Production of Sporangia and Oospores of *Phytophthora fragariae* in Roots of Strawberry Plants

T. F. LAW, Research Technician, and R. D. MILHOLLAND, Professor, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616

ABSTRACT

Law, T. F., and Milholland, R. D. 1991. Production of sporangia and oospores of *Phytophthora fragariae* in roots of strawberry plants. Plant Dis. 75:475-478.

Four cultivars of strawberry with different levels of resistance to *Phytophthora fragariae* were inoculated with encysted zoospores of four races of the pathogen. Plants were sampled at 2-day intervals up to 14 days after inoculation, at which time roots were excised and placed in nonsterile soil leachate for an additional 2- to 10-day incubation. Production of sporangia by *P. fragariae* was greatest when roots of susceptible strawberry cultivar Tennessee Beauty were incubated for a total of 8 days at 15 C after inoculation with isolate NC-1 of race Pf-2. An incubation period of 2–4 days in nonsterile soil leachate was necessary to induce maximum numbers of sporangia. Increasing the incubation period in nonsterile soil leachate beyond 2–4 days did not increase the number of sporangia produced. Oospore production was observed 4–6 days after inoculation and increased as the incubation period increased to 12 days. Optimum temperatures for the production of sporangia of the four races of *P. fragariae* tested were 12–20 C depending on the isolate. All races of *P. fragariae* produced more sporangia on roots of a very susceptible strawberry cultivar than on either moderately susceptible or resistant cultivars.

Red stele disease of strawberry (*Fragaria* × *ananassa* Duchesne), caused by *Phytophthora fragariae* C. J. Hickman (7), is a major factor limiting fruit production in North Carolina and most northern states. Roots of infected plants become necrotic, with a characteristic reddening of the root vascular system or stele. Infected plants become stunted, with reduced runner and fruit production. Oospores, not chlamydospores, are the primary survival structure in the field (7,11).

There are at least seven pathogenic races of *P. fragariae* (10) separated by their ability to infect and produce oospores in roots of differential strawberry cultivars. Disease severity of susceptible strawberry cultivars was quantified by the number of oospores produced within infected roots (10). It is not known if host genotype also affects the number of sporangia produced on strawberry roots by races of *P. fragariae*.

Optimum temperatures for production of sporangia of isolates of *P. fragariae* on agar media range from 5 to 17 C (2,7,9,15). Quantitative studies to evaluate the influence of temperature on production of sporangia on strawberry roots have not been reported. Effect of incubation period on the production of sporangia and oospores by *P. fragariae* in strawberry roots also has not been determined.

In these studies, we report on the production of sporangia by *P. fragariae* in roots of strawberry plants as affected by incubation period, sites of infection, temperature, host genotype, and isolate. The temporal relationship between production of sporangia and oospores in strawberry roots is also presented.

The research reported in this publication was funded in part by the North Carolina Agricultural Research Service.

Use of trade names does not imply endorsement by the North Carolina Agricultural Research Service of the products named nor criticism of similiar ones not mentioned.

Accepted for publication 24 October 1990 (submitted for electronic processing).

MATERIALS AND METHODS

Inoculum sources. Zoospores of isolates NC-1, A-4, A-8, and A-10 of *P. fragariae*, representing races Pf-2, Pf-3, Pf-5, and Pf-7, respectively (10), were used as inocula. Inocula were produced according to the procedure of George and Milholland (4).

Plant material and inoculations. Strawberry cvs. Aberdeen, Climax, Surecrop, and Tennessee Beauty were used in the experiments. The susceptibility of these cultivars was determined in previous tests (10). Primary runner plants were removed from stock plants in the greenhouse, potted in 5-cm-diameter clay pots containing Metro-mix 220 (W. R. Grace & Co., Cambridge, MA), sand, and soil (1:1:1, v/v/v), and placed under intermittent mist at 25–30 C for 7–10 days to promote root formation. Plants were removed from the soil and roots were rinsed thoroughly in tap water and then spray-inoculated according to the procedure of Milholland et al (10). A suspension of nonmotile (encysted) zoospores at 1.8–2.0 × 10^4 zoospores per milliliter was used in each test to ensure that zoospores were deposited on the entire root (4). Because motile zoospores are attracted up to 40–50 mm from the root tip of young strawberry roots (15), this inoculation procedure seemed the most appropriate.

