isolates of S. citri from various geographic areas in the USA from various host plants, in the epidemiology of stubborn disease of citrus. Determine transmissibility of isolates of S. citri from various host plants and geographical areas by vectors known to transmit to citrus in southwestern USA, and by other leafhoppers.

Determine the conditions under which various vectors species transmit S. citri to citrus in the fields in California.

Develop new serological methods for assaying for S. citri and for differentiating between strains based on monoclonal antibodies or molecular hybridization.

INTRODUCTION

The citrus stubborn disease (little leaf of citrus) has been known in Israel since 1928 (Reichert and Perlberger, 1928). Citrus groves were surveyed in 1964 for the incidence of stubborn and found to range between 5 and 50% (Pappo and Baumann, 1964). In certain years the damage was of economical importance especially in valencia and grapefruit. Mycoplasma-like organisms (MLO) were first observed in tissues of stubborn infected citrus trees (Igwegbe and Calavan, 1970; Zelcer et. al., 1971).

Until recently, little was known about the the natural vectors for

stubborn in the eastern Mediterranean. Also, little is known about the natural hosts of the pathogen.

Quite a different situation exists in California and Arizona due to intensive research conducted in the last 15 years.

Cultivation of S. citri from infected citrus and then from other hosts became a routine procedure since 1971 (Fudl-Allah et al., 1972; Saglio et al. 1973). Lee et al., (1973), reported cultivation also from leafhoppers.

Vectors for S. citri were obtained by injection of Euscellis plebejus (Markham et al., 1974). However, plant-to-plant transmission of S. citri by Circulifer tenellus was demonstrated by Oldfield et al., (1976) in laboratory and naturally inoculative leafhoppers were Scaphitopius nitridus and Scaphitopius acutus delongi, reported as vectors in California (Kaloostian et al., 1975).

The search for alternate hosts for S. citri in California yielded many non-citrus plants which were either naturally infected with the agent or have been inoculated in laboratory (Oldfield and Kaloostian, 1979; Oldfield and Calavan, 1981).

Presence of S. citri was determined by isolation of the organism in media.

Recently, transmission of S. citri was achieved with leafhopper from the Near East, C. haematoceps in Syria (Fos et al. 1986) C. haematoceps, was collected in small numbers in Syria on S. kali growing along the Mediterranean coast and in the desert on Matthiola annua cultivated as ornamentals (Fos et al., 1986). In Corsica this leafhopper was collected from Matthiola grown on the dry sandy east coast (Bove, 1986). Also, a leafhopper identified as C. haematoceps

was found to transmit curly top virus in sugarbeets in Turkey (Bennett and Tanrisever, 1957; and Iran (Kheyri and Alimoradi, 1969). This species, was later found to be able to acquire S. citri from Vinca rosea plants (Fos et al., 1986).

Attempts to transmit Israeli isolates of S. citri by N. haematoceps were unsuccessful (Bove and Lallemand, 1987).

The present diagnosis of S. citri in infected plants is based on symptoms appearance, isolation of the disease agent in medium and by ELISA (Clark et al., 1978; Clark et al., 1983). Recent progress in production of specific antibodies (mono and polyclonal) and development of sensitive serological procedures, are expected to reduce the duration of time needed for diagnosis from 7-10 to 1-2 days.

A major difficulty in the Mediterranean is the presence of complex groups of species, which are taxonomically similar or identical but biologically different. In order to recognize vector species from non-vector ones, there was a need to characterize different variants of leafhopper species which are related to the Circulifer spp. and determine their potential as vectors of S. citri.

The beet leafhopper, Circulifer tenellus has been known as one of the most destructive leafhoppers in several western states in the U.S.A. (Ball, 1907) since the beginning of the century. Almost every year this leafhopper arrives in very high population from desert vegetation and invades cultivated fields, causing direct damage by their intensive feeding and indirect damage by transmitting curly top virus to various plant species. Intensive research has been devoted to the study of its biology and the taxonomy of species related to this genus Oman (1936; 1948). In his study, Oman (1948), revised the

genus Circulifer, and included the two species haematoceps and tenellus under the genus Circulifer [However, the first leafhopper which was classified in this genus was Circulifer (Jassus) haematoceps (Mulsant and Rey). This leafhopper species served then as the genotype for the revision of this genus]. Young and Frazier (1954) developed a key for several species in this genus in which they divided the species into the haematoceps and tenellus groups. This grouping was justified on the basis of morphological differences in their genital plates. The tenellus group is characterized by oblong or oval plates, and the haematoceps group by triangulate plates (Young and Frazier 1954). Subdivision of different leafhoppers of the genus Circulifer into species is very difficult (Oman 1970; Young and Frazier, 1954). However, several species and subspecies of this genus were found in the Mediterranean area during a survey to find parasites and predators against Circulifer tenellus to introduce into the USA (Young and Frazier, 1954). The following species were characterized in that survey: C. tenellus (Baker), C. dubiosus (Matsumura) and C. hispaniae (Young and Frazier) in the tenellus group. For the haematoceps group, the included C. opacipennis (Lethierry), C. haematoceps (Mulsant and Rey), C. nausharensis (Pruthi) and C. nitridus (Young and Frazier) (Young and Frazier, 1954). Linnavuori (1962) reported a number of leafhopper species from Israel and included them in Circulifer (=Neoaliturus) spp. According to the classification of Oman (1970), part of the leafhoppers found in Israel should belong in groups of species either in C. haematoceps or in C. tenellus complexes. The rest should belong in genus Neoaliturus.

The species which were reported by Linnavuori in Israel are: N.

(=Circulifer) tenellus, N. (=Circulifer) haematoceps (opacipennis). N. fenestratus (Herrich-Schaffer), N. guttulatus (Kirschbaum), N. transversalis (Puton), N. inscriptus (Haupt), N. macchiae (Lindberg), N. pulchellus (Haupt), [=pulcher (Haupt)]; In 1987, Klein and Raccah reported species or subspecies found on different hosts varying in their host range and morphological features.

