Inoculation and Evaluation of Strawberry Plants with *Phytophthora fragariae*

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ABSTRACT

George, S. W., and Milholland, R. D. 1986. Inoculation and evaluation of strawberry plants with *Phytophthora fragariae*. Plant Disease 70:371-375.

Strawberry plants were inoculated effectively by spraying the roots with nonmotile (encysted) zoospores at 3×10^4/ml. Oospore formation in the stele was used to measure disease development. Infection increased on the susceptible cultivar Tennessee Beauty as inoculum density was increased, but the cultivars Stelemaster and Surecrop remained resistant even when inoculated with 10×10^4 zoospores per milliliter. The optimum temperature range for both zoospore germination on host roots and oospore development in host tissue was 15–20 C. A temperature of 25 C encouraged zoospore germination but greatly inhibited oospore formation. Both pathogen race and duration of flooding significantly influenced zoospore production in culture.

Red stele disease of strawberry (*Fragaria* × *ananassa* L.) incited by *Phytophthora fragariae* Hickman is a major factor limiting strawberry fruit production in many areas of North Carolina and around the world (13). The disease was found in 30 strawberry plantings in 15 counties of North Carolina in 1979 and 1980.

Symptoms on naturally infected roots include reddening of the stelar tissue and necrosis of the root tissue progressing upward from the tips. Infected plants are dwarfed, produce few runners and little or no fruit, and eventually die (1).

Various methods of inoculation have been employed to study the red stele disease, including planting in naturally infested soil, adding infected roots to the growing medium, dipping roots in zoospore or mycelial suspensions, and placing blocks of mycelium directly on the roots (1,9). Disease assessment procedures for red stele disease are generally subjective (2,9) and therefore frequently inconsistent. *P. fragariae* has been associated with reddened steles in strawberry roots since 1940 (6); however, it has never been shown that the fungus produces a specific substance that can induce this stelar reddening. Furthermore, Otterbacher et al (12) have shown that stelar reddening occurred in roots of both resistant and susceptible strawberry cultivars with application of sterile fungal filtrates, sterile basal medium, a streptomycin-captan mixture, and even distilled water. Although reddening of the stele in field-grown plants may be a preliminary diagnostic feature, the use of stelar discoloration as a major criterion to assess susceptibility may have contributed to the frequent variability observed in partially resistant plant material and the variation between successive pathogenicity tests of the same isolates on the same plant cultivars (2,9).

Montgomerie (8) reported that isolates varied in the rate at which sporangia were formed in culture, with one isolate requiring 7–8 days of flooding before a sufficient titer of zoospores was achieved. Peak zoospore production for *P. fragariae* race A-2 has been reported to occur 72 hr after flooding (7). The effect of producing mycelial mats in liquid rather than solid medium and extended periods of flooding on zoospore production of the predominant races of *P. fragariae* that occur in the eastern United States has not been reported.

Our studies were conducted to determine the effect of liquid culture and incubation period in soil leachate on zoospore production of the five major races in the eastern United States and to develop reliable inoculation and disease rating techniques to study host-pathogen interactions and to screen strawberry plants for resistance. The effect of temperature on zoospore germination, infection, and oospore development was also observed. Preliminary studies comparing inoculation methods with *P. fragariae* on strawberry have been published (4).

MATERIALS AND METHODS

Isolates of *P. fragariae*. Cultures of *P. fragariae* were obtained from two sources. Four isolates representing American races A-1, A-2, A-3, and A-4 were obtained from the American Type Culture Collection as ATCC 13973, 13974, 13977, and 11109, respectively, and a fifth isolate, race A-6, was kindly supplied by J. L. Maas, Beltsville, MD. Stock cultures were maintained in the dark on frozen lima bean agar (FLBA) at 20 C.

Paper 9932 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh 27695-7601. Portions of a thesis submitted by the first author in partial fulfillment of the requirements for the Ph.D. degree, North Carolina State University.