Effects of incubation period on the production of sporangia and oospores. Roots of the susceptible strawberry cv. Tennessee Beauty were spray-inoculated with isolate NC-1 of *P. fragariae* from North Carolina. Inoculated plants were placed in plastic bags in the dark at 15 C for 48 hr and then removed from the bags and planted into round plastic cylinders (25 cm wide × 9 cm deep) filled to a depth of 7.5 cm with Metro-mix 220 that had been saturated with tap water and the excess drained off. Plants were placed under fluorescent lights (80 $\mu E \cdot s^{-1} \cdot m^{-2}$, 12-hr photoperiod) at 15 C for up to 14 days. Three or four infected plants were removed from the cylinders at 2-day intervals and 10 root tips (8–10 mm long) per plant were excised and examined microscopically (×100) for oospores or eight root tips (50 mm long) per plant were excised and placed individually into each compartment of four-compartment 100-mm-diameter petri dishes. Each compartment contained 4 ml of nonsterile soil leachate, and plates were incubated for up to 10 days at 15 C to induce formation of sporangia. Soil leachate was prepared according to the procedure of George and Milholland (4). Mature sporangia containing zoospores on the periphery of each 10-mm section of entire 50-mm root segments were observed microscopically (×40) and counted every 2 days during the 10-day incubation period in soil leachate. Counts of sporangia were taken from three replicates for the experiment, each replicate consisting of two petri dishes containing four root tips (50 mm long). Sporangia data for each day after inoculation was analyzed by nested analysis of variance with replicate, petri dishes within replicate, roots within petri dishes and replicate, and distance from the root tip as factors. Oospore data were analyzed by analysis of variance in a completely random design with protected least square difference comparisons of the means. The experiment was repeated once with similiar results.

Temperature studies. Roots of Tennessee Beauty were inoculated with isolates

of races Pf-2, Pf-3, Pf-5, and Pf-7 of *P. fragariae*. Inoculated plants were incubated for 48 hr in plastic bags, and 10 root segments (8–10 mm long) were excised from each of three replicate plants per isolate, placed into individual petri dishes containing 5 ml of nonsterile soil leachate, and incubated at temperatures of 4, 8, 12, 15, 20, and 24 C. The number of sporangia per root segment was determined every 24 hr for 9 days as described earlier. The experimental design was a split-plot with temperature as the main plot factor and isolates within the chambers as subplots. Analysis of variance and orthogonal contrasts were performed on the counts of sporangia for isolates at the various temperatures. The experiment was replicated once over time.

Host genotype and isolate effects. Roots of cvs. Tennessee Beauty, Surecrop, Aberdeen, and Climax were inoculated with isolates of races Pf-2, Pf-3, Pf-5, and Pf-7 of *P. fragariae* and incubated in plastic bags as described earlier. After a 48-hr incubation, five root tips (40 mm long) were excised from each of four replicate plants and placed into 5 ml of nonsterile soil leachate to induce sporangia. Roots were observed microscopically (×40) 6, 8, and 10 days after inoculation, and the number of sporangia per root segment was recorded. The experiment was repeated once with similiar results.

RESULTS

Effects of incubation period on sporangia and oospores. Under the conditions of our study, mature sporangia of *P. fragariae* isolate NC-1, representing race Pf-2, were first observed on susceptible strawberry roots 6 days after inoculation (Fig. 1) and were greatest after 8 days. The total numbers of sporangia observed 6, 8, 10, and 12 days after inoculation were 57, 98, 84, and 41, respectively (Fig. 1). Production of sporangia was similiar ($P \geq 0.05$) when roots were incubated for 2 days in intact roots and 6 days in soil leachate after roots were excised, for 6 days before excising and placing in soil leachate for 2 days, or incubated for 4 days in intact roots and 4 days in soil leachate after roots were excised (*data not shown*). No sporangia were observed on roots after 0, 2, 4, and 14 days of incubation.

Sites of production of sporangia on inoculated roots were determined during an incubation period of 0–12 days (Fig. 1). After a 6-day incubation period, significantly higher ($P \leq 0.05$) numbers of sporangia were observed within 10 mm of the root tip than at 20–50 mm from the root tip. As the incubation period was increased to 8 days, sporangia were more uniformly dispersed over the root segment up to 40 mm from the root tip. However, relatively fewer mature sporangia were observed in the first 10 mm than were recorded in the same roots after a 6-day incubation period. After a 10-day incubation period, significantly fewer sporangia were observed at the root tip than at 20–40 mm from the tip (Fig. 1). In addition, mature sporangia were less apparent at 20 mm than at 30 mm and 40 mm from the root tip (Fig. 1). No sporangia developed at the 40–50 mm site until 8 days after inoculation. There was a general reduction in the number of sporangia observed overall after 12 days of incubation (Fig. 1).

In contrast to a decrease in the number of sporangia observed as the incubation period was increased, oospores were observed 4–6 days after inoculation and increased in number as the incubation period was increased to 12 days (Fig. 2).

