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STUDIES ON GREENING DISEASE TRANSMISSION BY THE CITRUS PSYLLA, *TRIOZA ERYTREAE* (HEMIPTERA : TRIOZIDAE)

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ABSTRACT

Before an acquisition feeding, some of the nymphs of the citrus psylla, *Trioza erytreae* may already be infected with the greening disease. ;mission of the disease. Nymphs in their second to he disease if they feed on greened citrus. A small percentage of nymphs in their fourth and fifth instars can transmit the greening disease while most can only do so when in the adult stage. This means that adult psylla that developed on greened leaves can transmit the disease without acquisition feeding. *Clausena anisata* (Willd.) Hook. f. ex Benth. (Rutaceae) can become infected with the greening disease and should be removed if they grow close to citrus orchards. No evidence was found to implicate three other indigenous tree species as carriers of the disease.

KEY WORDS: Citrus psylla, disease transmission, greening, *Trioza erytreae*, Triozidae

INTRODUCTION

The greening disease of citrus trees is of major importance in South Africa. Approximately 100,000 sweet orange trees were rendered commercially unprofitable in the early sixties (Oberholzer et al., 1965) and it was estimated in the late eighties that the annual loss in production due to greening in South Africa amounted annually to R35 million (Van den Berg et al., 1987).

The citrus psylla, *Trioza erytreae* (Del Guercio), was regarded as a major pest when McClean and Oberholzer (1965) found that the adult can transmit the greening disease. Studies by McClean and Oberholzer (1965), Catling (1969), Moll and Martin (1973) and Catling and Atkinson (1974) indicated that the adult citrus psylla can transmit the greening disease but that nymphs failed to do this. However, McClean (1974) found that citrus psylla nymphs can at times also acquire the infection when feeding on diseased tissue, but there may be some delay in the adult stage before such carriers are fully infective and capable of transmitting the disease.

The greening organism is apparently a Gram-negative bacterium (Moll and Martin, 1974; Bove, 1986). Passages from one generation to the next through the eggs have been demonstrated for a Gram-negative bacterium in a cicadellid (Purcell and Suslow, 1987), a virus in a delphacid (Raga et al., 1988) and rhabdoviruses in a number of aphids (Sylvester, 1980). This aspect has not yet been investigated with regard to the greening bacterium and the citrus psylla. Furthermore, the egg of this triozid seems to have an absorbant ability from the plant tissue (Moran and Blowers, 1967). The egg may therefore possibly be able to acquire the greening disease in this manner. These two possibilities need to be examined.

The citrus psylla feeds and breeds on citrus and also on indigenous Rutaceae plants (Van der Merwe, 1923; Catling and Annecke, 1968; Moran, 1968; Van Bruggen and Yilma, 1985). Adults were found on and probably also feed on some other non-host plants (Van den Berg and Deacon, 1989). Some of the latter plants may therefore become infected with the greening disease and act

as carriers. This possibility is strengthened by the statement of Moll and Van Vuuren (1982) that it would appear that citrus is not the ideal host for the greening organism and that continual re-infestation by citrus psylla seems to be required.

The aims of this study were to establish whether transovarial transmission of the greening bacteria by the citrus psylla is possible, to determine whether the immature stages can acquire and/or transmit the disease and whether some indigenous plants may act as carriers of the greening disease.

MATERIALS AND METHODS

Transovarial transfer of greening and/or transmission during oviposition

Branches of sweet orange trees that contained psylla nymphs in their final instar but no adults were collected in an orchard at Burgershall, Transvaal (25°07'S and 31°05'E) where practically every branch of every tree and all adult citrus psylla were infected with greening. The stems of the branches collected were placed in containers with water and three branches enclosed in gauze cages. Adults that emerged thereafter were collected and placed on greened sweet orange trees where they could mate and feed. The apical growth points of ten indicator plants (sweet orange seedlings that were grown in the absence of insects) (reference no. A) were placed individually in 2-l cages (Van den Berg, 1989). After 14 days, the mouthparts of 100 of the females kept on the greened sweet orange trees were severed under microscope with a small pair of scissors. Ten of these females were placed on each of the indicator plants where they oviposited. Adults that developed from these eggs were allowed to feed on the same plants for a further 30 days. The plants were then sprayed with a suitable insecticide to kill all the psylla. Thereafter, the plants were transferred to a glasshouse at 24°C (day) and 20°C (night). The plants were examined after six months for greening symptoms using the method of McClean and Schwarz (1970). To confirm the results, they were further analysed by thin layer chromatography using the method of Van Vuuren and Da Graça (1977).

