Relationship of Numbers of Zoospores of Phytophthora cryptogea to Infection and Mortality of Watercress

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ABSTRACT

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Suspensions of motile zoospores were added to individual, 2-wk-old watercress plants growing in flooded vermiculite. Percentages of infection after 7 days at 25 C in a growth room were 18, 26, 46, 56, 65, 81, and 100 at 50, 100, 200, 300, 500, 10^3, and 10^4 zoospores per plant. After 21 days percentages of dead plants were 30, 51, 67, and 81 at 10^3, 10^4, 10^5, and 10^6 zoospores per plant.

Additional key words: inoculum density, *Nasturtium officinale*.

Phytophthora cryptogea Pethyb. & Laff. causes a severe stem and root rot of watercress (*Nasturtium officinale* R. Br.) when it is grown commercially in central Florida in the summer (14). The continuous flow of water from artesian springs through shallow beds provides an efficient means of dissemination of zoospores of *P. cryptogea*. Dispersal of zoospores of other plant pathogenic phycomycetes or their secondary structures such as encysted zoospores, germ tubes, or microsporangia over distances greater than a few centimeters generally is dependent on moving water (5, 6, 7, 17).

Although information is available on the numbers of zoospores required for infection of plants or plant parts in vitro (1, 4, 5, 6, 10, 13, 15) and for levels of infection of plants after zoospores are added to sand or soil (3, 11, 12, 16), the literature is devoid of information on the quantities of zoospores required to infect specific numbers of plants under flooded conditions. The objective of this study was to determine quantitatively the relationship between numbers of zoospores of *P. cryptogea* and infection and mortality of watercress.

MATERIALS AND METHODS

The culture of *P. cryptogea* used in this study was isolated from the rotted stem of a commercially-grown watercress plant. It was maintained on V-8 juice agar and was transferred monthly.

Zoospores were produced by a method described previously (14) which consisted of flooding 7-day-old V-8 juice agar cultures with deionized water and incubating the cultures for an additional 7 days at 25 C under fluorescent light (2,000 lx at the level of the cultures). Zoospore suspensions were maintained at a stable pH of 6.2 throughout this study by the addition of 10^{-4}M 2-(morpholino)-ethanesulfonic acid (MES) as a buffer to deionized water; pH was adjusted to 6.2 with 1 N KOH. Four 3-ml samples of the zoospore suspension were agitated in test tubes for 60 sec on a Vortex mixer to induce motile zoospores to encyst (18). Concentrations of zoospores were determined by counting five fields for six samples from each of the four tubes on a standard hemacytometer. Since the preparation of dilutions leads to a reduction in the percentage of motile zoospores (2, 7), the concentration of motile zoospores in each dilution was determined. Eight 5- or 20-μliter drops from each dilution were placed on a glass slide coated with water agar, and the number of motile and nonmotile spores in each drop (usually one to five zoospores per drop) were counted microscopically.

Rooted cuttings of watercress were grown from 5-cm terminal stem segments from healthy stock plants. Each cutting was rooted in 5 g of vermiculite packed in a 50-ml polypropylene beaker (4.2 cm in diameter) that had three small holes at the base for water movement. The cuttings were watered daily and each plant was fertilized biweekly with 5 ml of Hoagland's solution (8). After 14 days in growth rooms at 25 C with 12 hr of light (4,000 lx at the level of the plants), the cuttings had developed large root systems and were about 10-15 cm tall. Fifteen beakers were placed in each of two nylon pans for each treatment; the pans were filled with a 10^{-4}M MES solution to a level that provided about 1 cm of standing solution above the surface of the vermiculite. In later experiments the trays were flooded with tap water because no differences in infection were obtained with tap or buffered-deionized water. One ml of 10^{-4}M MES solution containing various concentrations of zoospores was added to each beaker through a wide-mouth pipet. In an effort to maintain the highest possible level of motility, the zoospore suspensions and the trays of flooded watercress plants were maintained at approximately 20 C in cold water baths.

