The Role of Temperature and Inoculum Concentration in the Development of Tip Necrosis and Seedling Death of Beans Infected with Bean Yellow Mosaic Virus

J. C. TU, Research Station, Research Branch, Agriculture Canada, Harrow, Ontario N0R 1G0

ABSTRACT

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The role of temperature and virus concentration on tip necrosis and seedling death of bean yellow mosaic virus (BYMV) was investigated in growth chambers. The development of tip necrosis in BYMV-infected beans is controlled by temperature. The percentage of infected plants that developed tip necrosis increased between 16 and 24 C, but decreased drastically from 24 to 28 C. Temperatures higher than 28 C were inhibitory to the development of tip necrosis in BYMV-infected beans. Temperatures between 16 and 24 C that favored BYMV multiplication resulted in higher virus titer and contributed in part to the severity and percentage of tip necrosis. Death of BYMV-infected bean seedlings was strongly influenced by original inoculum concentration.

Bean yellow mosaic is an important disease of beans in southwestern Ontario, second only to bean common mosaic. The latter has been brought under control because major cultivars presently in use carry the resistant "I" gene (5). Consequently, the former has become more evident in this region (7). There are many strains of bean yellow mosaic virus (BYMV) (1). One strain that is prevalent in this region and in Michigan has been identified as a necrotic strain (5,6). Plants susceptible to this strain frequently developed tip necrosis and leaf abscission in addition to yellow mosaic in the field. Some inconsistencies were observed. For example, tip necrosis developed more uniformly and affected more plants in a greenhouse at 21 ± 3 C than in one in which temperature rose to as high as 35 C on a sunny afternoon. This observation suggested that the development of tip necrosis in BYMV-infected plants may be temperature-dependent.

Tip necrosis occurs in many virus-diseased plants. For example, in soybean (*Glycine max* (L.) Merr.) only the cultivar Columbia developed tip necrosis when infected with an ATCC strain of soybean mosaic virus (*author's unpublished data*). In navy bean (*Phaseolus vulgaris* L.) some strains of bean common mosaic virus induced systemic necrosis in some cultivars (1). Also in navy bean, the cultivar Kentwood was resistant to tip necrosis when inoculated with a necrotic strain of BYMV (6). Temperature has been proven to play a role in the systemic necrosis of some navy beans infected with bean common mosaic virus (3). Recently, Weststeijn (9) showed that a necrosis-inducing factor in *Nicotiana tabacum* L. 'Xanthi-nc' infected with tobacco mosaic virus was temperature-dependent. The infected plants remained symptomless when they were grown at 32 C, but developed necrotic lesions at 22 C. An investigation was initiated to determine if a similar temperature relationship occurs in tip necrosis of beans, and to further determine if 1) a delay in tip necrosis would occur when plants were subjected to a possibly unfavorable temperature (32 C) for virus multiplication for a varied duration before placing in a favorable temperature or 2) plants would fail to develop tip necrosis when they were subjected to a favorable temperature (24 C) for virus multiplication first and then transferred to an unfavorable temperature before the development of tip necrosis. Because virus titer and temperature played an important role in tip necrosis, the effect of inoculum concentration and temperature on seedling death (a tip necrosis that extended downward to the hypocotyl) was also investigated.

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MATERIALS AND METHODS

The necrotic strain of BYMV (5,6) used in this study was routinely maintained in *P. vulgaris* 'Bountiful' in the greenhouse at 21 ± 1 C. In order to obtain sufficient inoculum of the same titer, seedlings at the unifoliate stage were mechanically inoculated as previously described (8). When infected plants had reached the second trifoliate stage, fully expanded first trifoliate leaves were triturated and the resulting sap was strained through cheesecloth. The resultant crude sap was assigned an arbitrary concentration value of 1. This sap was then diluted with 0.01 M phosphate buffer, pH 7.0, to concentrations of 10^{-1}, 10^{-2}, 5×10^{-3}, and 10^{-3}. The dilution end point in BYMV is approximately 10^{-3} (4,5).