Accepted for publication 23 October 1985 (submitted for electronic processing).

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Zoospore production. Four 7-mm-diameter disks of mycelium were removed from the margins of 2- to 3-wk-old cultures on oatmeal agar and placed in 50 ml of autoclaved 4% V-8 juice broth in 250-ml flasks. Flasks were placed under continuous incandescent light (about 2,700 lux) at 11 C for 15 days. The broth was decanted, and the mycelial mats from each flask were transferred to a petri dish (100 × 20 mm) to which 20 ml of unsterile soil leachate was added. This soil leachate was decanted and replaced with an additional 20 ml of soil leachate after 24 hr. The soil leachate was prepared by placing 25 g of a loam topsoil in 2.5 L of deionized water, which was stored at 23 C for 3 days with daily stirrings, then filtered through a single piece of coarse filter paper just before use. Four petri dishes of each of the five isolates (ATCC 13973, 13974, 13977, 11109, and A-6) were evaluated 2, 5, 7, and 11 days after addition of the soil leachate for zoospore production. Zoospores in two separate samples from each dish were counted with a hemacytometer. One drop of 1% aqueous crystal violet stain was mixed with 1 ml of zoospore suspension to aid in counting. The experiment was repeated using mycelial disks from 2-wk-old cultures on FLBA.

Inoculation methods. Ten plants of the susceptible strawberry cultivar Tennessee Beauty were inoculated with a mixture of races A-2 (ATCC 13974) and A-6 of *P. fragariae* by one of the following procedures. Roots were immersed in a suspension of motile zoospores (3×10^4 zoospores per milliliter) for 48 hr at 13 ± 2 C in the light, immersed in a mycelial slurry of 3-wk-old FLBA cultures, or sprayed with a suspension of motile or nonmotile (encysted) zoospores (3×10^4 zoospores per milliliter). The mycelial slurry was prepared from 3-wk-old FLBA cultures (single culture of each race) blended with 60 ml of deionized water for 10 sec. The root systems of uninoculated control plants were either dipped in a slurry of FLBA or sprayed with deionized water. Nonmotile zoospores were obtained by placing 20 ml of a motile zoospore suspension in a test tube and allowing the suspension to vibrate for 1 min on a Vortex Jr. mixer (model K-500-J). The plants were placed on a paper towel saturated with deionized water, and the encysted zoospores were sprayed over the root system with a DeVilbiss atomizer (about 1.5 ml of inoculum applied to each plant). Plants receiving the mycelial treatments were transplanted immediately, whereas those sprayed with zoospores were wrapped in the toweling and placed in clear-plastic bags secured with twist ties, then placed under fluorescent light at 13 ± 2 C for 24 hr before planting. Plants in all treatments were planted in round, clear-plastic containers (25 × 9 cm) filled to a depth of 7.5 cm with Metro-Mix 220 (W. R. Grace & Co., Cambridge, MA) that had been saturated with tepid tap water and the excess drained off. Each container was placed inside a plastic bag under continuous fluorescent light at 13 ± 2 C. Roots were examined for oospore development after 2 wk. Analysis of variance (ANOVA) procedures were employed to calculate the protected LSDs used in mean separation. This test was then repeated with race A-6, minus the mycelial slurry treatment, using 12 (10-wk-old) Tennessee Beauty plants per treatment.

All treatments evaluated in these studies were arranged in a completely randomized design unless otherwise stated.

An additional test was conducted to determine the effect of centrifugation on zoospore encystment, germination, infection, and oospore development. Roots of six 4-wk-old Tennessee Beauty plants were inoculated with race A-6 of *P. fragariae* by immersion in a motile zoospore suspension (2.2×10^4 zoospores per milliliter), or the roots were sprayed with a suspension of nonmotile zoospores that had been either vortexed (2.6×10^4 zoospores per milliliter) or centrifuged (1.4×10^4 zoospores per milliliter). A suspension of 0.4×10^4 motile zoospores per milliliter was centrifuged at 3,300 rpm (1,840 *g*) for 1.5 min. After centrifugation, the suspension (1.4×10^4 zoospores per milliliter) was used as inoculum. Temperature ranged from 12 to 20 C during the 14-day incubation period. Six uninoculated plants were used as controls.

