Effect of Concentration of Inoculum and Method of Inoculation on Development of Verticillium Wilt of Sunflowers

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ABSTRACT

Hypodermic inoculation of sunflowers (*Helianthus annuus*) with *Verticillium dahliae* gave more uniform and predictable infection than root dip inoculation. There was a direct linear relationship between concentration of inoculum and the speed of development of leaf symptoms and degree of stunting of the susceptible inbred line CM 162. Stunting was not observed in the resistant line CM 144; leaf symptoms were less severe, developed more slowly, and at the lower inoculum concentrations affected fewer plants than in CM 162. Infection occurred in some susceptible plants inoculated hypodermically with a suspension averaging one spore per plant. Consistently uniform infections were obtained with an average of 10 spores per plant.

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Additional key words: minimum effective inoculum.

Wilt induced by *Verticillium dahliae* Kleb. is a destructive and widespread disease of sunflowers (*Helianthus annuus* L.) (3, 7), as well as many other hosts. We are interested in various aspects of development of Verticillium wilt. It is essential for our work to obtain predictable and uniform infection of inoculated plants. We report here the results of our comparisons of two methods of inoculation, and the effects of concentration of inoculum on wilt of sunflowers.

MATERIALS AND METHODS.—The sunflowers used were the *Verticillium*-susceptible inbred line CM 162 and the resistant line CM 144 (6), provided by J. A. Hoes, Canada Department of Agriculture Research Station, Morden, Manitoba. Seeds germinated in vermiculite were transplanted one per 10-cm diam pot of pasteurized soil and transferred to a greenhouse. They were inoculated when 3 wk old, at the four-to six-leaf stage. Inoculated plants were transferred to cabinets maintained at 25 C during a 16-hr light period and 20 C in the dark. Light intensity at plant level was about 20,000 lux, provided by cool-white VHO fluorescent tubes supplemented by incandescent bulbs. Humidity was maintained at about 80%.

The culture of *V. dahliae* used was our isolate V-58 from sunflower, which induces typical chlorosis, necrosis, and stunting in susceptible cultivars (7). Inoculum was prepared by washing conidia with sterile distilled water from 7- to 10-day-old cultures grown on potato-dextrose agar in petri dishes at 25 C. The conidial suspension was centrifuged and the pellets were resuspended in sterile distilled water. Conidial concentration determined with a haemocytometer was adjusted to 5×10^5 per ml; from this we prepared a logarithmic dilution series with 5×10^5, 5×10^4, 5×10^3, 5×10^2, 5×10^1, and 5×10^0 conidia per ml.

Root dip inoculations were made by a modification of Wellman's method (11). Plants were removed from pots and the soil was washed carefully from the roots with tap water. In preliminary experiments we found little difference in infection when roots were immersed for periods ranging from 30 sec to 60 min. In these experiments, the roots and hypocotyls were immersed in inoculum for 10 min. The whole range of dilutions from 5×10^5 to 5×10^0 was used in each test. Control plants were dipped in sterile water.

Hypodermic inoculations were made as described by Bugbee & Presley (2) for cotton. The conidial suspension was forced from the syringe to form a bead in the bevel of a 12-gauge needle. The needle was inserted into a sunflower stem just above the cotyledonary node until the bevel disappeared; as the needle was withdrawn, the drop of inoculum was drawn into the stem. As there were 50 such drops per ml, each plant was inoculated with 0.02 ml of spore suspension. Hypodermic inoculations were made with the dilution series 5×10^5 to 5×10^1. Control plants were inoculated with sterile water.

Each experiment was repeated twice, with four plants per treatment, in four replicates.

RESULTS AND DISCUSSION.—*Root dip inoculation.*—Leaf symptoms were conspicuous on many plants 11 days after inoculation by root dip (Table 1). Symptoms were severe on the susceptible CM 162 and light on the resistant CM 144. Severity of chlorosis and necrosis was comparable in all diseased plants within each inbred line, regardless of concentration of inoculum, although symptoms developed more slowly in plants exposed to the lower concentrations. Similar results were obtained by Phillips (5) with *Fusarium* on carnations. We confirm in part Talboys (9) general conclusion that differences in host resistance, virulence of the parasite, concentration of inoculum in soil, and environmental conditions result mainly in varying frequency of acute disease development, rather than variation in severity of Verticillium wilt.

