Epidemiology of *Spiroplasma citri* in the Old World

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ABSTRACT. Natural transmission of *Spiroplasma citri*, the causal agent of citrus stubborn disease, to periwinkle plants has been experimentally documented in Morocco and Syria. Leafhoppers have been collected in Morocco, Turkey, Syria and France (Corsica) and analyzed for infection with *S. citri*. *Neoaliturus* (*Circulifer*) *haematoceps* was the only leafhopper species found to be naturally infected with *S. citri* in Morocco, Syria, France (Corsica) and Iraq. *N. haematoceps* was reared in Bordeaux and shown to be able to acquire *S. citri* by feeding on *S. citri*-infected plants, to support multplication of the spiroplasma in its body and to transmit the stubborn agent to healthy periwinkles. Leafhoppers injected with a pure culture of *S. citri* were also able to infect healthy periwinkles. These experiments show that *N. haematoceps* is a vector of *S. citri*. In view of the paucity of *N. tenellus* in the Mediterranean area, *N. haematoceps* seems to be the major vector of *S. citri*. In Iran, *N. haematoceps* is also the major species, but *N. tenellus* seems to be more prevalent than in the Mediterranean area. Hence, in the Middle East, both *N. haematoceps* and *N. tenellus* can be vectors. Host plants of *N. haematoceps* have been identified in Syria and France (Corsica). Their occurrence along the Mediterranean coast explains recent epidemics in Syria. Their very wide distribution throughout Iran, explains the presence of *N. haematoceps* in or close to all major citrus growing areas. Natural transmission of *S. citri* in Iran has been documented and explains the presence of stubborn disease on local sweet orange trees as well as on Iranian nucellar trees. However, importation of *S. citri*-infected budwood from California or Mediterranean countries to Iran cannot be ruled out.

Index words. Stubborn, *Spiroplasma citri*, *Neoaliturus haematoceps*, Leafhopper-vector, Leafhopper host plants.

INTRODUCTION: DISTRIBUTION AND NATURAL SPREAD OF CITRUS STUBBORN DISEASE

Spiroplasma citri is the causal agent of citrus stubborn disease (CSD). On the basis of symptomatology, CSD extends throughout the Old World from Morocco on the Atlantic to Iran beyond the Caspian sea and the Gulf of Oman. Our group has cultured *S. citri* from citrus trees in the following countries during various surveys: Morocco, Algeria, France (Corsica), Turkey, Syria, Israel, Jordan, Saudi Arabia and Iran. The wide distribution of CSD in the Old World is not only due to the use of infected budwood; natural transmission has been suspected long ago from the fact that citrus seedling trees sometimes show symptoms of CSD. The occurrence of stubborn symptoms on seedling trees or plants is taken as evidence for natural spread of the disease because *S. citri* has never been found to be transmitted vertically through seeds, even though the seeds coats from infected citrus trees can carry *S. citri*.

Citrus is not the only host plant of *S. citri*. Many non-rutaceous hosts can become infected experimentally or naturally (9). Ornamental periwinkle seedlings were the first such hosts to be found naturally infected with *S. citri*, first in Arizona and California (1, 12), later in Morocco (4). More recently we have cultured *S. citri* from ornamental periwinkle seedlings collected in Dubaï (United Arab Emirates) and Nizwa (Sultanate of Oman). Periwinkle seedlings are good indicator plants for *S. citri* infection, and they have been used experimentally to gain information on natural transmission of the spiroplasma. By setting up periwinkle beds in various locations, we have shown that *S. citri* is naturally transmitted in Morocco and Syria (5, 6, 7).

THE LEAFHOPPER *NEOALITURUS HAEMATOCEPS* IS NATURALLY INFECTED WITH *S. CITRI*

In 1976, the leafhopper *Neoaliturus* (*Circulifer*) *tenellus* (Baker) was identified as a major vector of *S. citri* in California (13).

This discovery led us to search for *N. tenellus* in Morocco where its presence had been reported previously (11). In spite of many attempts, not only in Morocco but also in Turkey and Syria, *N. tenellus* was found in such low numbers that it did not appear as a major vector of *S. citri* in the Mediterranean area. We thus looked for alternative leafhopper vectors. Leafhoppers were collected with a D-vac aspirator, pooled according to species and analyzed for the presence of *S. citri* by ELISA (16) and the culture assay (3). Over 50 leafhopper species have been analyzed in this way on several surveys in Morocco, Syria, Turkey, France (Corsica) and Iraq between 1978 and 1986. Only one leafhopper species was found in all five countries to be naturally infected with *S. citri*, namely *Neoaliturus haematoceps* (Mulsant & Rey). Interestingly, this species is absent from the new world, but is very similar to the North American vector, *N. tenellus;* the two species can only be distinguished on the basis of their male genitalia. Of all *Neoaliturus* species encountered in the Mediterranean area, *N. haematoceps* was the most abundant, as already reported earlier (11).