Inoculum density. The efficacy of different concentrations of zoospores was evaluated by spraying the roots of susceptible (Tennessee Beauty) and resistant (Stelemaster) cultivars with a suspension of 1×10^3, 5×10^3, and 10×10^3 nonmotile zoospores per milliliter of race A-6. The same procedures were used for inoculating and planting as described before. Temperatures ranged from 12–18 C. Five plants of each cultivar were inoculated with each of the three concentrations tested.

The influence of inoculum density on infection and oospore development in the cultivars Tennessee Beauty and Stelemaster was also compared at concentrations of 10×10^3, 30×10^3, and 100×10^3 nonmotile zoospores per milliliter. Temperature ranged from 12 to 18 C for the 14-day incubation period. Ten plants of each cultivar were inoculated with each of the three concentrations tested. In addition, 10 plants of the resistant cultivar Surecrop were inoculated with 100×10^3 nonmotile zoospores per milliliter.

ANOVA procedures were employed to analyze overall differences between cultivars. Linear regression was used to analyze the reactions of individual cultivars to changes in inoculum density.

Effect of temperature on zoospore germination. Zoospores of race A-6 were vortexed for 1 min, then sprayed onto nine microscope slides with a DeVilbiss atomizer. Three slides were placed in a moist chamber at 8, 14, and 20 C under continuous incandescent light (about 2,700 lux). Two drops of aqueous crystal violet were added to each slide after 24 hr, and 100 zoospores per slide were examined for germination.

Excised root segments 15 mm long from 3-wk-old Tennessee Beauty plants were placed on a microscope slide, and the roots were sprayed with nonmotile zoospores (11×10^4/ml) of race A-6. Three slides were placed inside each moist chamber at 5, 10, 15, 20, and 25 C under continuous incandescent light (about 2,700 lux). Aqueous crystal violet was injected under the coverslip after 24 hr, and 100 zoospores per slide on or near the root segments were examined for germination.

Effect of temperature on oospore development. Roots of 24 (9-wk-old) Tennessee Beauty plants were inoculated with either nonmotile zoospores (8.6×10^4/ml) or a mycelial slurry of race A-6 (one 80-mm-diameter colony per 40 ml of deionized water) using the procedure described previously. Three plants were sprayed with nonmotile zoospores and incubated at each of five temperature regimes: 15, 20, and 23 C for 14 days or 15 C for 2 or 7 days followed by 20 C for 12 or 7 days. Three plants were dipped in a mycelial slurry and maintained at each of three temperature regimes; 15 and 20 C for 14 days or 15 C for 7 days followed by 20 C for 7 days.

A second test was conducted with roots of 10-wk-old Tennessee Beauty plants using the same procedures except that 3.2×10^4 zoospores per milliliter were used. Thirty plants were sprayed with nonmotile zoospores and six plants each were incubated at 5, 10, 15, 20, and 25 C for 14 days. The same number of plants was dipped in a mycelial slurry and incubated at 5, 10, and 15 C for 14 days, or they were placed at 10 and 15 C for 7 days, then 20 C for 7 days. Six uninoculated plants placed at each temperature regime served as the controls.

Disease evaluations. Root tissue from each applicable study was examined for oospore development 14 days after inoculation. Plants were removed from containers, roots washed, and 10 discolored (i.e., light brown) root tip segments 6–8 mm long were removed from each plant and mounted on a slide, then the coverslip (60 × 22 mm) was sealed with Permount (Fisher Scientific Co.). Visual observations of reddened steles and microscopic observations of the number of oospores per root segment as well as percentage of root segments containing oospores were recorded.