Temperature effects on production of sporangia in strawberry roots. The greatest number of sporangia per root segment was produced by isolate NC-1 of race Pf-2 at 15 C (Fig. 3). Optimum temperatures for single isolates of A-4, A-8, and A-10, representing races Pf-3, Pf-5, and Pf-7, were 12, 15, and 20 C, respectively. Production of sporangia by all isolates was reduced at 4 and 24 C, and no sporangia were produced by any isolate at 28 C. The isolate of race Pf-2 produced more sporangia per root segment than the other isolates tested at 4–24 C (Fig. 3). Fewer ($P = 0.0001$) sporangia were produced by the isolate of race Pf-7 than were produced by isolates of races Pf-2 or Pf-3 at most temperatures tested. Few sporangia were produced by the isolate of race Pf-5 relative to Pf-2 and Pf-3, but the number of sporangia produced by the isolate of Pf-5 did not differ ($P \geq 0.05$) from those produced by the isolate of race Pf-7 (Fig. 3).

Genotype and isolate effects. Higher numbers of sporangia were produced on roots of highly susceptible Tennessee Beauty by all isolates of *P. fragariae* tested than were produced on the other host genotypes (Table 1). Similiar numbers of sporangia were produced on roots of Aberdeen, Surecrop, and Cli-

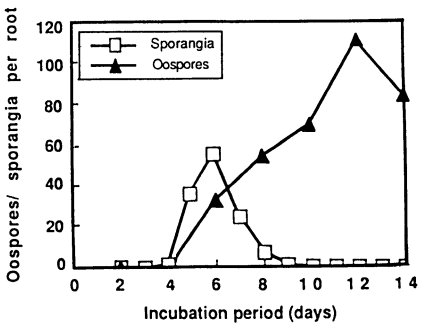

Fig. 2. Production of oospores and sporangia of race Pf-2 of *Phytophthora fragariae* in 10-mm root segments of susceptible strawberry cv. Tennessee Beauty after 0–14 days of incubation at 15 C. Roots were inoculated with a suspension of encysted zoospores of *P. fragariae* at 2×10^4 zoospores per milliliter.

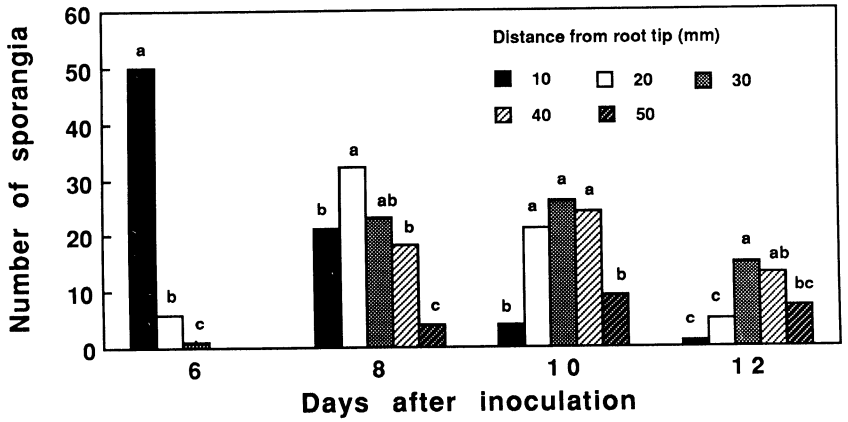

Fig. 1. Number of sporangia observed at various distances from the root tip in 50-mm root segments of susceptible strawberry cv. Tennessee Beauty 6, 8, 10, and 12 days after inoculation of the entire length of root with a suspension containing 2×10^4 encysted zoospores of race Pf-2 of *Phytophthora fragariae* per milliliter. The incubation period consisted of 2 days in plastic bags immediately after inoculation with 0–12 additional days of incubation in nonsterile soil leachate. No sporangia were produced 2, 4, or 14 days after inoculation. Means of the number of sporangia per root section are for eight roots per replicate and three replicates per distance per day. Bars with same letter at each day incubation are not significantly different ($P \geq 0.05$) by Waller-Duncan's *k*-ratio *t* test; $k = 100$, df = 8, n = 24.

