Occurrence and Populations of *Plasmodiophora brassicae* in Sediments of Irrigation Water Sources

L. E. DATNOFF, Graduate Student, and G. H. LACY, Associate Professor, Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg 24061, and J. A. FOX, Extension Plant Pathologist, Mississippi State University, P.O. Box 157, Decatur 39327

ABSTRACT

Datnoff, L. E., Lacy, G. H., and Fox, J. A. 1984. Occurrence and populations of *Plasmodiophora brassicae* in sediments of irrigation water sources. Plant Disease 68:200-203.

Cabbage plants with clubroot were found in seedbeds with no history of cabbage production in the southwestern Virginia counties of Carroll and Patrick. These beds had been irrigated from farm ponds receiving run-off water from *Plasmodiophora brassicae*-infested fields. Clubbed roots developed on cabbage seedlings planted in farm pond sediments, indicating that *P. brassicae* was present in the irrigation source sediments. When comparing standard curves of resting spore populations with symptom incidence, estimated sediment populations ranged from none detected to as many as 2×10^7 resting spores per gram of soil.

Additional key words: *Brassica oleracea* var. *capitata*, crucifers

Clubroot of cabbage (*Brassicae oleracea* var. *capitata* L.), caused by *Plasmodiophora brassicae* Wor., was first described in southwestern Virginia in 1909 by Reed (16) and continues to be the major disease problem causing economic losses for cabbage production in this area. In the past, the primary means of inoculum dispersal was believed to be *P. brassicae*-colonized transplants coming into southwestern Virginia; however, inspected transplants from the Eastern Shore of Virginia and Georgia were found to be clubroot-free (6). Also, clubroot has not been observed among commercially grown transplants from Georgia in the past 17 yr (J. Dan Gay, *personal communication*).

In 1978, Hutter (12) observed that some transplant seedbeds in southwestern Virginia with no history of cabbage production contained plants with clubroot symptoms. These seedbeds were irrigated with water from farm ponds and streams that received run-off water from fields infested with *P. brassicae*. Sediment from irrigation water sources has not been implicated specifically in the spread of propagules of *P. brassicae*; however, the role of irrigation water has been documented in the spread of *P. brassicae* (11) and other plant pathogens including fungi (23), bacteria (19), and nematodes (9). Recently, we reported observation of *P. brassicae* resting spore-like bodies in water from three of 18 ponds (7).

We now report the detection and estimation of populations of *P. brassicae* resting spores present in farm pond sediment. A preliminary report of this work has been presented (8).

Contribution 456 of the Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg.

Present address of first author: Department of Plant Pathology, University of Illinois, Urbana 61801.

Accepted for publication 29 August 1983.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Pond sediment collection. In June and July 1980, pond sediment was collected with a shovel, which was disinfested by flaming with ethanol between samples. For each pond (A–D), sediment was collected randomly at various points 90–180 cm from the bank near the irrigation pipe inlet site until a 19-L container was filled. Three ponds (A–C) drained fields with high incidences of clubroot in 1979. The fourth pond (D) drained cabbage fields without any detectable clubroot. Part of the sediment from each pond was steamed for 60 min at 121 C, incubated at room temperature (22–24 C) for 24 hr, and steamed again. Steamed and nonsteamed parts of each sample were air-dried for 5–7 days on a greenhouse bench. Sediments were either sand or sandy loams with pH values of 5.8–6.3.

Inoculum preparation. During the summers of 1978 and 1979, fresh, galled cultivar Market Prize cabbage roots were obtained from an infested field and stored at −20 C. *P. brassicae* isolated from the galled roots was designated 16/2/30 (13) based on reactions of standard European clubroot differential hosts (3). This designation is equivalent to race 6 (22). Resting spores were extracted, counted, and stored as described by Williams (22).

Sediment-Weblite mixture. Steamed or nonsteamed sediment from each pond was mixed with an equal weight (w/w) of steamed Weblite (Weblite Corp., Blue Ridge, VA 24064). Weblite is 50-mesh (2-mm-diam.) particle size, heat-expanded shale, dark greyish red (Munsell 10R 4/4), and has about half the specific weight of river sand (21). This sediment-Weblite mixture is designated SW.

Plant growth. Market Prize cabbage

seeds (Joseph Harris Co. Inc., Rochester, NY 14624) were planted in vermiculite (Terra-lite, W. R. Grace & Co., Cambridge, MA 02140) and seedlings were misted continuously in the greenhouse under natural light and ambient temperature (23 ± 2 C). Seedlings were removed from the flats after 22–25 days, washed with tap water, and placed into 6-cm clay pots containing SW.