The present project provided the first proof for natural transmission of a S. citri isolate from Israel by a variant of C. tenellus. The possibility that the haematoceps group comprises of more than one variant in Israel was investigated in the present study. In the process of the study of S. citri transmission in Riverside, it was found the Circulifer tenellus transmits MLO too which was designated as the beet leafhopper transmitted virescence agent (BLTVA) (Oldfield, 1982; Oldfield et al., 1977). In certain cases, BLTVA was transmitted by the C. tenellus together with S. citri. It was also found that coinfection by both BLTVA and S. citri, provides a certain protection and prevents fast decline of the infected plants. A coinfection of another MLO with S. citri was described by Saillard et al., 1984). In that cases too, coinfection resulted in a longer survival than by separate infection by each of the agents.

MATERIALS AND METHODS

Collections of Leafhopper in Israel

Collections of leafhoppers were made by using a gasoline operated portable suction trap constructed at the ARO. Several collection surveys were carried out during the summers of 1984 and 1986. In order to determine the range of hosts, leafhoppers were trapped from

individual wild plants. Collections from clusters of plants which included more than one species were avoided. Circulifer variants which were trapped on a variety of hosts (e.g. Atriplex spp. Portulaca oleraceae, Chenopodium spp. and Salsola spp.) and then, were transferred to the laboratory in jars containing small pieces of their hosts. The specimens were sorted for sex while still alive and several females from each of the hosts were caged on several experimental hosts in a growth chamber (see list in Table 1).

Culturing

Leafhoppers females deriving from their natural hosts were caged on a series of test host plants (see Table 1, 2).

The cages were transferred into a growth chamber with a constant temperature of $28 \pm 2^{\circ}\text{C}$ and a light regime of LD 16:8.

Taxonomy

Twelve males and six females from each host were used for taxonomic examination. The examination was based mainly on the morphology of the male genitalia and the 7th female sternite.

Preparations for microscopic observation

The abdomens of the leafhoppers were removed and transferred into small vials with 15% KOH solution in distilled water. The vials were put in a water bath and heated to near boiling, for clarification. The male genitalia or the female 7th sternites were removed from their respective abdomens under a dissecting microscope mounted in pure glycerol, and covered with a cover glass. The ten best genitalia and five sternites were observed under a microscope at a magnification of $\times 250$. Certain parts which were easy to measure were compared in both samples. Measurements were analyzed statistically by Student's test.

The agent: Spiroplasma citri.

The disease agent was isolated from aborted seeds of stubborn infected citrus trees grown in various regions in Israel.

Cultivation was made in a modification of the SMC liquid medium (Saglio et al., 1973). The modifications include a reduction of horse serum to 10%, and omission of DNA and fresh yeast. Culture were maintained at $30 \pm 2^{\circ}\text{C}$. Young S. citri culture (2 days old) were used for insect injection. Isolation of S. citri was made from several fruits for each infected tree. Several trees from various regions were selected for isolation of the organism. Isolates of S. citri in cultivation medium were kept at -60°C until used. Presence of S. citri in the media was verified by dark field light microscopy and ELISA.

Injections

The two last instar of nymphs or young adults in groups of 20, were anesthetized with CO_2 and arranged on their backs between two layers of parafilm which were stretched on ice. Injections were made using glass needles which were inserted through the parafilm into the thorax between the legs. A medium which contained S. citri was injected to 100 insects. Sterile medium was injected to an additional 100 insects as a control. After injection, the leafhoppers were caged for 16 days on beet (Beta vulgaris cv. Fodder) seedlings. Ten individual leafhoppers out of those which were injected with S. citri and 14 out of those which were injected with sterile medium, remained alive after 16 days. Each of the surviving insects was allowed a alternate sequential 24 hrs feedings on Matthiola incana and Vinca rosea seedlings until they died.

Serology

Antiserum against S. citri was prepared using an isolate of S. citri deriving from a stubborn infected Valencia orange at Bet Dagan.)5 ml of medium containing S. Citri were incubated in a medium in which the horse serum was replaced by rabbit serum/ Then, the S. citri was fractionated from the medium by a series of differential centrifugations. A formaldehyde (0.2%) treated preparation of the agent was mixed with an equal volume of Freund's incomplete adjuvant. The series of subcutaneous and intramuscular injections were carried out at two weeks intervals. The rabbit was bled one week after the last injection. ELISA (Enzyme linked immunosorbent assay (ELISA) was carried out as suggested by Clark and Adams, (1977) with the modification specifically advised for spiroplasma (Clark et al., 1978; Clark et al., 1983). Leaf extract (20mg plant tissue in 1 ml sample buffer). The presence of the organism in leafhopper was made by homogenization of the insects in an all glass homogenizer in sample buffer (1 ml.).

Acquisition access feeding from infected plants

Acquisition access feeding from Matthiola incana seedlings, took place by using the first batch of plant which were inoculated by S. citri injected leafhoppers. The plants have shown darker leaves with short internodes. The presence of the agent in the leaves was verified by ELISA and electron microscopy.

Additional plant species on which infective leafhopper were caged, became infected and served as source of acquisition too. Groups of 60 late instar nymphs (4th or 5th) were allowed an acquisition access feeding on each of 5 infected and 5 healthy M. incana plants for three days. Thereafter, the insects were transferred

to pathogen free MI for 20 days before being tested for their ability to inoculate. Some of the leafhoppers survived long enough to be tested for their vectorial potential. Those that survived, were individually and sequentially transferred every 2-3 days, to a new set of pathogen free test plants. Among the plant species tested, we included, M. incana, V. rosea, Raphanus sativus, Beta vulgaris, Citrus sinensis (cvs. Valencia or Mme. Vinous). All test plants, were tested by ELISA and confirmed by reculturing from plants. Symptoms were conspicuous in M. incana, R. sativus, but less so in V. rosea or in C. sinensis.

Acquisition on oranges:

Growth flushes from either stubborn infected or apparently healthy Valencia oranges were taken to the laboratory. The flushes were caged along with 60 nymphs (4th or 5th instar) for three days. The majority of nymphs, died in either treatment. The few insects that survived, were transferred to M. incana for recovery and completion of incubation in the vector for an additional 20 days. Surviving insects were serially transferred every 2-3 days to M. incana and V. rosea in alternation.