Transovarial transfer and/or ability of the egg to pick up greening from plant tissue

In this experiment, psylla that developed on greened trees were allowed to oviposit on the same trees. Four hundred and eighty first instar nymphs were taken from the leaves soon after they had hatched and before they started to feed. These were placed in equal numbers on 24 indicator plants. The nymphs on 13 of these plants (no. B) were allowed to feed on these plants for 7 days and then transferred to a second group of indicator plants (no. C). This procedure was repeated 7 days later. The nymphs on the latter plants and those on the remaining 11 plants were allowed to complete their development and the adults allowed to feed on these plants for a further 30 days. These 24 plants were all marked no. D. The plants were treated as described earlier and assayed for greening.

Transmission of greening by nymphs

Second and third instar nymphs that developed on greened plants were treated as follows. Each nymph was irritated with a brush until it withdrew its mouthparts from the leaf and crawled out of the gall. The nymphs were then removed and 20 of the same instar were placed on each of 56 indicator plants. After 4–7 days the nymphs on 19 plants (no. E) were transferred to a second group of indicator plants (no. F). These nymphs were transferred to a third group of plants 7 days later. The nymphs on the latter plants and those on the remaining 37 plants were left to complete their cycle. These were all marked no. G. Plants from which nymphs had been removed were sprayed and placed in the glasshouse. Plants with adults on them were kept in the laboratory for about 30 days, sprayed to kill the psylla and then placed in the glasshouse. The plants were tested for greening six months later.

Nymphs in their fourth and fifth instars were treated in the same manner as described for second and third instar nymphs. Twenty of the same instar were placed on each of 61 indicator plants. After 4–7 days nymphs on 22 plants (no. H) were transferred to new plants. These nymphs as well as those on the remaining 39 plants were left to complete their cycle. Thirty days later, the plants (no. I) were sprayed and then placed in the glasshouse.

Greening in other plant species

In a preliminary experiment, the following method was used to test the possibility of greening in other plants. However, this method could not distinguish between plants which were pre-infected with greening and psylla which might have been infected following transovarial transfer.

Ten branches of *Clausena anisata* (Willd.) Hook. f. ex Benth. and a branch of *Zanthoxylum capense* (Thunb.) Harv. (Rutaceae) which were infested with psylla nymphs were covered individually with gauze cages. The trees were growing in close proximity to a greening-infected citrus orchard. After the adults had emerged, 10 were placed on each of 12 indicator plants for 30 days. The plants were treated and tested for greening as mentioned.

In a second experiment three indicator plants were planted in each of five containers. Two of the plants in each of these containers were side-grafted to two branches of an indigenous tree in the field. Two *C. anisata* trees were used and one each of *Vepris lanceolata* (Lam.) G. Don, *Z. capense* (Rutaceae) and *Trichilia emetica* T. Roka. Chiov. (Meliaceae). Two branches from each of the trees were examined to ensure that they contained no psylla or other possible vectors. The branches were then side-grafted to two of the indicator plants in the same pot. The third indicator plant served as a control. All of the indicator plants and the side-grafted branches were sprayed thoroughly with a concentrated endosulfan spray to kill insects that might have been overlooked. They were then covered with a double gauze cage, which was sprayed as an added insurance against infestation. The cages were inspected twice a week to ensure that they were still effective in keeping insects out. The plants were watered as required. After 6 weeks, when the stems of the indicator plants and the side-grafted indigenous plants were thought to have formed callus, the branches of the latter were severed. The indicator plants, still in the gauze cages, were then brought to the glasshouse, taken out of the cages and tested for greening 6 months later.

RESULTS

Results of tests on the transmission of greening via the egg, during oviposition, and the ability of the eggs and nymphs to pick up and transmit the disease are summarised in Table 1. Evidence was found that females can transmit greening transovarially and/or to the plant during oviposition (Table 1, reference no. A). Furthermore, it has also been demonstrated (no. D) that greening can either be transmitted transovarially or be picked up by the eggs from diseased plants. In this experiment, greening was only transmitted after the second week of nymphal development (instar 5) and/or during the adult stage (21% of test plants).