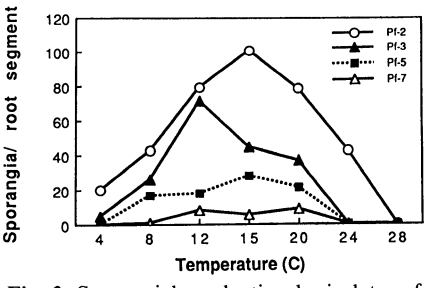

Fig. 3. Sporangial production by isolates of races Pf-2, Pf-3, Pf-5, and Pf-7 of *Phytophthora fragariae* on roots of susceptible strawberry cv. Tennessee Beauty at 4, 8, 12, 15, 20, and 24 C. Roots were inoculated with a suspension of encysted zoospores of *P. fragariae* at $1.8–2.0 \times 10^4$ zoospores per milliliter. Means of the number of sporangia per root are for 10 root segments per replicate and three replicates per temperature and represent cumulative totals of sporangia per root segment over a 9-day incubation period.

max regardless of which race was tested (Table 1).

DISCUSSION

We describe and quantify the chronological development of sporangia and oospores in roots of strawberry plants infected with *P. fragariae*. Sporangia first were observed on 50-mm root segments 6 days after inoculation and reached maximum numbers 8 days after inoculation. Mussel and Fay (12) observed sporangia on roots of strawberry seedlings 4–6 days after inoculation when held at day temperatures of 14–16 C and night temperatures of 11–13 C, and Wynn (15) noted that sporangia formed on roots of cv. Blakemore 5 days after inoculation. However, these studies did not quantify sporangia over time or define optimum incubation times for production of sporangia.

In our study, a period of 2–4 days in nonsterile soil leachate was necessary to induce maximal numbers of sporangia when the fungus was allowed to grow and develop for 2–10 days after inoculation. Few or no sporangia were formed when the incubation period consisted of 4–14 days in intact roots with no time in nonsterile soil leachate (T. F. Law, *unpublished data*). Agents in nonsterile soil leachate are known to stimulate sporangia formation with other species of *Phytophthora* (1,3). However, we also observed that maintenance of roots in soil leachate beyond 4 days for the total incubation period generally caused a decrease in the number of sporangia produced. It is possible that bacteria in nonsterile soil leachate may have caused a degradation of sporangia over time (3).

Oospores developed 4–6 days after inoculation in roots of susceptible strawberry cv. Tennessee Beauty. Goode (6) also observed that the early stages of oospore development occurred about 4 days after inoculation. In our studies, the number of oospores per root segment increased as the incubation period increased, reaching peak production after 12 days. This coincided with a significant decline in the number of sporangia produced on strawberry roots. We conclude that production of sporangia of *P. fragariae* may decline or cease after approximately 10 days as oospores mature in strawberry roots. Production of sporangia of other species of *Phytophthora* decreases with increasing culture age under axenic conditions (13).

The site of infection by *P. fragariae* on susceptible strawberry roots appeared to be within 10 mm from the root tip because more sporangia were produced initially in this area of the root, although the entire root was inoculated. According to Wynn (15), germ tubes from zoospores penetrated resistant cultivars as readily as susceptible cultivars and penetrated at any point along the length of a secondary root. In contrast, Goode (6) showed that penetration by encysted zoospores of *P. fragariae* occurred only within 5 mm of the root tips. Goode (6) also observed that advancing hyphae of *P. fragariae* grew away from the zone of penetration and became confined to the vascular system of the root in susceptible strawberry cultivars. As the incubation period increased, hyphae in cortical cells in the zone of penetration became devoid of protoplasm. We observed that sporangia developed at increasing distances from the root tip as the incubation period was increased, and further production of sporangia declined or ceased in portions of the root closer to the root tip where sporangia had been formed during earlier incubation periods. Preliminary studies in which only the root tips (10 mm) of intact roots (70 mm long) were inoculated with encysted zoospores showed that oospores developed within the vascular system of the root up to 50 mm from the point of inoculation after a 14-day incubation period (T. F. Law, *unpublished data*). Based on our observations of systemic growth of *P. fragariae* in strawberry roots, we are conducting histological studies to determine the rate of colonization and development of *P. fragariae* in the roots of susceptible and resistant host genotypes.

Minimum, optimum, and maximum temperatures for production of sporangia of *P. fragariae* in strawberry roots were within or slightly higher than the temperatures reported for production of sporangia in vitro by other workers (2,7,9,15). The two isolates of races Pf-2 and Pf-5 had an optimum temperature of 15 C, whereas the optimum temperatures for isolates of races Pf-3 and Pf-7 were 12 and 20 C, respectively. These low temperatures optimal for production of sporangia on roots support the observation that red stele disease of strawberry is most severe in cool, moist soils (5,14).