The duration of the latent period in the leafhopper was determined using ten groups of 60 nymphs (3 to 5th instar), which were caged for 3 days on 10 S. citri infected M. incana seedlings. Serial passage of a number of leafhoppers from each of the ten groups was carried out every 2-4 days to a fresh set of M. incana. The test plants, were sprayed with Nicotine sulfate, and kept in a green house for symptoms appearance. Every three weeks, all the test plants were tested by ELISA. This procedure allowed to determine the median duration of the

latent period of S. citri in the vector. All the insects which were used in these transmission experiments, were tested for the presence of S. citri after death. The insects were externally sterilized with 70% ethanol, and kept in sterile capsules at -20°C until being tested for S. citri presence by isolation of the organism from their body in liquid media.

RESULTS

Determination of stubborn presence in various regions in Israel.

Citrus has been planted in recent years in semi arid regions of the country. These regions include the Besor plains, the Arava and the Jordan Valley. In these regions, stubborn was prevalent at a higher incidence than in the coastal plains, the center of the Israeli citrus industry. In addition, groves with an elevated proportion of infected tress were recorded in the Hulah Valley in the upper Galilee.

Differences between variants of Circulifer spp.

1. Circulifer haematoceps group:

a. Host range

The plants used in attempts to breed the C. haematoceps are listed in Table 1. On the basis of these attempts, we could recognize two distinct variants of C. haematoceps, one from S. kali and another from C. album. The range of experimental hosts tested for culturing the two types indicated that the variants had no common hosts (Table 1) except for turnip plants. The Circulifer variant from C. album was cultured best on Matthiola incana plants, where it reproduces rapidly to very

high populations. The C. haematoceps variant from S. kali could not survive on M. incana. In general, hosts found to support the variant from C. album allowed good survival but not fair reproduction. The S. kali variant survived and reproduced on turnip for two generations only. Phytotoxic effects of the leafhoppers were noted for turnips even when they were caged with a single leafhopper. Apparently, phytotoxicity affected its reproduction on the host. The variant collected from S. kali has not yet been noted on any other Salsola species (unpublished data). S. kali grows in Israel only in the coastal area near the Mediterranean Sea, and almost all the other species of this genus are found in the desert areas of Israel (Zohary et al. 1954). Unfortunately, we could not use the native hosts for cultivation, as the S. kali from our region, germinated poorly in captivity, and the seeds of C. album were not available at the time of capture.

b. Taxonomy

The two variants of Circulifer on wild and experimental hosts, there were differences in the male genitalia and 7th female sternite (Fig. 1). The most conspicuous difference was in the styles (Fig. 1). In males of the S. kali leafhopper variant, the apices are aimed at a sharp angle, while in those from C. album they are only slightly bent. The size and width of the styles in the genitalia of the males from the S. kali variant were larger than those of the C. album variant, and the sinuses appearing near the base of the apex were significantly larger in the S. kali variants than in the C. album ones.

The sizes of distinct parts of the 7th female sternite and male

genitalia in the two variants of Circulifer (from S. kali and from C. album) show significant differences (Table 2). Although the average size of all the insect of both variants was the same, the S. kali variant was equipped with a larger 7th sternite and male genitalia than were observed for the C. album variant (Table 2, and illustrations 2, 3 in Fig. 1).

Description of insect structures depicted in the figures:

Fig 1: A general scheme of the parts measured in this study (see also Table 2) is given in detail in illustration 1. The part designated 'A' represent the 7th abdominal sternite; the parts designated B, C, D and E represent the parts of the male genitalia. Illustration 2 represents insects collected from S. kali and Illustration 3 represents those collected from C. album. Mean measurements ($\pm$ SD) of the various parts are given in Table 2.

Differences in Females:

The part designated 'd' for females collected on S. kali (part A, illustration 2) is much larger than that for females from C. album ($p < 0.01$) (Table 2). Other parts ('b' and 'c' in illustration 2 and 3) were also larger in females from S. kali than from C. album, but at a lower significance ($p < 0.05$) (Table 2).

Differences in males:

1. The part designated 'B' represent the adeagus and connectives. The parts of the adeagus marked 'a', 'c', 'e' and 'f' (see illustrations 2 and 3) are much larger in males collected from S. kali than from C. album ($p < 0.01$) (Table 2). Other parts of the adeagus and connective ('b', 'd', 'g' and 'i') are larger but at a lower significance in

males from S. kali than in males from C. album ($p < 0.05$) (Table 2).

2. The part designated 'C' represent the style. The parts of the style designated 'b', 'e', 'f' and 'i' are much larger in males collected from S. kali than in males from S. album ($p < 0.01$) (Table 2). The size of other parts did not differ significantly in leafhoppers from the two hosts.

3. The part designated 'D' represent the plate. The parts of the plate designated 'a', 'b', 'c', 'e' and 'f' are much larger in males collected from S. kali than from S. album ($p < 0.01$) (Table 2).

4. The part designated 'E' represent the base of the plates. The size of the base from the lower lateral part to its upper center ('b' in 2 and 3) is significantly larger in males from S. kali than from males of C. album ($p < 0.01$), while the width of the base ('a' in E) is about the same in the two leafhopper variants (Table 2).

In addition to the structural parameters which could be measured, there are some descriptive differences between the two leafhopper variants:

The bottom of the female abdominal depression (part 'A' in illustration 2 and 3) is much more indented in specimens from S. kali than from C. album. The rami of the aedeagus (part 'B' in illustration 2 and 3) are closed when removed from leafhopper bodies of males for the two variants. However, after the preparation of the aedeagi, those of males from S. kali are to be seen open while those of males from C. album are closed in the most of the individuals. The apex of the style in males from S. kali is strongly bent (see 'C' in illustration 2), while that of males from C. album ('C' in

illustration 3) is only slightly curved.

Differences in Color:

The C. haematoceps variants trapped on S. kali and C. album were similar in size and color at the time of capture. On the other hand, the variant of C. tenellus which was caught on A. halimus had a slightly different color from that trapped on P. oleraceae.

2. Circulifer tenellus group:

In survey trips made in 1984 and 1986, variety of variants which correspond to the description of Circulifer tenellus were found. In the present study we decided to focus only on variants which were prevalent in large number in nature.