To investigate the effect of inoculum concentration and temperature on tip necrosis, *P. vulgaris* 'Black Turtle Soup' was used because it is highly susceptible to tip necrosis when infected with BYMV. All inoculations were made on primary leaves at the unifoliate stage of the plant approximately 10 days after sowing. For each inoculum concentration, 50 plants were inoculated to accommodate five temperature treatments, 10 plants per treatment. To ensure the establishment of infection, a lag time of 4 hr was allowed after inoculation before each set of plants was transferred to one of five growth chambers at 16, 20, 24, 28, and 32 C. All growth chambers were programmed for 10 hr dark/14 hr light and were illuminated with cool-white fluorescent tubes supplemented with incandescent lamps that provided light at an intensity of 486 $\mu E \cdot m^{-2} \cdot s^{-1}$ at pot level. Observations on symptom development and disease progress began 4 days after inoculation. The experiment was done three times.

Virus titer in bean was determined by assaying the infectivity of sap extracted from infected plants onto the second fully expanded leaves of *Chenopodium amaranticolor* Coste & Reyn., which is a local lesion host of the BYMV (2) and is frequently used in quantitative assay for virus infectivity (10). For local lesion assay, a full-leaf technique was used instead of the conventional half-leaf technique because my past experience showed that if only leaves of *C. amaranticolor* of the same age on plants with the same growth stage were used the results from the full-leaf technique were sufficiently accurate for direct comparison without resorting to cross-reference of data points, as in the case of the half-leaf technique.

Assays were made 6 and 12 days after inoculation of the plants that were kept at different temperatures. Inocula were extracted from first trifoliate leaves of diseased plants. The crude sap was diluted with 0.01 M phosphate buffer, pH 7.0, to arrive at a concentration of 10^{-1}. The diluted sap was mechanically inoculated to *C. amaranticolor* by dipping a cotton-tipped stick in the inoculum and rubbing it once over the entire leaf surface that was predusted with Carborundum. Ten leaves were inoculated for each datum point. All experiments were done three times. The inoculated plants were kept in the same greenhouse. The number of local lesions on each leaf was recorded 7 days after inoculation. The virus titer was expressed

in terms of number of local lesions per leaf of *C. amaranticolor*. Each datum point and standard deviation was determined based on an average of three tests.

In order to determine whether the tip necrosis was related to high virus titer as well as incubation temperature, approximately 120 Black Turtle Soup plants were inoculated. Four hours later, they were divided into two groups (A and B), each with 60 plants. Groups A and B were placed in growth chambers at 32 and 24 C, respectively. Thereafter, 10 plants were moved from each chamber to the other every day for 6 days. As revealed in a preliminary test, these two temperatures represent two extremes: one favors virus replication and the other does not.

Inoculated plants were observed daily for symptoms of tip necrosis. The virus titers in the infected plants were assayed on *C. amaranticolor* 6 and 12 days after inoculation.

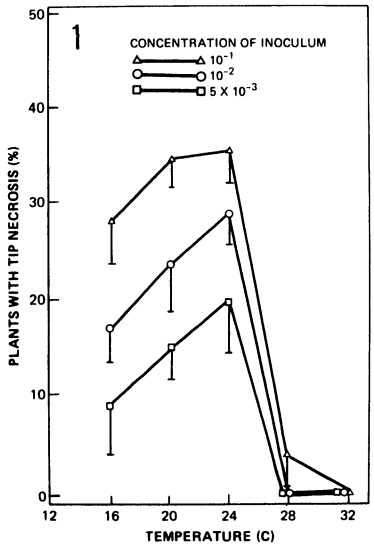

Fig. 1. Effect of inoculum (bean yellow mosaic virus) concentration on the development of tip necrosis when bean plants (*Phaseolus vulgaris* 'Black Turtle Soup') were inoculated at the unifoliate stage. Standard deviations (in one direction only) are shown for all data points ($N = 3$).