Pond sediment bioassay. Twenty-five 22-day-old seedlings were planted in 6-cm clay pots containing SW prepared from each steamed and nonsteamed sediment source. Pots were placed in the greenhouse under natural light and ambient temperature as described previously. Plants were fertilized weekly with Peter's Salts (A. H. Hummert Seed Co., St. Louis, MO 63103) containing nitrogen, phosphorous, and potassium in a 3:9:1 ratio by weight and watered as needed. Thirty days after planting, the plants were removed from the pots and their roots gently washed and rated for incidence and intensity of root clubbing.

Disease incidence and symptom intensity index. Disease incidence was designated as the proportion of diseased plants to total numbers of plants. Cabbage plants were rated for occurrence of root clubbing by a modification of the method of Seaman et al (17), where 0 = no clubs, 1 = club(s) on the taproot or primary root, 2 = club(s) on the secondary or lateral roots but not on the taproot, and 3 = clubs on both primary and secondary roots.

Sediment-Weblite infestation. Using a hand sprayer, 250 ml of distilled water containing a known number of resting spores was sprayed evenly with mixing into each steamed SW mixture representing each pond sample (A–D). The final populations ranged in 10-fold increments from 10^1 to 10^7 resting spores per gram of sediment.

Estimation of resting spore populations. The approximate populations of resting spores in nonsteamed pond sediment samples were determined by comparing disease incidence among plants in nonsteamed sediment with disease incidence in steamed and artificially infested sediment from the same pond. A standard curve was prepared by infesting steamed SW with different populations of resting spores. Ten 25-day-old cabbage seedlings were planted in 6-cm clay pots containing artificially infested SW. Each inoculum level was repeated once.

Water bioassay. A Styrofoam float (19 cm long × 14 cm wide × 2 cm thick) perforated with 20 evenly spaced 1-cm-diameter holes was placed in a plastic box (29 cm long × 15 cm wide × 9 cm deep) containing a 2,000-ml suspension of resting spores. Concentrations were zero and 10^1 to 10^6 resting spores per milliliter in 10-fold increments. Twenty 13-day-old cabbage seedlings were wedged into the holes in the Styrofoam with nonabsorbent cotton and the roots of the plants were immersed in the resting spore suspension. Plants were incubated in darkness for 36 hr at 25 C, then removed, potted in SW, and placed in the greenhouse. Thirty days after planting, roots were rated for incidence and intensity of root clubbing.

Data analysis. Disease incidence, symptom intensity, and standard curve data were analyzed by chi-square, Duncan's multiple range, and probit analysis, respectively. The 95% confidence limits were determined by Fisher and Yate's probit tables (10). Each experiment was replicated at least once.

RESULTS

Clubroot incidence and symptom intensity for four pond sediments. Among the four pond sediments tested, disease incidence and symptom intensity was highest in pond A (Table 1). Although disease incidence and symptom intensity did not differ significantly between ponds C and D, disease developed in plants exposed to sediment from pond C but not from pond D.

Population estimation. Populations of resting spores in naturally infested sediments were estimated by comparing disease incidence of plants in the nonsteamed sediments at the 95% confidence level with standard curves obtained from bioassay in artificially infested, steamed sediments (Fig. 1). The standard curves obtained in these artifically infested, steamed sediments were similar to the curves obtained by bioassay in infested water (Fig. 2). These estimates were zero, 10^0 to 10^3, 10^3 to 10^5, and 10^6 to 10^7 resting spores per gram of sediment detected in ponds D, C, B, and A, respectively. The $\log_{10}$ ED_{50}s for resting spores in artificially infested sediment from ponds A, B, C, and D, respectively, were 4.4, 4.8, 4.9, and 6.1. Semilogarithmic and $\log_{10}$-$\log_{10}$ plots of the estimated number of infections per cabbage plant are presented in Figure 3 and compared with the data of Colhoun

Table 1. Incidence and intensity of clubroot symptoms in plants grown in sediment from four irrigation ponds

Pond	Incidence (%)		Symptom intensity[y]	
	Trial 1	Trial 2	Trial 1	Trial 2
A	85.4 a[z]	85.7 a	2.3 a	1.8 a
B	59.0 b	50.0 b	0.9 b	1.0 b
C	8.5 c	2.0 c	0.1 c	0.04 c
D	0.0 c	0.0 c	0.0 c	0.0 c

[y] Symptom intensity rating index: 0 = no clubs, 1 = club(s) on taproot only, 2 = club(s) on the secondary (lateral) roots, and 3 = clubs on the taproot and secondary root.
[z] Values in the same column followed by the same letter do not differ significantly ($P \leq 0.05$) by chi-square distribution (for incidence) or by Duncan's multiple range test (for intensity).