The A. album C. tenellus:

a. Host range

The ability of C. tenellus deriving from Atriplex halimus to inoculate a range of host plants and survive on them is given in Table 3. The data in Table 3, allows a segregation of plant hosts in four categories:

1. Plants which can support feeding and reproduction.

(Cruciferous hosts: M. incana, R. sativus, Sinapsis spp., and Sysymbrium irio).

2. Plants that supported feeding but only a limited reproduction.

(Salsola vermiculata and S. foetida). Although few nymphs only were produced on these hosts, young nymphs which were transferred to them, developed well to maturity.

3. Hosts which allowed moderate reproduction and could maintain the leafhoppers for more than a week (Chrysanthemum coronarium, Plantago lagopus and beta vulgaris). Few leafhopper survived long enough to reproduce. However, few nymphs were seen in a few cages. A high incidence of mortality was recorded in the first days of caging the leafhoppers on plant in this category.

4. Plants that supported neither feeding nor reproduction.

Leafhoppers caged on either V. rosea or on Citrus sinensis have shown this reaction. Apparently, in nature, acquisition cannot be completed from these hosts.

From Table 3, is apparent that cruciferae are a much better host than any of other hosts tested even those which are the natural hosts of the insect. The ecological niche in the Jordan Valley, which is hot and dry, do not always allow a large population of leafhoppers in the winter months. In summer months the populations of leafhoppers have reached their peak, but by then, most the cruciferae were dead.

The list of plants tested as hosts for the second variant of C. tenellus deriving from P. oleraceae is given in Table 3. Although not all the plants which were tested for the A. halimus variant were yet tested for the P. oleraceae one, we can already discern a remarkable difference between them. They also differed in size and coloration.

In more details, The P. oleraceae variant did not develop well on M. incana but even less on other cruciferous hosts. The three host plants which were preferred by this variant were P. oleraceae, Emex spinosus and Beta vulgaris.

On the basis of their survival and host preference, the P. oleraceae variant which was trapped in Jordan Valley and Bet Dagan are

apparently identical.

3. Transmission tests using variants of Circulifer species.

a. Attempts to transmit S. citri by C. haematoceps

Presence of S. citri in field collected leafhoppers:

In 1986 and 1987, hundreds of leafhoppers belonging to 4 variants of both C. haematoceps and C. tenellus were trapped and tested for the presence of S. citri by allowing an inoculation access feeding on Matthiola incana. None of the insects was found infective nor could we isolate and detect the organism in their body.

Transmission by leafhoppers which were artificially inoculated with S. citri:

Large number of 5th instar nymphs of C. haematoceps which were collected on S. kali in nature, were injected with a culture of two S. citri isolates (One from the Arava valley (taken from Oroblanco) and another from the Modiin (Taken from grapefruit). Survivors after a period of 20 days (time necessary to complete the latent period in the insect) on turnip plants, did not transmit to any of the turnip or radish test plant to which they were exposed.

The C. haematoceps variant from C. album, was raised in captivity on M. incana. 100 individuals of 5th instar nymphs were injected for each of the two isolates of S. citri which were tested above. In this cases too, no transmission was obtained on any of the M. incana which were exposed to the injected leafhoppers. The absence of S. citri in the test plants on which the injected leafhoppers fed was verified by ELISA.

Attempts to transmit S. citri using leafhoppers which acquired the pathogen from plants:

No transmission was obtained in all the attempts in which the two variants of C. haematoceps were allowed acquisition access feeding on S. citri infected M. incana and turnip.

b. Transmission using C. tenellus from A. halimus:

Transmission using leafhoppers injected with S. citri:

Six of the 10 survivors of injection studies were capable to transmit S. citri to 6 M. incana plants and to 2 V. rosea plants. M. incana reacted to the presence of the disease 2-3 weeks after being exposed to injected insects. The earliest symptoms in M. incana plants were the appearance of short and narrow leaves and a change of their color from light to dark green, 2-3 weeks after being exposed to the injected insects. Later, the shortening of internodes, to a degree that all leaves seem to originated from one spot on the stem (Fig. 2). Finally, older leaves in infected plants, turned yellow and succulent in comparison to those of pathogen free plants. In all cases, detection by ELISA was possible before symptoms were visible. The presence of spiroplasma was confirmed in the thin sections of sieve cells of M. incana, V. incana, R. sativus and C. sinensis. The largest number of spiroplasma was seen in M. incana.

An additional confirmation was obtained by isolation of the agent from plants showing symptoms.

Isolation of S. citri was easier and faster from M. incana than from any other host found to harbor the disease agent. The rapidity of

multiplication of S. citri was measured by the time needed for the color change in the medium in which samples of hosts of same size were introduced. From M. incana, the color change was within 8 days. On the other hand, from citrus, the time needed was within 26 days.

From the above, we deduced the M. incana is the most appropriate host for S. citri in our system. For this reason, we have used M. incana as source plant for most subsequent experiments.

Transmission using leafhopper which acquired the pathogen from plants: The presence of naturally S. citri infected C. sinensis, S. irio and Hirschfeldia incana was already reported in Israel (Clark et al., 1978). Natural acquisition from infected citrus was not possible due to the high mortality on this host. None of the surviving nymphs transmitted the agent to test plants. On the other hand, during the period from 1985 to 1988, none of S. irio found in nature had shown S. citri symptoms, nor could be detect or isolate S. citri from the plant. For this reason, and for the period reported, we could evaluate the efficiency of the vector with laboratory host of S. citri only.

A series of host plants which were infected with S. citri had served as source plants in order to determine their suitability to serve as an acquisition host for the Israeli C. tenellus variant. The most efficient transmission was obtained by insect acquiring the agent from M. incana and R. sativus (more than 50% efficiency from each of these two hosts). For M. incana, the symptoms were visible in 27 out of the 53 which were positive by ELISA. On the other hand, for R. sativus, only 23 have shown symptoms out of the 53. In certain case, positive ELISA reactions were obtained from plants which showed no visible symptoms of stubborn (Table 4). Isolation of S. citri was

possible from all the plants which positively reacted by ELISA. C. sinensis which were exposed to infective leafhoppers, were positive by ELISA, but the isolation of S. citri in medium was unsuccessful, possibly because of the very low concentration of the S. citri in the petioles. Successful isolation of S. citri from petioles in oranges was reported. However, those successful isolation attempts were made from trees which were infected for several years.