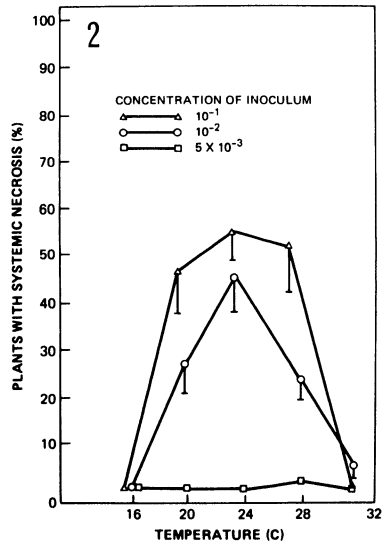

Fig. 2. Effect of inoculum (bean yellow mosaic virus) concentration on seedling death when bean plants were inoculated at the unifoliate stage and grown at five different temperatures. Standard deviations (in one direction only) are shown for all data points ($N = 3$).

RESULTS

Effect of inoculum concentration on tip necrosis. When seedlings at the unifoliate stage were inoculated with different concentrations of inoculum (i.e., 10^{-1}, 10^{-2}, and 5×10^{-3}), the percentages of seedlings that developed tip necrosis increased with increasing inoculum concentration (Fig. 1). On the other hand, when inoculum at a concentration of 10^{-1} was used to inoculate seedlings of different growth stages, the percentages of seedlings that developed tip necrosis were 54, 32, and 20% for seedlings inoculated at unifoliate, first trifoliate, and second trifoliate stage, respectively (*data not shown*).

Effect of temperature on tip necrosis. Temperature played an important role in the development of tip necrosis (Fig. 1). Percentages of plants with tip necrosis increased with increasing incubation temperature between 16 and 24 C. The highest percentage of plants that developed tip necrosis occurred at 24 C. At 28 and 32 C, little or no tip necrosis was observed, but the infected plants clearly exhibited yellow mosaic symptoms typical of BYMV infection, and the crude saps extracted from the leaves of infected plants were infectious.

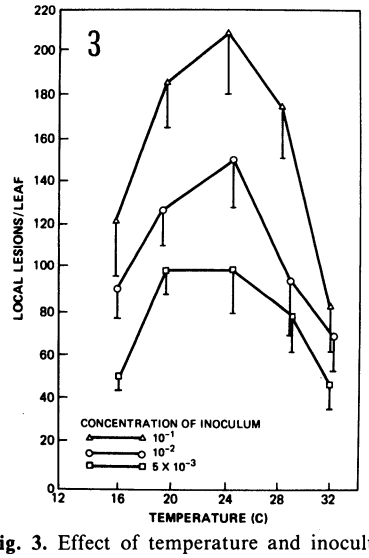

Fig. 3. Effect of temperature and inoculum concentration on bean yellow mosaic virus titer in the infected bean plants grown at different temperatures or inoculated at different temperatures and grown at the same temperature. Plants were inoculated at the unifoliate stage. Virus titer was determined assaying 10^{-1} crude sap dilution extracted from the first trifoliate leaves on leaves of *Chenopodium amaranticolor*. Standard deviations (in one direction only) are shown for all data points ($N = 3$).

Effect of inoculum concentration and temperature on seedling death. Death of seedlings occurred when the tip necrosis extended downward to the hypocotyl and only occurred when seedlings were inoculated at the unifoliate stage. No seedling death was observed in plants inoculated at the first or second trifoliate stage (*data not shown*).

The occurrence of seedling death was dependent on both inoculum concentration and temperature. As the inoculum concentration increased, the percentages of dead seedlings also increased (Fig. 2). The percentages of dead seedlings were 47, 57, and 51% at 20, 24, and 28 C, respectively, for seedlings inoculated with inoculum concentration of 10^{-1}. The percentages were derived from the sum of three repeated experiments. At 16 and 32 C, few seedlings died (Fig. 2).

Effect of temperature and inoculum concentration on virus titer. Both initial inoculum concentration and incubation temperature affected the virus titer in infected plants (Figs. 3 and 4). At a given temperature, higher virus titer was realized when higher inoculum concentration was used (Fig. 3). On the other hand, at a dilution of 10^{-1} crude sap, the virus titer was related to incubation temperature of the plants. For example, the virus titer in the first trifoliate was highest at 24 C with 210 local lesions per leaf followed by those at 20 and 28 C with 190 and 175 local lesions per leaf, respectively. Virus titers were least at 16 and 32 C (120 and 83 local lesions per leaf, respectively) (Fig. 3).

