TABASCO WILT:

NATURE OF HOST-VIRUS INTERACTION

by

Ali Zouba

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APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

Merritt R Nelson — July 29, 1977

MERRITT R. NELSON — Date

Professor of Plant Pathology

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

ABSTRACT

Tabasco pepper inoculated with tobacco etch virus (TEV) responded with a classic systemic mosaic infection when grafted on roots of Anaheim pepper. Conversely, Anaheim pepper grafted on Tabasco roots produced the classic wilt syndrome, identical in every detail to symptoms typically shown by TEV-infected Tabasco plants.

Increases in electrical conductivity of solutions surrounding roots of TEV-infected Tabasco was associated only with the occurrence of virus in roots, and a necrosis of phloem. The increased bathing solution conductivity was judged to be due to materials released from dead cells as a result of the hypersensitive response to virus infection.

Wilt symptom was also reproduced by inoculating Anaheim top on Tabasco root with pepper mottle virus (PMV). Tabasco top and roots react in a hypersensitive manner to PMV.

The disease "Tabasco wilt" therefore results from a hypersensitive response of the roots to TEV. The unique feature of the disease, wilt, is due to systemic multiplication of the virus in the upper portion of the plant, with a subsequent high concentration of virus moving to the root cells. These, due to the hypersensitive response, die. This is the feature that made this hypersensitivity disease an important field problem.

INTRODUCTION

Tabasco pepper (Capsicum frutescens L.) is used for the production of hot sauces. It is economically important in Iberia, St. Martin, and Lafayette Parishes in South Louisiana (Horn and Sinclair, 1959).

Growers in these regions have been affected by heavy losses due to wilting of Tabasco pepper crops. The disease was first reported in Louisiana in 1935 (Horn and Sinclair, 1959). Greenleaf (1953) proved that the Tabasco wilt disease is due to the infection of Tabasco pepper by tobacco etch virus (TEV).

While TEV infects a wide range of hosts, wilting is a unique response of Tabasco pepper to TEV infection (Fig. 1). Other cultivars develop vein-clearing and mottling of the young leaves but no wilt.

Vein clearing, preceding wilt, has been reported in Tabasco (White and Horne, 1965; Ghabrial and Pirone, 1967), with wilting usually occurring 5-20 days after inoculation, depending on the age of the plant and the concentration of TEV in the inoculum (Greenleaf, 1953). Approximately 24-28 hours after wilt symptoms appear, all roots of infected Tabasco become necrotic and die. A reddish-brown ring can be observed in cross sections of the large roots (White and Horne, 1965). Subsequently, the plants usually die.

Figure 1. Tabasco pepper infected with tobacco etch virus.

White and Horne (1965) made a histological study of Tabasco pepper infected with TEV. They found that roots are the only part that showed a histological modification, resulting in necrosis of the phloem and the cambium, and the degeneration of cortex cells and plastids. They also reported that wilted Tabasco plants recovered when stems were placed in water.

Ghabrial and Pirone (1967) found that phloem necrosis is associated with a permeability change in roots and a decrease in the respiratory rate. They also reported an enzymatic change in roots the first day of wilt. No such changes occurred in non-infected control plants or in plants infected with alfalfa mosaic virus or cucumber mosaic virus, which do not cause wilt of Tabasco plants. Similarly, no changes occurred with the TEV-infected California Wonder pepper cultivar.

Nelson and Wheeler (unpublished data, University of Arizona) observed that defoliated wilted Tabasco plants do not die. While little subsequent growth occurs, it is turgid and does not wilt.

Death of roots due to virus infection has been reported in several cases--e.g., tomatoes affected by curly top virus, tristeza, tea phloem necrosis (Holmes, 1964), and certain legumes when infected with tobacco necrosis virus (Teakle, 1962). In most cases, root death was shown to be a result of phloem necrosis (Holmes, 1964). Pepper mottle virus causes necrosis of stems and leaves of Tabasco pepper plants (Zitter, 1972) but no root necrosis was reported.

Many toxins of known or unknown chemical composition are presumed to cause wilt of plants (Diamond, 1955; Wheeler and Black, 1963). Also, Ghabrial and Pirone (1967) have suggested that root necrosis of Tabasco caused by TEV could result from a deficiency in potassium that leaked as a result of permeability changes.

My work seeks to more fully explain, with appropriate data, the possible mechanism of Tabasco wilt as caused by TEV.