Unlike in California, where V. rosea was found an efficient source plant for the acquisition (Granett et al., 1976), In Israel, only in 1 out of 29 attempts, was it possible to obtain an infected Vinca rosea plant. V. rosea was an indicator plant which served for monitoring the presence of natural transmission in nature (Bove et al., 1979; Granett et al., 1976; Raccach and Klein, unpublished).

Only one (MV 101) out of four isolates was transmitted to V. rosea by C. tenellus when several S. citri were compared in California (Liu et al., 1983). Among those which were not transmitted was one which derived from Morocco. Differences in the ability of C. tenellus to naturally infect V. rosea can be attributed to the extent of agent multiplication which may depend on both the V. rosea cultivar, and/or the S. citri isolate.

Sugar beets was found immune to S. citri. This was in a agreement with similar finding reported in California.

c. Transmission dynamics of infective leafhoppers

The median latent period (LP) [the median time required by a population of leafhoppers to become infective after acquiring a disease agent] was determined for 32 individuals of C. tenellus. Transmission results are given in Fig.3. The mean LP was 22.7 ± 5.4 days ranging between 12 and

40 days. Upon the termination of the LP, the leafhoppers remained infective for life (except for one leafhopper that serially transmitted the disease to three test plant only, and ceased thereafter. Some of the infective leafhoppers survived for more than 100 days, and infected more than 10 plants in a row. In comparison to the Lp reported for the S. citri isolated from horseradish (O'Hayer et al., 1983). From each of the infective leafhoppers, S. citri was isolated in liquid medium after their death. This included the insect that cease to transmit.

ABSTRACTS OF PUBLICATIONS ATTACHED TO THE REPORT (U.S.A)

4. Transmission and Host Range of the Beet Leafhopper Transmitted Virescence Agent (BLTVA) in Riverside, California.

Transmission and host reaction studies are summarized in three publications which are attached to this report. The summaries of these studies are given below.

a. Transmission characteristics of the beet leafhopper transmitted virescence agent (see attached publication by Golino, Oldfield and Gumpf, Phytopathology 77:954-957:1977)

Beet leafhopper transmitted virescence agent (BLTVA), which causes a disease with a presumed etiology by a mycoplasma-like organism, is vectored by C. tenellus in a pattern consistent with other leafhopper-borne plant pathogenic millicutes. The minimum acquisition access period (AAP) was 5 minutes, more than 50% of test plants developed symptoms after exposure to insects that had undergone a 4-hr AAP.

Transmission increased with increasing AAP until 100% of test plants became infected after an AAP of 1 or 2 days. The minimum demonstrated latent period in C. tenellus was 12 days. Maximum inoculation efficiency was reached at 26-27 days after which a gradual decline in efficiency occurred. An inoculation access period (IAP) of 5 min rarely resulted a transmission. IAPs of 2 days provided high rates of infection in test plants. Single C. tenellus transmitted to about 50% of the plants of Apion graveolens, Brassica geniculata, Catharanthus roseus, and Raphanus sativus. Groups of 5 or 10 inoculative insects gave nearly 100% transmission to the same host. Transmission efficiency was not related to sex; groups of 10 male and female leafhoppers transmitted 82 and 81% of test plants respectively.

b. New experimental hosts of the beet leafhopper transmitted virescence agent (BLTVA). (Golino, Oldfield and Gumpf, Plant Disease In press).

Fifty seven plants were tested as potential experimental hosts of BLTVA. Of them, 37 developed symptoms of infection. A wide range of host reactions were seen in infected plants including floral gigantism, floral induction, Floral proliferation, floral virescence, internode elongation, leaf mottling, proliferation of buds, stunting, tip necrosis, wilting and yellowing (Table G1). No non-symptomatic hosts were found. Sixteen plants species did not develop symptoms of infection after exposure to inoculative leafhoppers; this included all the monocots tested. A number of plants which have been reported as hosts of economically important MLO disease also proved to be hosts of BLTVA. Among them were Callistephus chinensis, Lycopersicon

esculentum, and Tropaeolum majus. The host induction response (HIR), the induction of flowering in plants grown under environmental conditions which would normally be non-inductive for flowering, was seen in 8 host species. None of the monocots tested developed syndromes of infection nor was the agent recovered from exposed plants during backpassage experiments with leafhoppers.

c. Induction of flowering through infection by beet leafhopper transmitted virescence agent: (see attached publication by Golino, Oldfield and Gumpf, Phytopathology 78:285-288:1988.

Circulifer tenellus transmitted the beet leafhopper transmitted virescence agent (BLTVA) to selected plant hosts, which included long-day plants grown under noninductive short days and cold-requiring annuals or biennials grown entirely in a warm greenhouse. Radish and dill infected with BLTVA bloomed. Infected spinach plants did not bloom, but stems grew to an average length of 2.8 cm compared to an average stem length of 1.1 cm in control plants, (different at $P=0.01$). A high percentage of flowering was observed in infected environmentally non induced Chinese cabbage, celery, and carrot. Control plants of each species, fed by uninoculative insects, did not bloom. These plant responses may be of particular significance since a plant growth substance, gibberellic acid (GA), is known to induce flowering in the specific plants tested. Catharanthus roseus, a host species with a GA-insensitive flowering rate, did not bloom prematurely when infected with BLTVA. Applications of two inhibitors of GA biosynthesis, CCC and AMO-1618, delayed the BLTVA-induced host induction response.

5. Attempts to differentiate strains of S. citri in California:

Complete summaries including all the information available on stubborn disease in California and how to effectively manage this disease are given by Gumpf (1988) and Gumpf et al. (1986a, 1986b, 1986c). In order to differentiate between the strains of S. citri as well as provide a very sensitive and rapid technique for identification, serological reagents were to be developed. As per the time schedule, polyclonal antibodies were prepared against various spiroplasmas as the initial phase of developing a better diagnostic method for S. citri. In all twelve different rabbit polyclonal antisera and one chicken polyclonal antiserum were produced. This phase was followed up by performing several hybridizations to produce monoclonal antibodies to a range of S. citri isolates. Two separate hybridizations involving two separate isolates were performed. From these hybridizations we selected four hybrids for detailed screening for specificity and suitability for use as a diagnostic reagent for detecting S. citri in citrus tissue. Unfortunately, most of the hybrids showed little specificity and sensitivity to the spiroplasmas and so that in diagnostic tests using citrus tissue there was little difference between healthy controls and infected. Because of the apparent lack of sensitivity using serological reagents, we then proceeded to prepare hybridizing probes. Our method of choice was to extract the DNA directly from cultured S. citri organisms, formamide treat this DNA and then end-label it with ³²P. We were unable to completely finish all the work with the probes.