RESULTS

Zoospore production. Both race and duration of flooding influenced zoospore production (Table 1). Race A-6 produced the greatest number of motile zoospores, whereas race A-3 produced the fewest. Incubation in soil lechate for 5 days resulted in the greatest zoospore production for race A-6, whereas 7 days was required for maximum production by races A-1 and A-2 and 11 days for races A-3 and A-4. Similar results were obtained when the experiment was repeated with 2-wk-old cultures on FLBA.

Inoculation methods. Inoculation of strawberry plants by dipping the roots in a suspension of motile zoospores and spraying the roots with a suspension of motile zoospores gave poor results (Table 2). However, the nonmotile or encysted zoospore inoculum gave the greatest percentage of root segments containing oospores and was just as effective as the mycelial slurry treatment in the number of oospores per root segment. When the experiment was repeated, minus the mycelial slurry treatment, the mean number of oospores per root segment and the percentage of root segments containing oospores with nonmotile and motile zoospore sprays and the motile zoospore dip were 18 and 43%, 4 and 17%, and 8 and 18%, respectively.

Inoculating strawberry roots with encysted zoospores obtained by centrifugation also produced excellent results. The mean number of oospores per root segment and the percentage of root segments containing oospores with centrifuged zoospores was 55 and 78%, respectively. The mean number of oospores per root segment and the percentage of root segments containing oospores for the nonmotile (vortexed) zoospore spray and the motile zoospore dip treatments were 55 and 92% and 92 and 87%, respectively. Oospores were not observed in the uninoculated control plants.

Inoculum density. Oospore development by *P. fragariae* in roots of susceptible strawberry plants varied with inoculum density (Table 3). There was no significant difference in oospore production or percentage of root segments containing oospores when Tennessee Beauty and Stelemaster were inoculated with 1, 5, or 10×10^3 zoospores per milliliter. The mean numbers of oospores per root segment for Tennessee Beauty and Stelemaster inoculated with 1, 5, or 10×10^3 zoospores per milliliter were 0.1 and 0.2, 3 and 0, and 6 and 0, respectively. The percentage of root segments containing oospores for these two cultivars at the same concentrations were 2 and 2, 6 and 0, and 11 and 0, respectively. In the second test, however, ANOVA revealed significant differences in oospore development between cultivars. For example, the mean number of oospores produced when susceptible Tennessee Beauty plants were inoculated with 10, 30, or 100×10^3 zoospores per milliliter was significantly greater ($P = 0.01$) than when resistant Stelemaster plants were inoculated with the same concentrations of zoospores. The percentage of root segments containing oospores was also greater ($P = 0.05$) for Tennessee Beauty plants. Linear regression analyses indicated that concentration of inoculum had no significant effect on mean oospore production in the susceptible or resistant cultivars, but differences ($P = 0.01$) did occur in the percentage of susceptible root segments containing oospores among the three concentrations tested. When the resistant cultivar Surecrop was inoculated with 100×10^3 nonmotile zoospores per milliliter, oospore development was similar to that in Stelemaster.

Effect of temperature on zoospore germination. Temperature and substrate had a significant influence on zoospore germination. Percent germination of zoospores placed on a glass slide at 8, 14, and 20 C was 26, 40, and 30, respectively. Zoospores applied to root segments had a greater percent germination than those placed on a glass slide at about the same temperature. Zoospore germination on or adjacent to strawberry root segments at 5, 10, 15, 20, and 25 C was 12, 79, 78, 80, and 83%, respectively.

Effect of temperature on oospore formation. A constant temperature of 15 C was optimum for oospore development when encysted zoospores were applied to roots of a susceptible strawberry cultivar (Table 4). Single degree of freedom orthogonal contrasts revealed that both production and percentage of root segments with oospores were significantly greater ($P = 0.01$) when nonmotile zoospores rather than a mycelial slurry were used to inoculate strawberry roots.

