

Transmission Studies on Little Leaf Mycoplasma of Brinjal

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Studies carried out on the transmission of little leaf mycoplasma of *Solanum melongena* by *Hishimonus phycitis* revealed that the minimum acquisition and inoculation feeding period was one hour each, while the optimum was 24 and 48 hr. respectively. A minimum incubation period of 15 days was observed to be essential in the vector for successful transmission. However, the vector reached the maximum transmitting ability after an incubation period of 23 days. The optimum incubation period of the mycoplasma in the host plant was recorded to be 35.67 days. There was no transovarial transmission of the mycoplasma. A single vector was able to transmit the causal organism, although the maximum percentage of infection in the test plants was achieved only when five vectors were used per plant.

Solanum melongena is an important vegetable widely cultivated in India, China, Japan, the Philippines and to a limited extent in the African countries. In India, one of the formidable set backs in the assured production of this vegetable is the incidence of 'little leaf' disease. This disease is widely prevalent in all brinjal growing areas and usually occurs at the productive stage of the crop. In the years of heavy incidence, it causes substantial loss in yield. The leaf-hopper *Hishimonus phycitis* (Distant) has been established as the vector for the causative agent (Anjaneyulu, 1969). Though the disease has been known for a long time, a perusal of literature revealed that attempts have not been made in adequate measure to study in detail the relationship of mycoplasma and the vector. This paper presents infor-

mation on the relationship of the vector and the mycoplasma in transmission.

MATERIALS AND METHODS

Vectors initially collected from gingelly (*Sesamum indicum* L.) plants and cultured subsequently on *S. melongena* in glasshouse were used for all transmission studies. About 30 days old brinjal seedlings (Pusa Purple Long) were used in the transmission experiments. The test plants were covered with glass chimneys or kept in glass cages and after inoculation, the plants were protected with insecticide and kept under insect proof conditions for further observations.

Acquisition feeding period: Young adults were collected from the healthy colony with an aspirator and allowed to feed on the diseased source

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for different periods *viz.*, 0, 1, 2, 4, 8, 16, 24, 48 and 72 hr. After acquisition feeding for prescribed periods, the leafhoppers were transferred to test plants at the rate of one leafhopper per plant. The leafhoppers were allowed to feed until death in all the test plants so as to complete the incubation in the vector and then transmit the causal agent. The development of disease symptoms in the test plants was observed daily and the results compared.

Inoculation feeding period: Fourth or fifth instar nymphs were collected from the healthy colony and were confined in the diseased plants for 26 days. Then the leafhoppers were allowed to feed individually on test plants for different periods *viz.*, 0, 1, 2, 4, 8, 16, 24, 48 and 72 hour with a view to find out the minimum inoculation feeding period.

Incubation period in the vector: A batch of fifth instar nymphs or young adults were collected from the healthy colony and allowed to feed on the fully diseased plants. Starting from the 5th day, the leafhoppers were transferred to eight test plants per day for inoculation of causal agent at the rate of one leafhopper per plant. The vectors were allowed to feed on the test plants for 24 hr intervals. This was continued till 27th day.

Congenital transmission: Infective leafhoppers were collected from the diseased plants and released on *Sesamum indicum* L., a resistant plant to the etiologic agent (Anjaneyulu, 1969). After feeding for about a week,

all the leafhoppers were removed. The nymphs which emerged within 9 to 12 days were allowed to grow on the same plant. All the freshly emerged adults were collected periodically and transferred to test plants in batches of 3 leafhoppers per plant. These leafhoppers were allowed to feed on the same plants until death. The plants were observed for development of disease symptoms upto 80 days.

Incubation period in the host plant: Viruliferous vectors were allowed to feed on test plants for a period of 24 hr for inoculation of etiologic agent, the plants were sprayed immediately with an insecticide and kept under observation, for the appearance of disease symptoms in insect proof conditions.

Optimum number of infective vectors: The leafhoppers were allowed singly and also in groups of 2, 3, 4, 5 and 6 giving them 24 hr of inoculation feeding after making sure that they had completed the incubation period.

RESULTS AND DISCUSSION

Acquisition feeding period: Under the minimum acquisition feeding period tried (30 min) none of the eight plants tested was inoculated. While 12.5 per cent of the plants were infected when the acquisition feeding period was one hr, there was no infection under two hr period. The vector was found to infect maximum number (62.5 per cent) of test plants when they were permitted to acquire the mycoplasma for 24 hr. Increased acquisition feed-

TABLE I. Acquisition and inoculation feeding periods

Period (hr)	Percentage of infection	
	Acquisition feeding	Inoculation feeding
1	—	—
1	12.5	25.0
2	—	25.0
4	37.5	25.0
8	50.0	37.5
16	37.5	37.5
24	62.5	50.0
48	37.5	62.5
72	50.0	50.0

No. of plants tested in each period - 8.

ing period over this limit did not bring about any increased infection (Table I).