Zoospore motility and germination percentages were determined after each inoculation time by placing 15, 5-μliter drops from each of several dilutions on each of 10 petri plates of a selective medium which contained 10 mg

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pimaricin (Delvocid, 50%, Gist-Brocades N.V., Delft, Holland), 250 mg ampicillin (Polycillin-N, 81%, Bristol-Meyers Co., Syracuse, NY 13201), 10 mg rifampicin (Rifamycin SV, 100%, Sigma Chemicals Co., St. Louis, MO 63178), and 17 g Difco cormeal agar in 1 liter of deionized water. The drops were observed microscopically for numbers of motile zoospores; the plates were subsequently incubated for 48 hr in the dark at 28 C and observed for the number of colonies growing from drops that had contained zero to two (average of one) zoospores per drop.

The trays were maintained in the flooded condition for 3 hr after the addition of the zoospores. The water then was drained through the vermiculite to prevent the production of secondary inoculum on infected stems. The plants were returned to the growth room for 7 days to allow time for the establishment of the fungus in host tissue or 21 days to provide time for the infected plants to die. The vermiculite was maintained in a moist condition by retaining a shallow level of water in the bottom of the trays.

At the termination of each experiment, the entire root system plus about 3 cm of the stem of each plant was washed in running tap water, dipped in 70% ethyl alcohol for 15 sec, rinsed three times in sterile deionized water, dried on paper towels, and plated on the selective medium. The plates were observed for growth of *P. cryptogea* from the roots or stems after 48 hr incubation in the dark at 28 C. Plants were considered dead when roots and stems were decayed and the tops were yellow and permanently wilted. The data presented in this paper are means of experiments with 30 plants for each of 10 inoculum levels per experiment; the experiment was repeated six times.

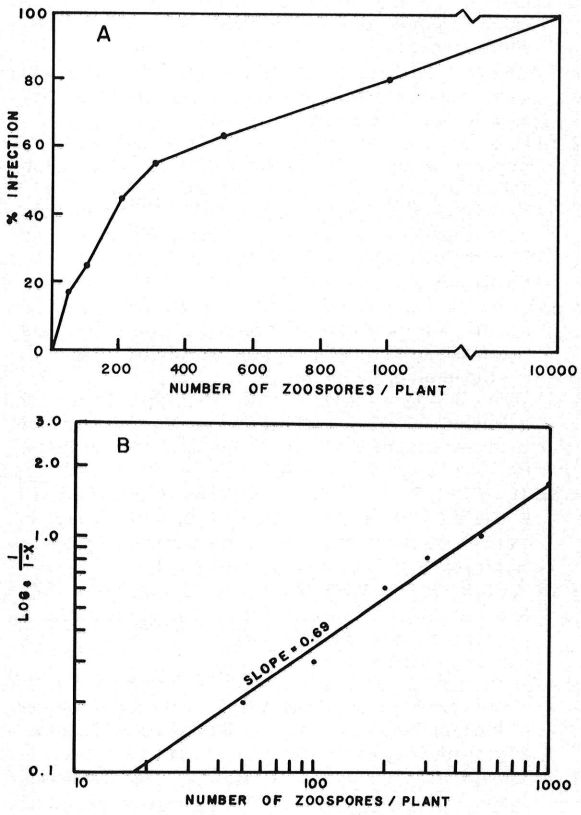

Fig. 1-(A, B). The relationship of incidence of infection in watercress plants to concentration of zoospores of *Phytophthora cryptogea* per plant: **A)** percentage infection (arithmetic) and zoospore concentration (arithmetic); **B)** percentage infection adjusted for multiple infections (logarithmic) and zoospore concentration (logarithmic).

RESULTS

Many of the zoospores observed for motility in 5- or 20-μliter drops encysted soon after they were placed on the selective medium, but at least 72 to 90% of the zoospores added to the flooded beakers in different tests were motile. After 24-48 hr of incubation, small colonies of *P. cryptogea* grew from 84 to 93% of the drops that had contained approximately one zoospore per drop.

Percentages of infection and mortality of watercress cuttings increased with increasing levels of zoospores per plant, but much higher levels of zoospores were required to kill the plants than were required to initiate infection (Fig. 1-A, Table 1). Only 18% of the plants were infected after exposure to 50 zoospores per plant, and 100% of the plants were infected after exposure to 10^4 zoospores per plant. No changes in mortality occurred when the plants were held for 6 wk instead of 3 wk.