The results indicate that psylla nymphs may already be infective when emerging from the eggs, having an influence on the following transmission results. However, because of the increase in percentage of infectiveness, namely from 10% to 43%, the following deductions may still be made: During their second, third, fourth and fifth instar, nymphs can probably also become infected with greening. It is, however, mostly during the adult stage that they can transmit the disease as shown by 39% and 43% of the indicator plants (no. G and I), respectively. One indicator plant became infected with greening after nymphs in their fourth and fifth instar fed on it (no. H).

In the preliminary study performed to determine whether greening was present in other plant species, none of the indicator plants with psylla that developed on *C. anisata* (10 indicator plants) or *Z. capense* (2 indicator plants) were infected with greening.

Of the four indicator plants that were side-grafted to *Clausena anisata* trees in the field, one

TABLE 1
Studies on greening disease transmission by the citrus psylla: transovarial, during oviposition and by eggs or nymphs

Stage (and ref. no. as used in Materials and Methods)	Period in days on indicator plants as Eggs		Nymphs		Adults	Indicator plants (n)	Positive transmission in stage and %
Eggs (A)	±7	+	±20	+	±30	10	1 (10.0%)
Nymphs							
Crawlers							
1st week (B)	0		7		0	13	0
2nd week (C)	0		7		0	13	0
3rd week (D)	0		±7–21	+	±30	24	5 (20.8%)
Second and third instar							
1st week (E)	0		4–7		0	19	0
2nd week (F)	0		11–14		0	19	0
Nymphal and adult stage (G)	0		18–21	+	±30	56	22 (39.3%)
Fourth and fifth instar							
1st week (H)	0		4–7	+	0	22	1 (4.5%)
Nymphal and adult stage (I)	0		4–14		±30	61	26 (42.6%)

developed the symptoms and positively-tested for greening. The control indicator plants remained uninfected as was the case with those that were side-grafted to *V. lancolata*, *Z. capense* and *T. emetica*.

DISCUSSION

It has been found that greening can be transmitted transovarially and/or during oviposition or that greening can be picked up from the plants by the eggs. It has been demonstrated that Gram-negative bacteria (Purcell and Suslow, 1987), rhabdoviruses (Sylvester, 1980) and viruses (Raga et al., 1988) can be transmitted through the eggs from generation to generation in a cicadellid, a number of aphids and a delphacid. Because of these results, transovarial transmission of the greening disease in the citrus psylla seems the most likely. The results obtained do not exclude the possibility that greening can be transmitted by oviposition rather than by feeding. If this is true, this will be an interesting form of transmission and ought to be investigated further.

The present results indicate that nymphs can probably become infected with greening which is in agreement with the work of McClean (1974). However, in other studies (McClean and Oberholzer, 1965; Catling, 1969; Moll and Martin, 1973; Catling and Atkinson, 1974) no evidence was found that nymphs can acquire greening disease. It has also been found that nymphs of the Asian citrus psylla, *Diaphorina citri* Kuwayama (Psyllidae), can also become infected with the greening disease (Nariana and Singh, 1971 according to McClean, 1974; Xu et al., 1988).

The observation that the immature stages of *T. erytreae* may become infective with greening implies that when the adults develop, some of them will already be infective and greening can be spread without an acquisition feeding period. The duration of the acquisition period plus the period before the insect is able to transmit the disease has previously been given as 21 days (Moll and Martin, 1973), but recent results (Van Vuuren et al., 1986) indicate this to be only 2 days. The present results and those of McClean (1974), therefore, indicate that some adults are immediately infective, not needing any time to acquire the greening disease. For this reason it is essential to reduce the greening inoculum by removing infected trees (Schwarz, 1967; Green and Schwarz,

1970) and assessing psylla populations more precisely as suggested by Van den Berg et al. (in press a).

Citrus psylla nymphs feed exclusively on young plant tissue (Van der Merwe, 1923; Moran, 1967; Catling, 1972). When leaves turn hard, however, the nymphs, which are partly sedentary, can move to younger flush (Van den Berg et al., in press b). Although it may not occur often, the movement may be from a greened to an uninfected branch and greening can then be spread by nymphs in this manner.

As *C. anisata* can become infected with greening, these trees ought to be eliminated from the proximity of citrus orchards. This has also been recommended because of the apparent increase in psylla populations when these trees grow in the vicinity of citrus groves (Van der Merwe, 1923; Van den Berg et al., in press a). Further research is necessary to determine whether other indigenous host and non-host plants of the citrus psylla may be carriers of the disease and, if they are, they should also be removed from the proximity of citrus orchards.

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