N. HAEMATOCEPS IS A VECTOR OF *S. CITRI*

Natural infection of *N. haematoceps* with *S. citri* prompted us to investigate the potential of this species to act as a vector. *S. citri*-free leafhoppers were collected in Corsica and raised in Bordeaux on ornamental stock (*Matthiola incana* L.). An isolate of *S. citri*, cultured from a pool of *N. haematoceps* leafhoppers collected in Palmyra (Syria), was used in the transmission studies. Leafhoppers injected with the Palmyra isolate were able to multiply the spiroplasma to titers of 10^5-10^6 CFU per insect, and to transmit it to healthy periwinkle indicator plants, which began to show symptoms 6 weeks after infection. *N. haematoceps* leafhoppers were also able to acquire *S. citri* by acquisition feeding on periwinkle plants previously infected with the Palmyra isolate, and to transmit it to periwinkle plants. The efficiency of transmission seems high since 60% of individual leafhoppers were able to transmit the spiroplasma to periwinkles after a latent period of 20 days on stock (10). Both male and female individuals transmit *S. citri*. No evidence for vertical transmission has been obtained.

In previous experiments, we failed to get transmission with the leafhopper *Euscelidius variegatus* (Kirschbaum), the *S. citri* isolate used being a strain (Israel) that had been maintained by graft inoculations in periwinkles for over 10 yr. We have recently examined the ability of *N. haememathoceps* to transmit the Israel and the Palmyra isolates of *S. citri*. The spiroplasmas were used in parallel experiments. The spiroplasma cultures had the same low passage numbers. No transmissions were obtained after acquisition by feeding or injection with the Israel isolate, whereas high transmission rates were observed with the Palmyra isolate. These experiments seem to indicate that *S. citri* loses the ability of being transmitted by leafhoppers when it is kept too long in plants without undergoing a cycle through the insect. The loss of transmissibility is independant of pathogenicity to plants, since the Israel isolate continues to induce severe symptoms in plants. These observations might indicate that old, symptomatic "stubborn" trees do not function as sources of *S. citri* inoculum for transmission by *N. haematoceps*.

S. KALI, A MAJOR HOST PLANT OF *N. HAEMATOCEPS*

One of the major results of the work carried out in Syria was the discovery of the Chenopodiaceae, *Salsola kali* L., as a major host plant of *N. haematoceps*. Interestingly, this plant, also named *S. pestifer* (Russian thistle, tumbleweed), is a major host of *N. tenellus*, the principal vector of *S. citri* in California and Arizona. In the Old World the natural distribution of *S. kali* covers the Mediterranean zone and most of the Irano-Turanian regions. It has spread over all arid and semi-arid areas in the warm temperature zones. The species is extremely common in Morocco, Syria, Iraq and Iran, particularly on disturbed habitats (ruderal places in towns, villages, road sides), on irrigated and non-irrigated arable land, and on seashores; it extends from the hot lowlands up to more than 2,000 m, with highest densities in the hot and arid to semi-arid lowlands (Prof. Helmut Freitag, personal communication). In Syria, *S. kali* is very common along the seashores and the coastal citrus areas. Both *S. citri* infected and uninfected *N. haematoceps* leafhoppers are frequently collected on *S. kali* plants. In Iran, not only *N. haematoceps* but also *N. tenellus* occur, even though the latter is much less widespread than the former. The distribution of these leafhoppers throughout Iran correlates well with the presence of *S. kali*. In Iraq, *S. kali* was the only plant on which *N. haematoceps*, with and without *S. citri* infection, could be collected. In Cyprus, *S. kali* is very frequent along the southern shores and a search for *Neoaliturus* sp. is underway. The situation in Corsica, where *S. kali* also occurs on the coast, is described elsewhere (8).

Besides *S. kali*, ornamental stock and wild stock (*M. sinuata* L.) are good cruciferous hosts of *N. haematoceps*. In Syria, hundreds of *N. haematoceps* leafhoppers, most of them infected with *S. citri*, were collected on a few ornamental stock plants in the Orontes valley, a sugar beet, but not a citrus, region. In Corsica, the leafhopper overwinters on wild stock.