In the second test, 15 C was also

Table 1. Zoospore production by five races of *Phytophthora fragariae* after incubation in a soil leachate for different periods of time

	Race				
Days	A-1	A-2	A-3	A-4	A-6
2[a]	7.6[b]	19.5	0.1	0.1	41.5
5	14.6	26.4	0.3	2.9	72.3
7	16.0	53.1	0.5	6.8	41.3
11	5.0	19.0	1.8	7.4	26.0

[a] Number of days mycelial mats were flooded with nonsterile leachate.
[b] Number of zoospores per milliliter $\times 10^3$; data averaged over two runs of the experiment for a total of eight plates for each race/day combination.

Table 2. Influence of inoculation method with *Phytophthora fragariae* on oospore development in roots of the susceptible strawberry cultivar Tennessee Beauty[a,b]

Treatment	Oospores per root segment (no.)	Root segments containing oospores (%)
Nonmotile zoospore spray[c]	35.00 j	80 x
Motile zoospore spray	0.02 k	2 y
Mycelial slurry dip	48.00 j	64 x
Motile zoospore dip	1.00 k	10 y

[a] Data are means of 10 plants per treatment (five root segments per plant). Inoculum concentration was 3×10^4 zoospores per milliliter. Root segments were 6–8 mm long.
[b] Means in the same column followed by the same letter are not statistically different ($P = 0.05$). Protected LSD for mean number of oospores per root segment and percentage of root segments containing oospores is 22.7 and 20.9, respectively.
[c] Zoospores were made to encyst (nonmotile) by vortexing suspension for 1 min.

Table 3. Influence of inoculum density of *Phytophthora fragariae* race A-6 on oospore development in strawberry roots of susceptible (Tennessee Beauty) and resistant (Stelemaster) cultivars 14 days after inoculation with encysted zoospores[a]

	Oospores per root segment (no.)		Root segments containing oospores (%)	
Zoospores/ml ($\times 10^3$)	Tennessee Beauty[b]	Stelemaster[b]	Tennessee Beauty[c]	Stelemaster[b]
10	17	0.2	19	8
30	22	0.6	36	9
100	29	0.9	51	16

[a] Data are means of 10 plants per treatment (10 root segments per plant).
[b] Slope of linear regression model was not different from zero ($P = 0.05$).
[c] Slope of linear regression model was different from zero ($P = 0.01$).

optimum for oospore production and percentage of root segments containing oospores. The mean numbers of oospores per root segment that developed 14 days after inoculation with encysted zoospores at 5, 10, 15, 20, and 25 C were 0, 5, 29, 12, and 0.02, respectively. Percentages of root segments containing oospores for these same temperatures were 0, 23, 40, 37, and 2, respectively. The data for mean oospore production and percentage of root segments containing oospores showed significant quadratic trends ($P = 0.072$ and $P = 0.052$, respectively). The extreme temperatures (5 and 25 C) were both highly detrimental to oospore formation in inoculated strawberry roots. No oospores developed in uninoculated control plants or in plants dipped into a mycelial slurry of *P. fragariae*.

Disease evaluations. Evaluating disease development by means of oospore formation in strawberry roots proved to be an effective procedure not only to compare methods of inoculation with *P. fragariae* but also to determine the influence of inoculum densities and temperature on oospore development (Fig. 1).

Results of greenhouse inoculation studies revealed that of the more than 500 root tip segments examined that had reddened steles, 70% did not contain oospores. Furthermore, of the 142 root tip segments containing oospores 14 days after inoculation, 78% had no stelar discoloration.