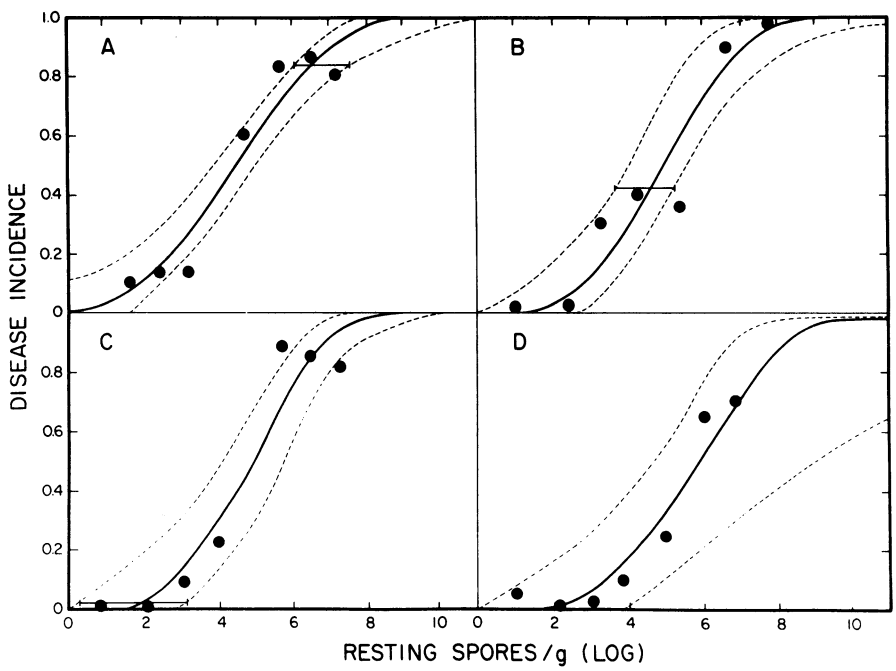

Fig. 1. Populations of resting spores in naturally infested sediments of ponds A–C (horizontal bars) estimated from the standard curves for the disease incidence in steamed sediments artificially infested with *Plasmodiophora brassicae*. Each datum point represents averaged observed incidence of disease in the artificially infested sediments. Dotted lines are 95% confidence limits for the standard curves. There were no resting spores detected by bioassay in sediment from pond D. The $\log_{10}$ ED_{50} for resting spores per gram of artificially infested sediment from ponds A, B, C, and D were 4.4 (2.5×10^4), 4.8 (6.3×10^4), 4.9 (7.9×10^4), and 6.1 (1.2×10^6), respectively.

(5). These data were transformed to $\log_e (1/1-y)$, where y = symptom incidence (1). For each artificially infested pond sediment, the slope and R values, respectively, were 33 and 0.99 for pond A, 0.29 and 0.61 for pond B, and 0.72 and 0.97 for pond C.

Although the fields draining into ponds A, B, and C were not used for cabbage culture after 1979, as determined by bioassay, sediments from these ponds were infested in June and July 1980 (this study) and October 1981 (T. K. Kroll and G. H. Lacy, *unpublished*).

DISCUSSION

Resting spores of *P. brassicae* were present in irrigation water sediment. Although resting spores were detected in the same ponds 2 yr after cabbage culture was discontinued in the drainage areas for these ponds, whether the spores survived there during that time could not be determined because later deposition in these sediments could not be controlled (18). Previously, only surface drainage water (11) or flood water (15) has been implicated in dissemination of *P. brassicae* propagules within and among fields.

Because of the differences in disease incidence and symptom intensity ratings, we believe that resting spore populations varied from pond sediment to pond sediment. Other investigators have reported that with increases in resting spore populations, there is an increase in incidence of clubbed plants (14). Colhoun (4,5) determined that the resting spore concentrations could be estimated by comparing disease incidence in naturally infested soil with incidence in artificially infested soil if the plants were grown under the same environmental conditions. He found, however, a greater incidence of disease at lower resting spore densities than reported here. When his data and ours were compared, slopes of curves in semilogarithmic and $\log_{10}$-$\log_{10}$ plots of resting spore density versus symptom incidence were similar to the slopes of curves based on his data, even though similar symptom incidence occurred at a higher resting spore density than he observed (Fig. 3).

Variation from expected disease threshold values is not unknown for clubroot. Vanderplank (20) found that for Colhoun's data (5), disease development and inoculum density were not directly proportional, possibly because of environmental conditions and host nutrition. These factors could also explain why our threshold levels were lower than current theory. Consequently, it would be difficult to determine if a rhizoplane or rhizosphere effect is taking place even though our slopes were 0.33, 0.29, and 0.72 for ponds A, B, and C, respectively.