DISCUSSION

In the USA, the major effort was made in order to determine the transmission characteristics and host range of the Beet Leafhopper Transmitted Virescence Agent (BLTVA). This agent complicates the epidemiological situation of stubborn disease being transmitted by the same vector, namely, Circulifer tenellus. Moreover, BLTVA frequently naturally infects plants which are infected also with Spiroplasma citri. Evidence exists that such doubly infected plants survive longer than plants infected only with S. citri and doubly infected plants remain sources from which S. citri is acquired by leafhoppers for comparatively long periods. Although the extent of knowledge of the ecology of S. citri in North America is much greater than it is in Old World citrus growing areas, the development of ecologically sound management strategies required further basic information on the ecology of both disease agents, including their relationship to various hosts and vectors.

An additional objective was to develop sensitive and accurate serological means which will allow to differentiate between the strains of S. citri was less successful. Limited success was only achieved in this direction. Unfortunately, most of the monoclonal hybrids showed little specificity and sensitivity to the spiroplasmas and so that in diagnostic tests using citrus tissue there was little difference between healthy controls and infected. This has been a consistent problem preventing our use of serology as a method of disease detection. Rarely if ever can we obtain any kind of positive serological test for stubborn directly on citrus whether using polyclonal or monoclonal antibodies. Alternatively, preliminary

attempts were made to use recombinant DNA technology for detection of S. citri. Results indicate the DNA probes are extremely sensitive even to the point of detecting S. citri in individual insects. However, the probes did not allow to distinguish between strains or isolates of the agent.

The present project added important information on the potential leafhopper vectors of S. citri in Israel. In the Mediterranean region, there is an economical importance to identify the species of the genus Circulifer as they might serve as vectors for S. citri, the causative agent of the citrus stubborn. Unlike in the U.S.A., a variation of species were discerned in the region, and we anticipate that more than one will be capable of transmitting the pathogen. Knowledge about the identity and occurrence of the potential vectors and their breeding hosts, might provide means for the control. Results which were obtained in our laboratory has shown at least two variants of C. tenellus which transmitted S. citri from and to M. incana, Raphanus sativus and from M. incana to Citrus sinensis (Klein et al., 1988; 1989).

Differentiation of the subspecies of C. tenellus on the basis of male genitalia, was impossible and additional means of identification were needed in order to clarify the complexity of the C. tenellus group. This problem was not resolved since the study of Young and Frazier (1954) and Bindra et al., (1970), where variations in male genitalia and in the 7th abdominal sternite of females were described within species of Circulifer. From their and our descriptions, it is

not entirely clear whether the specimens found of the above species should be referred to distinct species or to variants of species.

Variations in genitalia and host plants supporting survival and/or reproduction, suggest that the Circulifer haematoceps trapped on S. kali is different from that caught on C. album. A similar phenomenon was seen for C. tenellus group, where a variant which was trapped on A. halimus differs from that trapped on P. oleraceae.

Young and Frazier (1954) have recorded C. haematoceps from a relatively small area in Europe - Sicily, Sardinia and Spain - and only on the host Cistus sp. On the other hand, they reported C. opacipennis on a very wide host range and in many countries of Western Europe, Russia; the Near East and North Africa. They pointed out that this species is extremely variable and postulated that it may comprise more than one species (Young and Frazier, 1954).

Another case of presence of variants of C. opacipennis can be deduced from the findings of Bindra et al., (1970). In their description, Indian specimens of C. opacipennis, were found on various hosts. These C. opacipennis specimens also differed in male genitalia. One of them has shown apices with slight tendency to bend while the other exhibited laterally bent apices.

As for the two C. tenellus variants from A. halimus and from P. oleraceae, although they differed from each other in color and in host preference, they were both identified as C. tenellus by Prof. P. Oman.

The conclusion that can be drawn from the data above is that in the Middle East, variants which differ in host range, in conferring phytotoxicity to certain hosts and morphological variations in the genitalia, coexist. Therefore, we suggest to consider this region as

one of the places where species of this genus evolved. However, the differences recorded in this study by themselves are not sufficient to allow a reclassification of the variants. A common host which allows continuous breeding and mating of both variants of C. haematoceps group is still lacking. For C. tenellus the variants share the M. incana as a common host. However, cross hybridization between the variants are still to be made in order to determine if they should belong in two distinct species of Circulifer. Preliminary sound signals for both the variants of C. haematoceps and for the variants of C. tenellus have shown, unequivocal differences.

Both variants of C. tenellus described above were found capable to transmit S. citri to citrus. Other variants or species which belong in the Circulifer/Neoliturus complex were not characterized yet, and this was beyond the scope of this proposal.

The role of additional variants of the Circulifer/Neoliturus complex in the epidemiology of stubborn is still unknown. Although natural spread of stubborn was recorded in the Jordan Valley in former years, none of the leafhoppers of the C. tenellus variant from the Jordan Valley were found to carry S. citri or to transmit it to test plants. One possible explanation might be the absence of S. citri herbaceous plants in certain years and presence in other. The erratic nature of stubborn outbreaks was recorded in this region in the past, but no direct proof is available yet for its cause. Another explanation suggests that the leafhoppers species found capable of transmitting S. citri in Israel are within the limit of the migratory range of the insect. In certain years, individuals will migrate further and in greater number from the east than in others, depending on

meteorological conditions. This hypothesis assume that the sources of infection are found in deserts further east, from which they migrate when the vegetation dries. If condition allow a long distance migration, larger number of leafhoppers will reach the citrus growing region in Israel. In other years, few or none will make it. Unfortunately, little is known on the vegetation and the S. citri presence east of the Jordan Valley, and this hypothesis could not been ascertained.