The most obvious difference attributable to varying concentrations of inoculum was in plant

TABLE 1. Effect of inoculum concentration on rate of development of Verticillium wilt symptoms on sunflowers

Method of inoculation	Inoculum concentration (conidia/ml)	Percentage of plants with foliage symptoms Days after inoculation									
		5		7		11		15		21	
		CM 162	CM 144	CM 162	CM 144	CM 162	CM 144	CM 162	CM 144	CM 162	CM 144
Root dip	5×10^5	0	0	0	0	100	100	100	100	100	100
	5×10^4	0	0	0	0	50	100	50	100	100	100
	5×10^3	0	0	0	0	50	75	75	100	100	100
	5×10^2	0	0	0	0	0	0	0	25	75	25
	5×10^1	0	0	0	0	25	0	25	0	50	25
	5×10^0	0	0	0	0	0	0	0	0	50	0
	Water	0	0	0	0	0	0	0	0	0	0
Hypodermic	5×10^5	50	0	100	75	100	100	100	100	100	100
	5×10^4	25	0	75	50	100	100	100	100	100	100
	5×10^3	0	0	75	0	100	25	100	50	100	50
	5×10^2	0	0	0	0	75	0	100	25	100	25
	5×10^1	0	0	0	0	0	0	25	0	25	0
	Water	0	0	0	0	0	0	0	0	0	0

height (Table 2). There was severe stunting in plants dipped in the two heaviest suspensions, but none at the lightest concentration (5×10^0). The effect was not completely uniform within treatments, however, nor was it quite a straight-line relationship.

There was no accurate way to determine the number of conidia lodging on each plant, although in the concentration 5×10^0 it may have been as few as one to five spores. Schnathorst (8) noted a direct relationship between concentration of inoculum and symptom severity in cotton plants dipped in a logarithmic series of suspensions containing from 10^6 to 10^2 conidia per ml. He did not report symptoms at the lowest concentration nor estimate the minimum number of conidia required to establish *Verticillium* infection in cotton. Correlations have also been reported between numbers of *Verticillium* microsclerotia per g of soil, and speed of appearance and severity of symptoms in potatoes (10) and mint (4).

Hypodermic inoculation.—The time required for development of leaf symptoms in 3-wk-old sunflower plants inoculated by hypodermic injection was directly related to concentration of inoculum (Table 1). Symptoms were less severe, developed more slowly, and on fewer plants of resistant CM 144 than on susceptible CM 162.

Plants of CM 144 in the respective series showed little difference in height, so were not measured. Inoculation with heavy suspensions markedly reduced the height of CM 162 plants; the degree of stunting bore a straight-line relationship to concentration of inoculum (Table 2), and was uniform within treatments. Plants inoculated with the lightest concentration were slightly but not significantly taller than the noninoculated controls. Light and incompatible infections with *Verticillium* have been reported to stimulate growth of some plants; isolate V-58 was one of the cultures found to have some gibberellin-like activity (1).

TABLE 2. Effect of inoculum concentration on stunting of sunflowers (CM 162) induced by Verticillium infection

Method of inoculation	Inoculum concentration (conidia/ml)	Average height of plants 21 days after inoculation (% of noninoculated controls)
Root dip	5×10^5	46
	5×10^4	45
	5×10^3	65
	5×10^2	91
	5×10^1	91
	5×10^0	100
	Water	100
Hypodermic	5×10^5	54
	5×10^4	66
	5×10^3	73
	5×10^2	84
	5×10^1	105
	Water	100

As each plant was inoculated with 0.02 ml of suspension, those in the series with 5×10^1 conidia per ml received on the average only one spore. Allowing for nonuniform distribution of spores in the suspension, the occurrence of symptoms in 25% of the plants in this series indicates that infection by one spore may be enough to induce disease. Inoculation with an average of 10 spores per plant in the 5×10^2 series resulted in uniform infection and conspicuous stunting.

This uniformity and predictability of infection is extremely important in our studies on factors influencing disease development, particularly because the space available in growth cabinets often limits us to four or even fewer pots per treatment in any one replicate. It is equally important for the work in other laboratories.

The hypodermic inoculation method has additional advantages. It avoids the transplanting

shock suffered by plants inoculated by root dip. Hoes (3) found that it permitted differentiation of high, moderate, and low resistance of sunflowers to *Verticillium*, whereas root dip distinguished only between high and low levels. Adjusting concentration of inoculum to the appropriate dilution would almost certainly permit even finer discrimination.

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