Inoculation feeding period: Under 30 min inoculation feeding period the vectors did not infect any of the test plants. Maximum percentage (62.5) of infection occurred, when the vectors were allowed to inoculate the causal agent for 48 hr (Table I).

Incubation period in the vector: The observations revealed that a minimum incubation period of 15 days was essential in the vector for successful transmission. While the percentage of transmission ranged from 25.0 to 50.0 at incubation periods of 16 to 27 days the vector reached the maximum transmitting ability (50 per cent) after an incubation period of 23 days (Table II). Increased incubation period over this level did not increase the percentage of infection.

Congenital transmission: The mycoplasma was not transmitted through the eggs of the vector as evidenced by the vectors failing to trans-

mit the causal organism in all the 25 plants tested.

Incubation period in the host plant: The mean incubation period in the host plant was found to be 35.67 days. The minimum and maximum period of incubation of the etiologic agent in the host plant was recorded to be 31 and 39 days respectively.

TABLE II. Incubation period in the vector

Days after acquisition*	Percentage of infection
16	37.5
17	25.0
18	—
19	25.0
20	37.5
21	25.0
22	25.0
23	50.0
24	37.5
25	25.0
26	50.0
27	50.0

No. of plants tested under each period - 8

* There was no transmission upto 15 days of acquisition feeding.

Optimum number of viruliferous vectors required for transmission: A single vector was observed to infect 41.67 per cent of test plants. However, the maximum percentage (66.67) of infection was achieved only when five vectors were used per test plant (Table III).

Increased percentage of transmission with longer acquisition feeding time was reported by Lee (1962) with *Macrostoteles fascifrons* Stal, transmitting aster yellows mycoplasma. The aster leaf-hopper, *M. fascifrons*, transmitted the eastern strain of aster yellows most

TABLE III. Optimum number of infective vectors required for transmission

Number of vectors used	Percentage of infection
1	41.67
2	33.33
3	50.00
4	58.33
5	66.67
6	66.67

No. of plants tested - 12

effectively only when it had 48 and 72 hr acquisition feeding (Granados and Chapman, 1968). Increased percentage of infection consequent to prolonged acquisition feeding period was also reported in the case of tungro virus transmitted by *Nephotettix virescens* (Rivera and Ou, 1965; Ling, 1966). However, the infective capacity of *H. phycitis* given an acquisition feeding for a longer period than the optimum level did not improve the transmission any further. Similar views have also been expressed by Ling (1966) while working on tungro virus. Bindra (1973) reported an acquisition threshold of 8 hr for *H. phycitis* and pre-fasting the vectors for 3 to 4 hr did not have any effect on the acquisition efficiency.

Chiu *et al.* (1968) showed that even though 5 to 19 min inoculation feeding was effective in transitory yellowing by *Nephotettix apicalis* (Motschulsky), 24 hr inoculation was observed to produce 90 per cent of infection in test plants. Sahambi (1970) also reported that an optimum of 24 hr

inoculation feeding period was essential for greater percentage of transmission of *Sesamum* phyllody by *Orosius albicinctus* Distant. Thirty minutes inoculation threshold period has been reported for *H. phycitis* transmitting little leaf of *S. melongena* (Bindra, 1973).

The reports of extended incubation period in the leafhopper vectors transmitting mycoplasma were presented by several workers (Granados and Maramorosch, 1967; Palomar and Rivera, 1967; Bindra, 1973). In general, most of the mycoplasma transmitted by leafhoppers were not transmitted transovarially. The etiologic agent of clover club leaf was reported to be transmitted transovarially by Black (1948). However, recently the causative organism of this malady was suspected as rickettsia or Chlamydiae (Windsor and Black, 1972).

A relatively long incubation period in *S. melongena* plant was comparable with those of other mycoplasma infecting several plants and transmitted by leafhoppers (Palomar and Rivera, 1967; Sahambi, 1970; Bindra, 1973). Increased percentage of infection with increased number of vectors was also reported by Edison (1973) in the grassy shoot disease of sugarcane.

The senior author is thankful to the Tamil Nadu Agricultural University, Coimbatore for according permission to publish this research finding which formed part of a dissertation submitted for the award of M. Sc. (Ag.) degree.

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