DISCUSSION

Zoospore production of the five isolates of *P. fragariae* increased when mycelial mats were grown in liquid rather than solid media coupled with extended incubation periods in soil leachate. Researchers have reported problems with consistent production of zoospores by *P. fragariae* (3,11), and the results in some of our preliminary studies with various solid media, using modifications of the method of Mussell and Fay (11), were equally unreliable. During these preliminary studies, mycelial growth from FLBA disks was certainly adequate, but very few sporangia and zoospores were produced, even with extended periods of incubation in soil leachate that was changed daily.

Montgomerie (8) found that some isolates required several days of flooding before sufficient numbers of zoospores were produced. Differences in zoospore production by various races of *P. fragariae* have also been reported by other investigators (7,13). Maas (7) reported that race A-2 reached maximum zoospore production after 3 days of flooding, whereas all isolates in our study required a minimum of 5 days of incubation in soil lechate for maximum production. This difference in incubation requirements could be due to the type of media or composition of flooding liquid.

A real need has existed for an inoculation method that was both reliable and that would permit more accurate quantification of inoculum densities. Strawberry roots are usually inoculated by dipping them in a mycelium-agar slurry or a suspension of motile zoospores (3,5,10). However, neither method permits accurate quantification of the number of propagules reaching individual roots. Furthermore, Draper et al (3) found infection by motile zoospores produced in culture to be inconsistent, with almost complete failures in some years. In our studies, when roots of strawberry plants were immersed in a motile zoospore suspension, results were also erratic. If roots were sprayed with a suspension of motile zoospores, consistently poor results were obtained. Excellent results were obtained, however, when roots were sprayed with a suspension of nonmotile (vortexed) zoospores. A similar method employing nonmotile (centrifuged) zoospores also showed promise, particularly for races such as A-3 that are known to be poor producers of zoospores. Both of these inoculation methods permit much more accurate quantification of inoculum densities applied to individual roots than do previously employed methods.

Goode (5) reported that zoospore attraction, encystment, and penetration of epidermal cells was similar in susceptible and resistant strawberry cultivars. However, penetration does not progress beyond the epidermal layer except in the susceptible cultivars. Thus, spraying inoculum on the roots of susceptible and resistant cultivars is an entirely appropriate technique to study cultivar resistance, the influence of inoculum density, or other host-pathogen studies.

The study with defined concentrations of inoculum of *P. fragariae* indicated that infection increased as inoculum density was increased, which agreed with previous reports (7,8); however, there was no breakdown of resistance even at the highest inoculum concentrations tested.

Exudates from excised roots were probably responsible for the increase in germination observed for the zoospores applied to the root segments. The optimum temperature range for both zoospore germination and oospore development was 15–20 C. A temperature of 25 C also encouraged zoospore germination but greatly inhibited oospore development.

Montgomerie (9) discussed the undesirable variability frequently observed within and between experiments when screening strawberry plants for resistance to *P. fragariae* and the need for more controlled screening procedures. A disease rating scheme based primarily on root necrosis and extent of reddened steles has been the most commonly employed method to evaluate red stele disease for the past two decades (2). This rating system is based, to a large extent, on the premise that disease is highly correlated with the presence and extent of reddened stelar tissue. Bain and Demaree

Table 4. Effect of inoculation method, temperature, and incubation period on oospore development in roots of susceptible strawberry cultivar Tennessee Beauty by race A-6 of *Phytophthora fragariae*

Temperature/ incubation period	Oospores per root segment[a] (no.)	Root segments containing oospores[a] (%)
Zoospores[b]		
15 C (14 days)	89	87
20 C (14 days)	41	83
23 C (14 days)	43	63
15 C (2 days) + 20 C (12 days)	32	77
15 C (7 days) + 20 C (7 days)	28	47
Mycelium[c]		
15 C (14 days)	7	10
20 C (14 days)	1	7
15 C (7 days) + 20 C (7 days)	14	27

[a] Means based on three plants per treatment (10 root segments per plant). Root segments examined were 6–8 mm long.
[b] Inoculum concentration was 8.6×10^4 nonmotile zoospores per milliliter.
[c] Inoculum concentration was one 80-mm-diameter colony per 40 ml of water.