It is however apparent that citrus never serves as sources of infection (at least for the variants of the leafhopper species which were tested in the present study) and all of that derives from the external by leafhoppers visiting groves in their attempts to look for feeding hosts.

Another leafhopper species which was reported to transmit the disease in the region, namely C. haematoceps, was not capable to transmit the S. citri which served for the transmission by C. tenellus either after injection or after being allowed an acquisition access feeding on M. incana. In Syria, C. haematoceps transmits S. citri to C. sinensis (Bove and Lallemand, 1987). The syrian isolate of S. citri and the isolates used in this study may be different in their affinity for a vector species. It is also possible that the C. haematoceps of Syria is of a different biotype from that trapped in Israel, as they seem to have a different preference for host plants.

In Israel, we found so far at least two variants of C. tenellus , differing in their capacity to breed on hosts. In 1982, Klein et al., (1982) described a C. tenellus variant which cannot breed on sugar beet in the laboratory. On the other hand, more recently, the variants

collected and used in this study, survives well and reproduces on this host. We therefore assume that Frazier collection from the Mediterranean basin did not include these two variants (Frazier, 1953).

Transmission studies indicate that there was a long time difference between the time of the positive ELISA reaction to the presence of S. citri and the time when first symptoms became conspicuous. In one case only, we could isolate the organism from an infected seedling prior of seeing symptoms in the test citrus host.

Isolation from leaves of well developed stubborn infected trees, indicates that these trees were probably infected long time ago. In most cases we failed to inoculate V. rosea with our isolates of S. citri. This shows that V. rosea is not a suitable test plant for the disease in Israel.

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TABLES

Table 1. Survival and reproduction of field females of Circulifer collected from Salsola kali and Chenopodium album on various host plants in the laboratory

Survival and reproduction				
Host plants	Females from Salsola		Females from Chenopodium	
	Survival	Reproduction	Survival	Reproduction
Beta vulgaris	-	-	++++	+++
Chenopodium amarinticolor	-	-	+++	++
Turnip	+++	++	++++	++
Sinapis	-	-	++++	++
Brassica oleracea	-	-	++++	+
Matthiola incana	-	-	+++++	+++++
Phaseolus vulgaris	-	-	++	-
Portulaca oleracea	-	-	++	+
Chrysanthemum	-	-	++++	+++
Carthamus tinctorius	-	-	++	+

* - Insect survive on these hosts not more than two days, did not reproduce

** + Insect survive more than two days and reproduced

(3) Matthiola incana plants were given +++++ as all females from Chenopodium reproduced on this host and more than 60 larvae emerged per female per week. This host plants served for comparison to the best host.

Table 2. Differences in mean sizes ($\bar{x}$ ($\pm$ SD)) of distinct parts in female seventh sternite (A) and male genitalia (B to E) of two leafhoppers of the Circulifer opacipennis complex.

Females		Males											
		Adeagus and connective (B)				Style (C)		Plate (D)		Plate Base (E)			
		<u>S. kali</u>		<u>C. album</u>		<u>S. kali</u>		<u>C. album</u>		<u>S. kali</u>		<u>C. album</u>	
Seventh sternite (A)													

Description of symbols (capital letters A to E) and lower cases (a to i) are given in Fig. 2.

1. Circulifer species trapped on Salsola kali in the coastal area of Israel

2. Circulifer species trapped on Chemopodium salbum in the Golan Heights

V.S. - Not significant $P > 0.05$

S - Significant $P > 0.05$

V.S. - Very Significant $P > 0.05$

Table 3. Host plants tested in the laboratory for their ability to support two variants of Circulifer tenellus trapped on Atriplex halimus and on Portulaca oleraceae

Plant species	Botanical family	PERFORMANCE			
		<u>C. tenellus</u> from <u>A. halimus</u> ¹		<u>C. tenellus</u> from <u>P. Oleraceae</u>	
		Feeding	Breeding	Feeding	Breeding
<u>Atriplex halimus</u> L	Chenopodiaceae	++	-		
<u>Salsola vermiculata</u>		+++	+		
<u>S. foetida</u>		+++	+		
<u>Beta vulgaris</u> cv. Red					
<u>Beta vulgaris</u> cv. Fodder		+++	++	+++	++
<u>Chenopodium amaranticolor</u>		-	-	-	-
<u>C. quinoa</u>		-	-	-	-
<u>Matthiola incana</u>	Cruciferae	+++	+++	+	+
<u>Raphanus sativus</u>		+++	+++	-	-
<u>Sinapsis</u> spp.		+++	+++	-	-
<u>Sysymbrium irio</u>		+++	+++	-	-
<u>Phaseolus vulgaris</u> (cv. Blackeye).	Leguminosae	+	-	-	-
<u>Malva nicaensis</u>	Malvaceae	-	-		
<u>Gossypium hirsutum</u> (cv. Acala SJ2)		-	-	-	-
<u>Emex spinosus</u>	Polygonaceae	++	-	+++	++
<u>Erodium rotundifolium</u>	Geraniaceae	-	-	-	-
<u>Carthamus tinctorius</u>	Compositae	-	-		
<u>Chrysanthemum coronarium</u>		++	++		
<u>Statice sinuata</u>	Plumbaginaceae	-	-		
<u>S. thouni</u>		-	-		
<u>Plantago lagopus</u>		++	++	-	-
<u>Sesamum orientale</u>	Pedaliaceae	-	-	-	-
<u>Vinca rosea</u>	Apocynaceae	-	-	-	-
<u>Citrus sinensis</u> (cvs. Valencia and Mee. Vinous)	Rutaceae	-	-	-	-
<u>Portulaca oleraceae</u>	Portucalaceae	-	-	+++	+++

- Leafhoppers died within days after confinement on the host.
- + Leafhopper survived up to 7 days, poor reproduction (single nymphs only hatching on host).
- ++ Leafhopper survived for more than a week but less than one months on the host, Medium reproduction (less than 20 nymphs per female hatched on each plant).
- +++ Continuous survival and abundant reproduction (more than 20 nymphs per female on breeding host).

1. Leafhoppers were trapped at Gilgal, the Jordan Valley, on A. halimus in shaded places. An additional variant of C. tenellus from A. halimus which was trapped in sunny places, fed only on B. vulgaris and A. halimus and reproduced on A. halimus only.
2. Leafhoppers were collected at Bet Dagan, Israel.