MATERIALS AND METHODS

Virus Strains

The viruses TEV and pepper mottle virus (PMV) were maintained in Anaheim pepper. The identification of the two viruses was established using serological and pathogenicity tests.

Correlation Between Wilt and TEV Presence in Roots

Seeds of Tabasco pepper were sown in vermiculite. Seedlings in the 2 to 2-leaf stage were transplanted to a standard soil mixture (one part Canadian peat moss, one part red Mesa top soil, and one part sand). Fifty 10-15-cm tall plants were inoculated by rubbing the four oldest leaves with a virus inoculaum prepared by grinding infected Anaheim leaves in a mortar with phosphate buffer pH 7.0 at a ratio of 1:5 (w/v). At 24 hr intervals after inoculation, five infected Tabasco plants were severed at the base of the stem, roots were ground in a mortar, and the extract was used for inoculation of three healthy Tabasco plants. The cut ends of the severed plants were dipped in Rootone and replanted. A parallel experiment was also run using old, woody Tabasco plants.

Girdling of Infected Plants

Long distance movement of plant viruses is mostly known to occur through the phloem (Matthews, 1970). Destruction of the phloem therefore could prevent the translocation of the virus to the roots and then wilt symptoms.

In one experiment, 35 young Tabasco plants (about 20-cm tall) were inoculated by rubbing the four youngest leaves with a suspension of TEV. The plants were then divided into seven groups of five plants. At 24-hr intervals over 6 days, a group was girdled; the last group was not girdled and served as a control. Plants were observed for the appearance of wilt symptoms. At the same time 20 other plants were divided into two even groups. Plants in the first group were girdled and inoculated; the remaining plants were only inoculated. Roots of the 20 plants were assayed for TEV each 24 hrs over 10 days. A duplicate experiment was conducted using old Tabasco plants with woody stems.

Solanaceous plants, including peppers, have internal primary phloem (esau, 1969). Since girdling would not remove this phloem, virus particles could still be basipetally translocated in the internal phloem. To determine this, 15 plants were girdled and a small hole was made in the exposed xylem. A wire was inserted through the hole and the tissues internal to the xylem were destroyed by scraping. Thus, the only remaining part of the stem at the level of girdling was the xylem. Leaves above the girdling were then inoculated. Control

plants were inoculated without girdling. In all experiments, one-two shoots were left below the point of girdling. The plants were observed for the appearance of wilt and mosaic symptoms.

Grafting

To determine the effect of the aerial portion of the plant on roots of Tabasco pepper that may result in wilt symptoms, other cultivars of pepper that do not wilt when infected with TEV were grafted on Tabasco roots. Reciprocal grafts were also made. The cultivars used for this purpose were: Anaheim, STD Mercury, Yellow Wonder, Gold Spike, and Bell Boy peppers. The experiment was repeated at least two times for each top-scion construction, using ten plants in each test.

Two grafting methods were used: Chip grafts and approach grafts. In the former method, woody plants were used. Buds of the desired cultivars were placed in small cuts on the stem of the cultivar serving as root stock. The bud was thoroughly taped to the stem. The tape was removed 2 weeks later. The new growth from the grafted bud was inoculated after 6 weeks (Fig. 2). In the latter method, one Tabasco plant and one plant from the desired cultivar were transplanted into one pot. When they were about 10-cm tall, a small part of the bark of both plants was removed and the wounded areas were put together and thoroughly taped. The tape was removed 10 days later and the desired top cultivar was severed below the point of graft (Fig. 3). Many attempts were made before getting successful grafts.

Figure 2. Chip graft.

Figure 3. Approach graft.

Introduction of a portion of eggplant (Solanum melongena L.) stem between the top and the roots of Tabasco pepper was done by approach grafting two Tabasco plants to an eggplant. After removing the tape, one of the Tabasco plants, serving as the top, was severed below the graft. The experiment was repeated two times, using 5 plants in each test.

The tops of all combinations were inoculated with either TEV or PMV and roots were assayed for TEV.

Defoliation of Wilted Tabasco Plants

This experiment was done to confirm the observation of Nelson and Wheeler (unpublished data) that defoliation of wilted Tabasco plants induced their recovery. Twenty Tabasco plants were inoculated with TEV. When wilt occurred in all plants, fifteen plants were defoliated. The other five plants served as control.

To observe roots of recovered Tabasco plants, fifteen young plants were grown in a nutrient solution (Osmo-coat fertilizer and water). The solution was changed every 3 days. When roots were well developed, leaves were inoculated with TEV. Plants were defoliated after wilt symptoms appeared in all plants. Roots were observed for regrowth and necrosis.