Fig. 1. Oospores of *Phytophthora fragariae* in the stelar tissue of the susceptible strawberry cultivar Tennessee Beauty 14 days after inoculation with encysted zoospores.

(1), however, stated that the most dependable proof of infection is the microscopic demonstration of oospores of *P. fragariae* in diseased strawberry roots. In February 1985, 343 field-grown strawberry plants (12 cultivars) suspected of being infected with *P. fragariae* were obtained from a North Carolina nursery and examined for red stele symptoms and oospore development (R. D. Milholland and R. K. Jones, *unpublished*). When about seven roots per plant were examined, 18% of these plants had reddened steles, but none of the roots with discolored steles contained oospores. In particular, 46% of the 50 Red Coat plants examined did show reddened steles, but none of the roots showed evidence of oospore development. Thus, although the presence of stelar reddening can be a useful preliminary indication of infection in field-grown plants, it is most important that microscopic confirmation of oospores of *P. fragariae* be used as positive proof of infection.

In addition to determining the number of oospores per root segment, we feel it is also very important to ascertain the percentage of root segments containing oospores. This adds greatly to the overall assessment of the resistance or susceptibilty of a cultivar. We also suggest that a cultivar known to be highly susceptible to the race(s) under consideration be included in each inoculation test to act as a base of reference, with percentages of the infection present in the known susceptible used in delimiting categories of resistance.

The method of resistance evaluation proposed herein should prove particularly useful in race determination and confirmation of the resistance or susceptibility of advanced breeding lines or known strawberry cultivars.

LITERATURE CITED

1. Bain, H. F., and Demaree, J. B. 1945. Red stele root disease of the strawberry caused by *Phytophthora fragariae*. J. Agric. Res. 70:11-30.
2. Converse, R. H., and Scott, D. H. 1962. Physiologic specialization in *Phytophthora fragariae*. Phytopathology 52:802-807.
3. Draper, A. D., Scott, D. H., and Maas, J. L. 1970. Inoculation of strawberry with *Phytophthora fragariae*. Plant Dis. Rep. 54:739-740.
4. George, S. W., and Milholland, R. D. 1985. A comparison of inoculation methods for evaluating strawberry plants for resistance to *Phytophthora fragariae*. (Abstr.) Phytopathology 75:500.
5. Goode, P. M. 1956. Infection of strawberry roots by zoospores of *Phytophthora fragariae*. Trans. Br. Mycol. Soc. 54:351-360.
6. Hickman, C. J. 1940. The red core root disease of the strawberry caused by *Phytophthora fragariae* n. sp. J. Pomol. Hortic. Sci. 18:89-118.
7. Maas, J. L. 1976. Stimulation of sporulation of *Phytophthora fragariae*. Mycologia 68:511-522.
8. Montgomerie, I. G. 1966. Pathogenicity of British isolates of *Phytophthora fragariae* and their relationship with American and Canadian races. Trans. Br. Mycol. Soc. 50:57-67.
9. Montgomerie, I. G. 1977. Red core disease of strawberry. Hortic. Rev. 5, Commonw. Agric. Bur., Farnham Royal. 47 pp.
10. Mussell, H., and Fay, F. E. 1971. A method for screening strawberry seedlings for resistance to *Phytophthora fragariae*. Plant Dis. Rep. 55:471-472.
11. Mussell, H., and Fay, F. 1973. Aseptic zoospore production by *Phytophthora fragariae*. Phytopathology 63:1081-1082.
12. Otterbacher, A. G., Powell, O., and Zych, C. C. 1969. The induction of stelar reddening in strawberry roots. Plant Dis. Rep. 53:794-797.
13. Wynn, W. K. 1968. Development of controlled conditions for the study of red stele disease of strawberries. Contrib. Boyce Thompson Inst. 24:95-102.