Table 4. Transmission of Spiroplasma citri to several host plants by the Jordan Valley variant of Circulifer tenellus.

Test plant species	Symptoms	Rate of transmission	
		ELISA reaction	Cultivation in medium
Vinca rosea	1/29*	1/29	0/1**
Matthiola incana	27/53	33/53	5/5
Raphanus sativus	23/53	39/53	5/5
Beta vulgaris	0/12	0/12	0/5
Citrus sinensis			
cv. Valencia	0/9	3/9	0/3
cv. Mme.Vinous	0/15	6/15	0/6

All leafhopper were allowed an acquisition access feeding on Matthiola incana *S. citri* infected source plants. Transmission were made after the termination of 20 days of latent period.

* Numerator, number of test plants with a positive reaction for disease presence; Denominator, number of plants exposed to inoculation access feeding to leafhoppers which completed acquisition feeding on S. citri infected hosts.

** Isolation and cultivation was made up to 6 plants from each host. Test plants which reacted positively (showing symptoms or by ELISA) were selected. In cases where there was no reaction, the test plants were taken at random.

Legend for figures:

Fig. 1. Description of the 7th abdominal sternite of female (A) and genitalia: aedeagus and connective (B), style (C), plate (D) and plate base (D). Specific parts in each of the above are designated by lower case (a to i). (1) General scheme (2) male and female leafhoppers of Circulifer spp. trapped on Salsola kali in the coastal plain. (3) Male and female leafhoppers of Circulifer spp. trapped on Chenopodium album in the Golan Heights.

Fig. 2. Healthy (right) and infected (left) Matthiola incana test plants. The plants were exposed in the laboratory by the Circulifer tenellus variant from Atriplex halimus. The symptoms include typical little leaves.

Fig. 3. Cumulative curve of the proportion of the infective Circulifer tenellus variant from Atriplex halimus. Legend for figures:

Fig. 1

Fig. 2

Fig. 3

C. Evaluation of the research achievement with respect to the original research proposal and its objectives.

1. CHANGES IN THE PROJECT:

All the changes that occurred in this project were an outcome of failures to proceed along the aims which were planned in the original proposal. In Israel, all transmission were carried out with laboratory infected hosts, as no S. citri infected herbaceous hosts were found in nature. This was probably the reason for the lack of inoculative leafhoppers in nature. In California, the closure of the Boyden Entomology Laboratory, had a direct effect on the activity of the principal investigator Dr. George Oldfield. However, in order to ensure maximum benefit of BARD funds, Dr. Oldfield applied for the transfer of \$12,000 of the USDA budget to Israel. The application was approved by BARD. This transfer, allowed extension of the research in Israel.

In the University of California, little progress was achieved in the direction of development of isolate specific antibodies. A change was needed, and the one chosen was to use DNA probes. Those too, were so far found nonpsecific.

2. EVALUATION OF THE RESEARCH ACHIEVEMENTS:

In the Boyden Entomology Laboratory, Dr. Oldfield in cooperation with Dr. Gumpf contributed to the characterization, vector transmission and host range of the BLTVA, which often complicates the S. citri situation.

In the University of California laboratory at Riverside, Dr. Gumpf, conducted preliminary experiments in order to use DNA for detection of the stubborn agent in plant and insects.

In Israel, an intensive work was done to clarify the complex of the Circulifer spp. in this region. Also, polyclonal antibodies were raised in rabbits against several isolates of S. citri. A certain distinction between the S. citri isolates which are present in Israel was possible using polyclonal antibodies in Ouchterlony tests.

Despite difficulties to obtain infective leafhoppers in nature, two variants of the C. tenellus which became inoculative by injection, could later acquire and transmit S. citri between herbaceous hosts and to oranges. This was the first insect transmission of israeli stubborn isolates by israeli variants of the beet leafhopper. The knowledge acquired in Israel, fulfilled three of the objective

D. Description of cooperation:

Cooperation took place between Dr. Oldfield and the laboratory in Israel and between Dr. Oldfield and the laboratory of Prof. Gumpf in the University of California. On the other hand, cooperation between the Israeli laboratory and that of Prof. Gumpf in UCR was most limited. This lack of cooperation did not allow to compare the S. citri isolates in Israel with those in California.

Dr. Oldfield visited in Israel and assisted the Israeli team in recognition of the C. tenellus and C. haematoceps preferred flora. Also, assisted in searching for S. citri infected plants in various areas of Israel. His help to the progress of the project in Israel was remarkable.

Dr. Oldfield and Dr. Gumpf were the advisors of Ms. D. Golino and cooperated on the study of the BLTVA. Also, Dr. Oldfield provided the Laboratory of Prof. Gumpf with S. citri isolates to initiate the monoclonal study.

In 1987, Dr. Klein visited Riverside, where he was introduced to the vector studies and to the S. citri infected flora in Riverside and in the Boyden Entomology Laboratory. In January 1988, Dr. Raccah visited Riverside and went out to field trips with Prof. Calavan and Dr. G. Oldfield to get an insight into the native breeding sites of C. tenellus.

F. List of publications:

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Gumpf, D.J., Calavan, E.C. and Pehrson, J. (1986b) Spread of stubborn disease in California citrus. *Citrograph* 71:136-137.

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Klein, M. and Raccah, B. (1987) Morphological variations in a Neolaliturus spp. (Cicadellidae) from various host plants in Israel. in: Wilson, M.R. and Nault, L.R. [Eds.] Proc. 2nd Int. Workshop on LEAFHOPPERS AND PLANTHOPPERS OF ECONOMIC IMPORTANCE. Provo, Utah, USA., CIE. London. p. 267.

Klein, M. and Raccah, B. (1989) Vectors of Spiroplasma citri the causal agent of the stubborn (little leaf) disease of citrus in Israel. *Phytoparasitica* 17:3.

Klein, M. Rasooly, R. and Raccah, B. (1988) Transmission of Spiroplasma citri the agent of citrus stubborn by a leafhopper of the Circulifer tenellus complex in Israel. - p. 49, Abstracts, Sixth International Citrus Congress, Middle East, held at Tel Aviv, Israel, March 6-11, 1988.