Permeability of Roots of Virus Infected and Defoliated Tabasco

Young seedlings of Tabasco and Anaheim pepper were grown in a solution (10g/1) of fertilizer (Osmo-coat) until they were 3-4-cm tall. Individual plants then were placed in 25 ml. of distilled water. Three days later leaves of 15 Tabasco plants and five Anaheim plants were inoculated. Five control Tabasco plants were rubbed with distilled water. Infected Tabasco plants were divided into three even groups. Two groups were respectively defoliated 48 and 72 hr after inoculation. The third group was not defoliated and served as a control. The electric conductivity of the distilled water in which roots were suspended was measured at 24-hr intervals over 9 days using a conductivity bridge (Kemetrex, type 70).

RESULTS

Correlation Between Wilt and TEV Presence in Roots

Root extracts of Tabasco plant roots, assayed for TEV 3 days after inoculation, induced wilt in Tabasco plants used for the detection of TEV. Roots of Tabasco plants assayed 24 and 48 hr after inoculation showed no infectivity. Most Tabasco plants started to wilt 5-6 days after inoculation. Wilt symptoms, therefore, appeared 2-3 days after the virus was detected in roots. In the second experiment, using old plants, the virus was detected in roots 72 hr after inoculation; however, wilt occurred 4-5 days after virus detection.

Girdling of Infected Plants

When young Tabasco plants were used, no difference was observed between the time of the first appearance of wilt in girdled and non-girdled plants. Also, virus was detected 3 days after inoculation in roots of both groups. In the parallel experiment, using old woody plants, the first appearance of wilt in plants girdled 24 hrs after inoculation and in non-girdled plants was respectively 13 and 9 days after inoculation. The virus detection in roots was respectively 7 and 3 days after inoculation. Girdling of woody plants therefore delayed the translocation of the virus into the roots for at least 4 days. The old

plants girdled the second day after inoculation showed also a 4-day delay in the appearance of symptoms. Plants girdled 3 days or more after inoculation wilted at the same time as non-girdled plants.

In the second experiment, when the continuity of the internal and external phloem was destroyed, no wilt occurred in twelve of the fifteen plants. Leaves of the non-wilted plants above the girdles showed a typical mosaic symptom, those below the girdling point remained symptomless. These results suggest that the virus passes through the internal and external phloem of the stem to the roots.

Grafting

All cultivars grafted on Tabasco roots and inoculated with TEV wilted. When reciprocal grafts were made and the Tabasco leaves were inoculated, however, wilt did not occur (Fig 4 and Table 1). The cultivar developed typical, systemic mosaic symptoms. The introduction of the eggplant stem between the roots and tops of Tabasco prevented the translocation of TEV to the roots and no wilt occurred. TEV, therefore does not move in the eggplant tissue.

Pepper mottle virus induces local and systemic necrosis in the leaves and stems of Tabasco plants while Anaheim plants are infected systemically (Zitter, 1972). When Anaheim plants were grafted on Tabasco roots and leaves were inoculated with PMV, all plants wilted.

(a) (b)

Figure 4. Effect of TEV on (a) Tabasco grafted on Anaheim root; and (b) Anaheim grafted on Tabasco root.

Table 1. Symptoms on different pepper scion-root combinations caused by pepper mottle virus (PMV) and tobacco etch virus (TEV)

	Symptoms				
Virus	Tabasco	Anaheim	Tabasco on[a] Anaheim	Anaheim on Tabasco	Tabasco on Eggplant on Tabasco
PMV	Local lesions and systemic necrosis	Mosaic	Local lesions and systemic necrosis	Wilt and death	- - -
TEV	Wilt and death	Mosaic	Mosaic	Wilt and death	Mosaic

[a]Tabasco grafted on Anaheim root.

Figure 5. Effect of pepper mottle virus on Anaheim grafted on Tabasco root.

(Fig. 5). Tabasco roots showed a severe necrosis identical to that caused by TEV.

The virus was detected in roots of all top-scion combinations except for eggplant-Tabasco combinations.

Defoliation of Wilted Tabasco Plants

Tabasco plants inoculated with TEV recovered following defoliation; however, there was little growth. This was slow in developing, but remained turgid. Mild mosaic appeared on the new leaves (Fig. 6). Inoculum from leaves of recovered plants induced wilt on Tabasco plants; however, reinoculation of recovered plants did not induce further wilt.