N. haematoceps was also collected on *Alhagi camelorum* along the Syrian coast where this legume often occurs in association with *S. kali*. In southeast Iran, *A. camelorum* and *A. persarum* can be seen within citrus orchards.

Finally, plants of the typical Mediterranean vegetation "maquis" harbor *N. haematoceps* in Corsica (8).

EPIDEMICS OF STUBBORN DISEASE IN SYRIA

In the Mediterranean area, stubborn disease was first reported in Palestine in 1931 by Reichert and Perlberger (15), who indicated that the disease was in an epidemic form and causing serious damage to young citrus orchards. More recently, in the Ghor (Jordan River valley), young citrus plantings, established in a noncitrus growing area, showed a high incidence of stubborn infection (M. Bar-Joseph, personal communication). Epidemics of stubborn are also known in California where they occur in certain years, 1973 for instance. A stubborn epidemic has occurred in Syria at El Annadeh, south of Lattaquia and at Tartous in the late 1970's, and was responsible for infection with *S. citri* of many young citrus trees from two important nursery projects. The Syrian epidemic has been the incentive to a FAO project to study natural transmission of *S. citri* in Syria. Work carried out from 1982 to 1985 has shown that the nurseries were established in areas along the coast where two major host plants of *N. haematoceps* occur: *Salsola kali* and *Alhagi camelorum*. *S. citri*-infected *N. haematoceps* leafhoppers are frequently collected on these hosts. The land for the nurseries was cleared in 1978 and the natural vegetation including *S. kali* and *A. camelorum*,

was removed. This left barren, sandy land with no windbreaks around the plots to be planted with citrus. The young trees that were planted in this open land probably served as alternate host plants on which leafhoppers were practically forced to feed. In short, it is believed that land clearing operations under semi-desert conditions favored leafhopper migration and feeding on young trees resulting in local epidemics. At the El Annadeh project, new land was prepared in 1985 to extend the nursery. In June 1986, *Salsola kali* was growing again amid the young nursery trees and will probably help to attract *N. haematoceps* for a new epidemic.

STUBBORN IN IRAN

In Iran, CSD is widespread throughout the citrus growing areas. The first *S. citri* isolate from an Iranian citrus sample was cultured in Bordeaux as early as 1974. Since then, *S. citri* was isolated from citrus trees in many areas of southern, southeastern and northern Iran (14; Bove, unpublished). Both *N. tenellus* and *N. haematoceps* occur in the country (17). Their distribution throughout Iran has been well studied insofar as they are vectors of sugarbeet curly top virus. *N. haematoceps* occurs in all citrus growing areas of the county. *N. tenellus* is less widespread than *N. haematoceps*, but it also is present in areas where citrus is grown. Two of the major host plants of the above leafhoppers are well known and common in Iran: *Salsola kali* and *Alhagi camelorum* or *A. persarum*. Their distribution undoubtedly explains that of the two *Neoaliturus* species. Hence, there seems to be a close correlation between the occurrence of *N. haematoceps-N. tenellus*, the presence of *S. kali* and other major host plants of the leafhoppers and the distribution of CSD in the major citrus growing areas of Iran.

CONCLUSION

Natural spread of CSD has been demonstrated in several countries of the Old World. *Neoaliturus haematoceps* has been identified as a major leafhopper vector of *S. citri*. This species is related to *N. tenellus*, a major vector in the southwestern USA. The biology of the two leafhoppers is similar. While *N. haematoceps* does not occur in the New World, *N. tenellus* is present in the Old World. In the Mediterranean area, *N. tenellus* does not seem to be a major vector in view of its scarcity. In Iran, it might play a larger role. Major host plants of *N. haematoceps* have been identified. Some of these, such as *Salsola kali*, are also major hosts of *N. tenellus* in California and Arizona. Epidemics of CSD in Syria have been correlated with the presence of *S. citri*-infected *N. haematoceps* leafhoppers and the host plant, *S. kali*. The extremely wide distribution of plants such as *S. kali*, which extends from the Atlantic to the Himalayas, favors the presence of *S. citri* leafhopper vectors over all arid and semi-arid areas in the warm temperature zone where citrus is often grown, and is probably one of the reasons why CSD is present throughout much of the Old World. It has been roughly estimated that CSD-affected trees represent about 1 to 5% of all sweet orange trees. This percentage is probably not higher because *N. haematoceps* and *N. tenellus* are polyphagous insects that visit citrus only rarely.

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