The relationship between virus titer and seedling death is seen by comparing Figures 2 and 3.

Effect of temporary high incubation

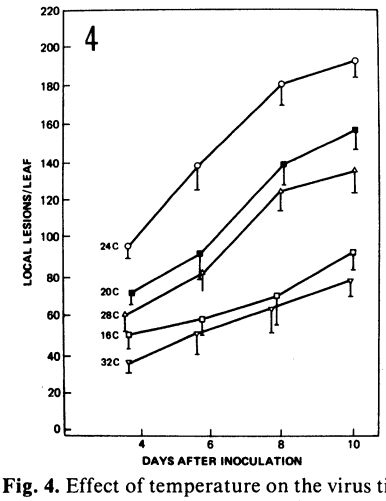

Fig. 4. Effect of temperature on the virus titer in bean plants (*Phaseolus vulgaris* 'Black Turtle Soup') inoculated with bean yellow mosaic virus on primary leaves at the unifoliate stage. Virus titer was determined using 10^{-1} crude sap dilution prepared from the first trifoliate leaves of bean (cv. Bountiful). Standard deviations (in one direction only) are shown for all data points ($N = 3$).

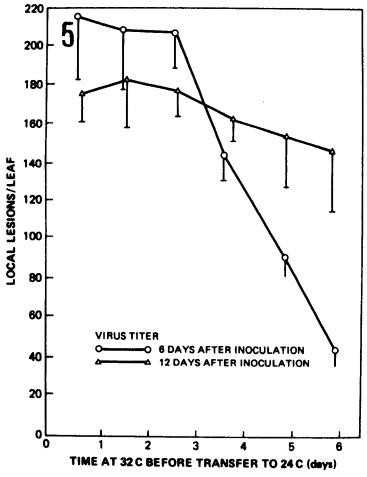

Fig. 5. Effect of varying duration (1–6 days) in which bean plants (*Phaseolus vulgaris* 'Black Turtle Soup') were incubated at 32 C before being moved to 24 C on bean yellow mosaic virus titer and effect on tip necrosis development. Virus titer was assayed on *Chenopodium amaranticolor* 6–12 days after inoculation. Standard deviations (in one direction only) are shown for all data points ($N = 3$).

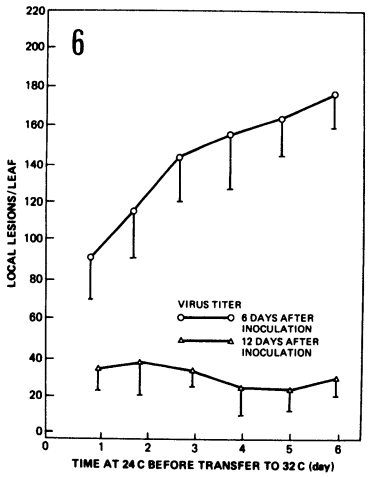

Fig. 6. Effect of varying duration (1–6 days) in which bean plants (*Phaseolus vulgaris* 'Black Turtle Soup') were incubated at 24 C before being moved to 32 C on bean yellow mosaic virus titer and effect on tip necrosis development. Virus titer was assayed on *Chenopodium amaranticolor* 6 and 12 days after inoculation. Standard deviations (in one direction only) are shown for all data points ($N = 3$).

temperature on virus titer and tip necrosis development. Results of inoculated plants that were moved progressively for 1–6 days from 32 to 24 C and assayed for virus titer 6 and 12 days after inoculation (Fig. 5) showed that 1) virus titer in plants that were kept 1–3 days at 32 C and assayed 6 days after inoculation had minimal effect on virus titer, but a duration of 4–6 days at 32 C resulted in a precipitous decrease in virus titer—the longer the duration at 32 C, the lower the virus titer (Fig. 5) and 2) virus titer in plants subjected to 1–6 days at 32 C and transferred to 24 C for up to 6 days before assaying (to make a total of 12 days incubation after inoculation) resulted in a gradual and slight decrease in virus titer (Fig. 5).

By comparing 1 and 2 above, it becomes apparent that the 6 days of posttreatment incubation at 24 C had essentially restored the lost virus titer (Fig. 5).