Growth of roots of recovered, defoliated plants was stimulated but necrosis occurred before the new roots reached 2-3 mm in length. Similarly, stems of wilted Tabasco plants which were severed at the base, dipped in Rootone, and transplanted produced only few roots which were continuously killed.

Permeability of Roots of Virus Infected and Defoliated Tabasco

The conductivity of the distilled water in which roots of Tabasco and Anaheim were placed was measured approximately at 24-hr intervals. A marked increase in the conductivity, indicative of a release of electrolytes, was detected 72 hr after inoculation and 48-72 hr before non-defoliated plants started to wilt (Fig. 7). No

Figure 6. Recovery of wilted Tabasco after defoliation.

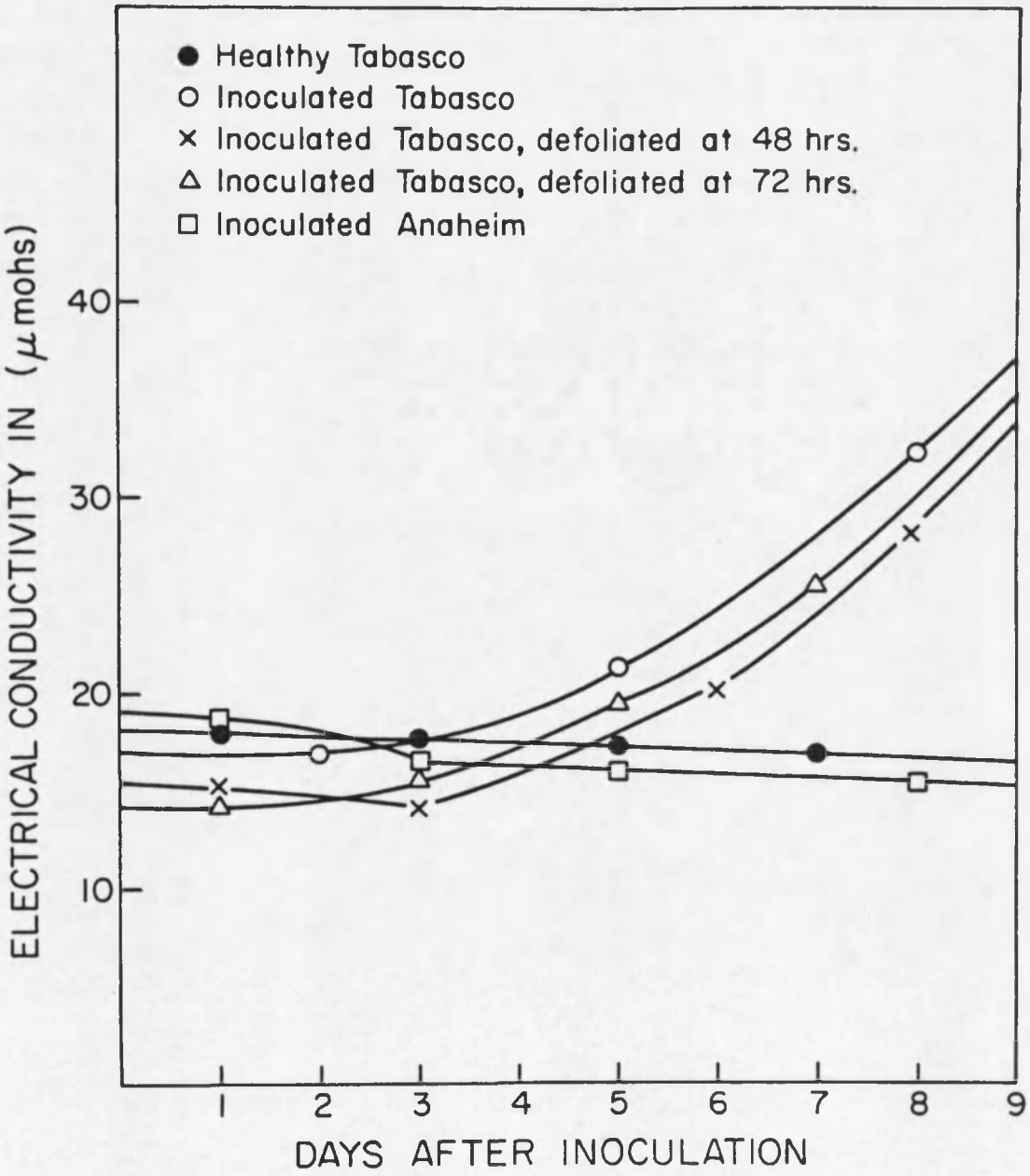

Fig. 7. The electrical conductivity of solutions bathing roots of healthy Tabasco, tobacco etch virus inoculated Anaheim and Tabasco, and inoculated, defoliated Tabasco pepper plants. -- Each point represents an average based on five plants tested.

difference in the electric conductivity of the bath solution was observed between defoliated and non-defoliated plants. No such increase was observed with infected Anaheim plants or with healthy Tabasco plants.