When percentages of plants infected were transformed to $\log_e 1/(1-x)$ to adjust for multiple infections (19) and $\log_{10}$ [$\log_e 1/(1-x)$] was plotted against $\log_{10}$ of the number of zoospores per plant, points lay in a straight line between 50 and 10^3 zoospores per plant (Fig. 1-B). The slope determined by linear regression analysis was 0.69, and the number of zoospores required to infect 50% of the cuttings (ID_{50}) was interpolated to be 276 zoospores per plant. Approximately 10^4 zoospores per plant, however, were required to kill 50% of the plants (Table 1).

DISCUSSION

Infection increased as inoculum increased in this study, but the ratio of infection to inoculum decreased as the amount of inoculum increased. Van der Plank (19) interpreted such a relationship to represent infection without interaction between spores but with competition for susceptible sites on the host. Although zoospores of several pythiaceous fungi are attracted to and aggregate on certain sites of plant roots and stems (5, 6, 10), it is probable that the lack of direct proportionality of infection to disease is attributable to several or many

TABLE 1. The effect of concentrations of zoospores of *Phytophthora cryptogea* on percentages of infection and mortality of watercress plants 7 and 21 days, respectively, after inoculation

Zoospores/plant[a]	Infection (%)	Mortality (%)
10^3	83	30
10^4	100	51
10^5	100	67
10^6	100	81

[a]Thirty plants inoculated with each zoospore concentration.

factors. The slope of 0.69 thus may reflect not only competition for susceptible sites but also factors that reduce the dissemination, duration of motility, or survival of zoospores.

Zoospores are induced to encyst by such factors as collisions with zoospores or solid surfaces, changes in hydrogen ion concentration or temperature, variations in water currents (rheotaxis), and variations in nutrients or ions (2, 4, 5, 6, 7, 9). Since some or all of these factors may be involved in the inoculation of plants and since fewer motile than encysted zoospores are required for the same incidence of infection (1, 11), extreme care must be used in attempting to determine the numbers of motile zoospores that are required for specific levels of infection. Although inoculations in this study were completed as rapidly as possible and factors that induce encystment of zoospores (eg changes in pH or temperature, or rigorous handling of dilutions) were avoided, up to 30% of the zoospores had encysted in some of the tests by the time they were added to the flooded beakers. Since the beakers were drained after 3 hr to prevent the production of secondary inoculum, it is assumed that, although the encysted zoospores rarely acted in stem infection, they were still infectious if they were carried by draining water to positions where germ tubes could reach susceptible roots. *Phytophthora cryptogea* grew from roots of some of the infected plants on the selective medium, but the main points of infection in over 90% of the infected plants were located on the stems at or near the level at which the surface of the water had been maintained during inoculation. Thus the data are assumed to reflect the approximate numbers of motile zoospores required for various percentages of infection under flooded conditions.

It is probable that death of plants resulting from infection by zoospores of *Phytophthora* spp. involves one or more of three basic mechanisms: (i) rapid, severe disease development caused by initial infection by large numbers of zoospores (probably 10^3 zoospores per plant or more); (ii) initial infection by low levels of zoospores and a subsequent slow development of disease; or (iii) infection by low levels of zoospores with secondary spore production and massive secondary infection of the same plant. The results of this study are in general agreement with work with *P. parasitica* var. *nicotianae* on tobacco (3), *P. palmivora* on papaya (11, 12, 16), and *P. citrophthora* on milkweed vine (D. Mitchell, *unpublished*) in which 10^4 to 10^5 zoospores per container of plants growing in sand or soil were required to approach 50% mortality of host plants. It is improbable, especially in flowing water, that such high levels of initial zoospore inoculum would be amassed around the stems of individual plants in the field, and thus the latter two mechanisms are more likely to occur under natural conditions. When zoospores are added in drops of water directly to susceptible plant tissues such as cotyledons, hypocotyls, stems, or roots, infection may result from one or a few zoospores per drop (1, 5, 10, 13, 15). Greater numbers of zoospores are required for infection of such tissues in soil or floating in water (1, 4, 5); Halsall (4), for example, found that more than 400 zoospores of *P. drechsleri* were required to infect 50% of eucalypt cotyledons floating on the surface of zoospore dilutions. The present study demonstrates that almost 20% of the watercress plants exposed to 50 zoospores per plant were infected, and 276 zoospores per plant were required for 50% infection.

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