Similarly, in inoculated plants kept at 24 C and moved progressively for 1–6 days to 32 C and assayed for virus titer 6 and 12 days after inoculation, the results showed that virus titer in plants assayed 6 days after inoculation had a steady increase with the duration of incubation at 24 C, whereas virus titer in plants assayed 12 days after inoculation had little change. It is obvious that the 6 days of posttreatment incubation at 32 C had nullified the effect of the initial treatment (Fig. 6).

Daily observation of inoculated plants that were kept at 32 C for 1, 2, 3, 4, 5, and 6 days before being moved to 24 C showed that the development of tip necrosis was delayed for 1, 2, 3, 3, 4, and 4 days, respectively. The present study shows that all infected plants develop systemic mosaic in the temperature range of 16–32 C.

DISCUSSION

Tip necrosis in BYMV-inoculated bean seedlings appeared to increase from 16 to 24 C, and then decrease drastically between 24 and 28 C. Assays of virus titer showed that tip necrosis was closely related to the virus titer that increased from 16 to 24 C, peaked at 24 C, and declined drastically thereafter. It was noted that the virus titer in infected plants incubated at 16 and 32 C was approximately the same (Figs. 3 and 4). It was also noted that higher inoculum concentrations resulted in more tip necrosis (Fig. 1).

The results indicated that the virus titer of the infected plants kept at 16 and 32 C were approximately the same. Yet at 16 C, substantial numbers of plants developed tip necrosis (Fig. 1), in contrast to none at 32 C. This is a clear indication that high temperature (28 C or higher) is inhibitory to the development of tip necrosis.

As mentioned previously, seedling death is an extension of tip necrosis that progresses downward beyond the cotyledons. The development of tip necrosis is determined by temperature and enhanced by virus concentration in situ in infected plants. Higher inoculum concentration and favorable temperature should lead to quicker increase of virus concentration in infected plants and, consequently, quicker progression of disease and more seedling death. This is reflected in the results where seedling death was high (47%) at 20 C, peaked at 24 C (57%), and declined slightly at 28 C (51%).

This study has clearly demonstrated that the development of tip necrosis in BYMV-infected bean plants in the growth chamber is inhibited at temperatures of 28 C or higher. The results also help to explain the inconsistency of tip necrosis development in the field where temperature fluctuates.

LITERATURE CITED

1. Drijfhout, E., Silbernagel, M. J., and Burke, D. W. 1978. Differentiation of strains of bean common mosaic virus. Neth. J. Plant Pathol. 84:13-26.
2. Gibbs, A. J., Harrison, B. D., and Murant, A. F. 1970. Bean yellow mosaic virus. No. 40 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England.
3. Grogan, R. G., and Walker, J. C. 1948. The relation of common mosaic to black root of bean. J. Agric. Res. 77:315-331.
4. Pierce, W. H. 1934. Viruses of the bean. Phytopathology 24:87-115.
5. Tu, J. C. 1980. Occurrence and identification of a flexuous rod virus from a mosaic complex of white beans in Southern Ontario. Phytopathol. Z. 99:163-174.
6. Tu, J. C. 1982. Inheritance of *Phaseolus vulgaris* cv. Kentwood of resistance to a necrotic strain of bean yellow mosaic virus and to a severe strain of tobacco ringspot virus. Can. J. Plant Pathol. 5:34-35.
7. Tu, J. C. 1988. Bean yellow mosaic: Now the most severe virus disease of white beans in southwestern Ontario. Bean Improv. Coop. 31:143.
8. Tu, J. C., and Ford, R. E. 1968. Effect of maize dwarf virus infection on respiration and photosynthesis of corn. Phytopathology 58:282-284.
9. Weststeijn, E. A. 1984. Evidence for a necrosis-inducing factor in tobacco mosaic virus-infected *Nicotiana tabacum* cv. Xanthi-nc grown at 22° C but not at 32° C. Physiol. Plant Pathol. 25:83-91.
10. Yarwood, C. E. 1964. Assay of infectivity. Pages 79-110 in: Techniques in Experimental Virology. R. J. C. Harris, ed. Academic Press, New York.