DISCUSSION

Permeability changes of Tabasco roots following TEV infection has been considered to be the major factor leading to Tabasco wilt (Ghabrial and Pirone, 1967); the other symptoms were assumed to be secondary effects. Wilt was reported to be due to a blockage of water which could result from (1) sealing of injured cells preventing the passive absorption of water, and/or (2) a decrease of the active absorption due to a decreased rate of respiration of roots. Ghabrial and Pirone (1967) also suggested that cell injury could be due to a deficiency of potassium which results from permeability changes.

Permeability changes following infection have been reported in many cases of fungal and bacterial wilt diseases. The pathogens usually produced certain "toxins" that cause death of the host tissue. The presence of toxins in the case of the Tabasco infected with TEV seems to be unlikely, since our data show that: (1) root necrosis occurs only when the virus is present in the roots, (2) preventing the translocation of the virus into Tabasco root prevents wilt, (3) defoliation of infected Tabasco did not prevent death of roots, and (4) Tabasco pepper grafted on Anaheim root does not wilt when infected with TEV.

Altered membrane permeability has been reported to be associated with hypersensitive reactions (Gianinazzi, Vallie and Martin,

1972; Addy, 1976; Ohashi and Shimoura, 1976). The change of permeability was reported to be a secondary development. A high correlation was found between the leak of electrolytes and the phenol content of apple leaves infected by Erwinia amylovora (Addy, 1976). Ethylene has been associated with virus-induced local lesion development, a hypersensitivity phenomenon (Ross and Williamson, 1951; Pritchard and Ross, 1975). This chemical is known to cause alteration of membrane permeability and induction or increase of certain enzymes (Gahagan, Holm, and Abeles, 1968). These phenomena suggest that the change in permeability of Tabasco roots when infected with TEV is a secondary development due to hypersensitive reaction and cellular death.

Further support for this hypothesis is provided by a model of the TEV Tabasco wilt syndrome using the same hosts and grafting techniques but a different virus, PMV. Pepper mottle virus reaction on Tabasco pepper differs from TEV in that both tops and roots react hypersensitively to infection. When Anaheim pepper on Tabasco roots is inoculated with PMV, the result is wilt and death identical to the reaction to TEV. Tabasco inoculated with PMV ordinarily does not wilt. This is probably due to a low virus titre. When Anaheim is the scion, PMV multiplies in the Anaheim component. Virus then is translocated in massive quantities to roots where the hypersensitive reaction occurs and wilt of top ensues. There appeared to be identical reactions following infection of Tabasco by TEV.

Tabasco wilt can be explained by the failure of dead roots to absorb water. Survival of wilted Tabasco after defoliation suggests that the passive transportation of water is not altered even after death of roots. Recovery of defoliated plants could result from a reduction of stomata such that a small quantity of water is able to maintain a much reduced plant without wilt. The continuation of the passive transportation of water may explain why an additional inoculation of recovered, defoliated plants does not cause death.

Resistance to TEV in Capsicum spp. does occur (Greenleaf, 1956; Greenleaf et al., 1970). Barnes, Maskar, and Black (1971) studied the source of resistance in two Capsicum frutescens cultivars, LP-1 and Almeda. They showed that resistance in these two cultivars follows the pattern of simple Mendelian inheritance with resistance being dominant. LP-1 was found to be homozygous for resistance to the mosaic and wilt symptoms, while Almeda was wilt resistant and mosaic susceptible. Capsicum frutescens, therefore, is homozygous for susceptibility to mosaic and wilt symptoms since Tabasco top grafted on Ahaheim roots showed mosaic symptoms.

In summary, the truly unique feature of the disease, "Tabasco wilt," as caused by tobacco etch virus, is the fact that the disease results from a situation where the plant top responds in a classic systemic mosaic infection while the root responds in a purely hypersensitive fashion.

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