Effectiveness of the Different Life Cycle Stages of Brevipalpus Phoenicis Geijskes in Leprosis Transmission

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ABSTRACT. The ability of the instars of the false spider mite, *Brevipalpus phoenicis*, in transmitting leprosis was investigated. Non-viruliferous colonies of the mite were reared on healthy citrus fruits under laboratory conditions. Then they were transferred to, and confined on, disinfected leprotic leaf tissues where they were allowed to feed for 1, 3 or 5 days at 15C-17C. After each period 1, 3 or 5 mites per plant were transferred according to their life cycle stage, larva, nymph or adult, to young healthy potted seedlings of sweet orange. Plants were individually protected under glass jars at room temperature. Larvae transmitted leprosis at 48.3%, whereas nymphs and adults transmitted at 8.7%. Although the results obtained were insufficient to evaluate the influence of the number of mites and feeding period on leprosis transmission, they show that larvae are more efficient vectors than nymphs and adults.

The first indication that a mite was associated with leprosis in Brazil was in 1942 (1). Before that time Frezzi (8) and Vergani (19) demonstrated that the mite *Tenuipalpus pseudocuneatus* Blanchard, later identified as *Brevipalpus obovatus* Donn., was associated with "lepra explosiva", prevalent in Argentina. Later Knorr (10, 11) reported that *B. californicus* (Banks) was associated with leprosis in the United States (Florida). Following Bitancourt's work, Rossetti, *et al.* (14) and Musumeci, *et al.* (12) reported *B. phoenicis* Geijskes as the mite species closely associated with leprosis in Brazil. Further investigation carried out by Rossetti (16) showed that *Brevipalpus* mites from leprosis-free regions were unable to transmit the disease unless they had access to affected tissues. Although these results, which strengthen the assumption of the viral nature of leprosis, clearly show that *Brevipalpus* mites can induce the disease, the authors did not mention the effectiveness of the different mite stages on transmission. The purpose of the research reported in the present paper was to determine a closer relationship between the mite and the disease by studying the influence of the different life cycle stages of the false spider mite *B. phoenicis* in transmitting leprosis.

MATERIALS AND METHODS

Colonies of *B. phoenicis* that in previous tests did not transmit leprosis, and which we assumed were not viruliferous, were reared on green fruit of Pera sweet orange or of Rangpur lime. In order to prevent rapid desiccation of the fruit, they were coated with a paraffin film with exception of an area about 5 cm in diameter that was surrounded by a barrier of "Stickem Special" (Michel & Pelton Co., Femeryville, Calif.)* to prevent the mites from escaping. Mites were allowed to colonize this area in an incubator at 25C. After the colonies were well established, mites were transferred to detached leaves or to leaf pieces showing symptoms of leprosis. This leaf material was previously disinfected with 70% alcohol and examined under a stereomicroscope to be sure that it was free from any

* Mention of a trademark or proprietary product does not imply its approval to the exclusion of other products which may also serve the same purpose.

arthropods. Then the leaf piece was conditioned by hanging it from a thin nylon thread fixed to the inner part of a glass cover, so that it hung freely in a glass cylinder where drops of water prevented it from drying. The glass with the leaf material was kept at 15C-17C and the mites were allowed to feed for 1, 3 or 5 days. At this temperature, the molting process is retarded so that after 5 days larvae and nymphs could be found easily before molting. After each acquisition period, mites were transferred, according to their life stages, to young healthy potted plants of sweet orange using 1, 3 or 5 individuals per plant. Infested plants were individually protected with glass cylinders covered with a thin nylon net and kept at room temperature in glasshouse compartments. Control potted plants were similarly treated using mites fed on healthy citrus leaves. Three transmission experiments were undertaken separately at different times over several months (table 1).

RESULTS

Forty to 50 days after the experimental infestation the first symptoms of leprosis appeared on leaves of plants which received mites previously fed on affected tissues. Control plants remained symptomless.

Table 1 shows the results of the experiments involving *B. phoenicis* instars. The results show that there is a difference between the larvae, nymphs, and adults in their efficiency in transmitting leprosis. In preliminary experiments (5), larvae were shown to be the most efficient vectors and transmitted in 52% of the attempts as compared with nymphs (12.5%) and adults (8.6%). Later, other experiments with a larger number of plants confirmed this tendency and showed a transmission rate of 48.3% for the larvae, 8.7% for the nymphs and 7.6% for the adults (table 1). Many mites died during transfers to, and while feeding on, detached leaves, which made it difficult to determine the best acquisition time and the number of mites to use to obtain a high transmission rate.

DISCUSSION

Transmission of plant viruses by mites has been well explored in the case of eriophyid mites (13, 18). On the other hand, there are few references to viruses and virus-like diseases transmitted by tenuipalpid mites. Apparently, the only virus that has been shown conclusively to be transmitted by a tenuipalpid mite (*Brevipalpus phoenicis*) is coffee ringspot virus (2, 3), although there is strong evidence supporting the viral nature of leprosis (4, 6, 7, 9) which is also transmitted by *B. phoenicis*. The first research referring to a close relationship between mites and leprosis was that of Knorr (11) who reported that leprosis could be transmitted through eggs, nymphs, and adults of *B. obovatus* and of *B. californicus* but not those of *B. phoenicis*. However, no experimental feeding of any mite instar was carried out in that case.

The present data show that *B. phoenicis* is an effective vector of leprosis especially in the larval stage. In a previous report (4), instars of the same species of mite, originally from coffee plants, were unable to transmit leprosis even after feeding on affected citrus tissues. These findings suggest that the leprosis agent is adapted to strains of *B. phoenicis* prevalent on citrus. Also the same species of mite which also transmits citrus zonate chlorosis (15) and coffee ringspot virus (2) did not transmit either leprosis or zonate chlorosis to coffee, or coffee ringspot to citrus (17).

The greater efficiency of the

TABLE 1
TRANSMISSION OF LEPROSIS BY DIFFERENT LIFE CYCLE STAGES OF *BREVIPALPUS PHOENICIS*

Mite stage	Acquisition period (days)	No. of mites per plant* 1	3	5	Total*	Transmission (%)
Larva	1	4/5	3/7	—‡	15/31	48.3
	3	2/5	2/7	0/1		
	5	3/3	—	1/3		
	1†	—	0/3	—	0/8	0
	3†	—	0/5	—		
Nymph	1	0/5	0/2	—	2/23	8.7
	3	0/7	1/2	0/1		
	5	0/2	0/2	1/2		
	1†	—	0/3	—	0/8	0
	3†	—	0/5	—		
Adult	1	1/5	2/6	—	3/33	7.6
	3	0/5	0/4	0/2		
	5	0/2	0/4	0/5		
	1†	—	0/3	—	0/8	0
	3†	—	0/5	—		

* No. of plants showing leprosis symptoms/total number of infested plants.
† Control, with non-infected mites.
‡ No data.

larvae in leprosis transmission could be related to a more favourable condition for the agent in the digestive tract of this instar. Electron microscopic studies of viruliferous mites could offer a means to support this assumption and disclose other aspects of the agent in the vector.

The results obtained strengthen evidence for the viral nature of leprosis since only the mite instars which have fed on diseased citrus leaf tissues, and not those fed on comparable healthy tissues, could transmit the disease. Although the present data give additional information on the vector-disease relationship much remains to be learned about acquisition and retention of the leprosis agent by *B. phoenicis*.

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