High numbers of sporangia were produced in roots of susceptible Tennessee Beauty by the highly virulent isolate of race Pf-2 and the weakly virulent isolate of race Pf-3. The other highly virulent isolate used in this study, race Pf-5, and the other weakly virulent isolate of race Pf-7 produced very few sporangia on the roots of Tennessee Beauty. Maas (8) also observed few sporangia produced by a highly virulent isolate on agar cultures, but a weakly virulent isolate used in his test produced abundant sporangia. Our isolate of race Pf-5 (A-8) also produced few sporangia on Aberdeen, Surecrop, and Climax. These results were unexpected, as these cultivars were rated as highly susceptible to race Pf-5 when oospore formation in roots was used as an index of disease severity (10). Similarly, few sporangia were formed on roots of Surecrop by the isolate of race Pf-2 and on roots of Aberdeen by the isolate of race Pf-3, although both cultivars were determined to be susceptible to these respective races in the earlier study (10). Apparently, production of sporangia of *P. fragariae* and virulence are not correlated. The number of oospores formed in roots of susceptible strawberry cultivars, therefore, appears to be a better measurement of virulence of *P. fragariae* isolates (10).

Table 1. Number of sporangia[y] produced by isolates of four races of *Phytophthora fragariae* on roots of selected strawberry cultivars

Cultivar	Race			
	Pf-2	Pf-3	Pf-5	Pf-7
Tennessee Beauty	77.2 a[z]	46.9 a	25.0 a	13.7 a
Aberdeen	15.2 b	0.4 b	8.2 ab	0 b
Surecrop	0.5 b	0.2 b	4.9 b	0 b
Climax	0 b	0 b	0 b	0 b

[y] Means of five-root replicate, 12 replicates per treatment, represent the cumulative number of sporangia per 50-mm root segment at 6, 8, and 10 days after inoculation.

[z] Means for isolate and cultivar followed by the same letter within columns are not significantly different ($P > 0.05$) by Waller-Duncan's k-ratio t test; $k = 100$, df = 42, n = 12.

ACKNOWLEDGMENTS

We thank M. K. Beute, J. B. Ristaino, and H. D. Shew for critical review of the manuscript, and R. A. Fong and M. E. Daykin for technical assistance.

LITERATURE CITED

1. Ayers, W. A., and Zentmeyer, G. A. 1971. Effect of soil solution and two soil Pseudomonads on sporangium production by *Phytophthora cinnamomi*. Phytopathology 61:1188-1193.
2. Bain, H. F., and Demaree, J. B. 1945. Red stele root disease of strawberry caused by *Phytophthora fragariae*. J. Agric. Res. 70:11-30.
3. Broadbent, P., and Baker, K. F. 1974. Association of bacteria with sporangium formation and breakdown of sporangia in *Phytophthora* spp. Aust. J. Agric. Res. 25:139-145.
4. George, S. W., and Milholland, R. D. 1986. Inoculation and evaluation of strawberry plants with *Phytophthora fragariae*. Plant Dis. 70:371-375.
5. George, S. W., and Milholland, R. D. 1988. Seasonal occurrence and recovery of *Phytophthora fragariae* in infected strawberry roots. HortScience 22:1254-1255.
6. Goode, P. M. 1956. Infection of strawberry roots by zoospores of *Phytophthora fragariae*. Trans. Br. Mycol. Soc. 39:367-377.
7. 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.
8. Maas, J. L. 1972. Growth and reproduction in culture of ten *Phytophthora fragariae* races. Mycopathol. Mycol. Appl. 48:323-334.
9. McKeen, W. E. 1958. Red stele root disease of the loganberry and strawberry caused by *Phytophthora fragariae*. Phytopathology 48:129-

132.
10. Milholland, R. D., Cline, W. O., and Daykin, M. E. 1989. Criteria for identifying pathogenic races of *Phytophthora fragariae* on selected strawberry genotypes. Phytopathology 79:535-538.
11. Montgomerie, I. G. 1977. Red core disease of strawberry. Hortic. Rev. 5, Commonw. Agric. Bur., Farnham Royal. 47 pp.
12. Mussell, H., and Fay, F. 1971. A method for screening seedlings for resistance to *Phytophthora fragariae*. Plant Dis. Rep. 55:471-472.
13. Ribeiro, O. K. 1983. Physiology of asexual sporulation and spore germination in *Phytophthora*. Pages 55-70 in: *Phytophthora*: Its Biology, Taxonomy, Ecology, and Pathology. S. Bartnicki-Garcia and P. H. Tsao, eds. American Phytopathological Society, St. Paul, MN.
14. Temple, C. E. 1939. Red stele root rot of strawberry. Trans. Peninsula Hortic. Soc. 53:141-149.
15. Wynn, W. K. 1968. Development of controlled conditions for the study of red stele disease of strawberries. Contrib. Boyce Thompson Inst. 24:95-102.