Several factors, including differences in soil type, lighting conditions, inoculum potential, race of pathogen, host nutrition, and host cultivar, probably contributed to the differences between Colhoun's results and ours. Because the soil used for our standard curve was steamed whereas Colhoun used non-steamed soil, this may also contribute to the differences observed. In steamed soils, readily available nutrients might cause spontaneous germination and lysis of resting spores. Consequently, the initial resting spore population may have been reduced and may explain why higher inoculum concentrations of resting spores were required in this study to cause disease incidences similar to those reported by Colhoun.

In this study, however, to reduce the possible effect of germination and lysis on resting spores, cabbage seedlings were placed into the artificially infested soil immediately after infestation. Furthermore, symptom incidence-resting spore density slopes calculated using steamed soil were similar to those with water culture inoculations (Fig. 2), indicating that disease incidence for the suscept and pathogen system employed here occurred at higher resting spore populations than reported by Colhoun.

However, there are several factors that make direct comparisons of the water culture system with the soil infestation system difficult. The first is that the resting spore concentration and spatial geometry in the water phase of the SW mixture are different from those in the water culture system. Second, water is a small part of soil weight; the resting spore concentration in water may have been less than that in the water phase of the SW mixture. Also, diffusion of root exudates, known to influence resting spore germination (2), occurs in the capillary water films of the SW mixture and would, therefore, reach higher concentrations in extremely localized areas than in the bulk water of the water culture system. Third, resting spores settled to the bottom of the container in the water culture system. Fourth, resting spores may not behave in the water culture system as they would in SW. Spontaneous germination may occur in the water culture system because there is no soil fungistasis to inhibit the spores and stimulatory root exudates, albeit more dilute than in the water phase of SW, are free to diffuse throughout the water system.

In conclusion and considering these experimental limitations, results of this investigation indicated that resting spores of *P. brassicae* were present in high populations in irrigation water sediment. Even though the population estimates were high compared with the data of Colhoun (5), the populations of *P. brassicae* resting spores found in these pond sediments definitely pose a threat for spreading clubroot into pathogen-free seedbeds and fields by pumping water for irrigation from these sources. Datnoff (6) established that these resistant propagules remain able to cause disease on cabbage after passing through a pump and being aerated and impacted on filters. This may explain why clubroot continues to spread in southwestern Virginia despite use of disease-free transplants and dis-

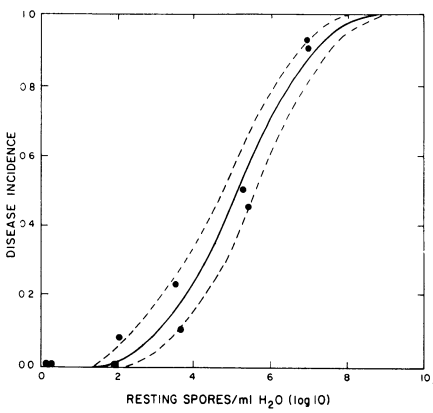

Fig. 2. Incidence of disease observed in cabbage seedlings exposed to water infested artificially with resting spores of *Plasmodiophora brassicae*. Dotted lines are 95% confidence limits. The $\log_{10}$ ED_{50} for resting spores per milliliter in water was 5.1 (1.2×10^5). The slope and the R value for the $\log_e 1/1-y$ transformation are 0.40 and 0.78, respectively.

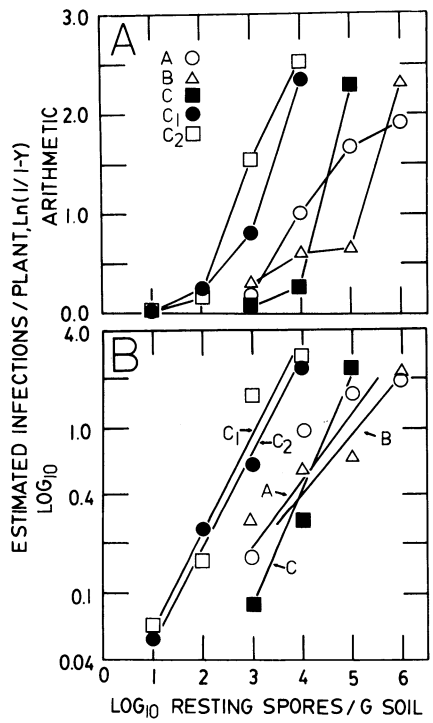

Fig. 3. (A) Semilogarithmic plot of the estimated number of infections caused by *Plasmodiophora brassicae* resting spores per cabbage plant [$\log_e (1/1-y)$, in which y = proportion of symptomatic plants] versus inoculum density in artificially infested soils. C_1 and C_2 are data of Colhoun (5) and A, B, and C are data from this study. **(B)** The $\log_{10}$-$\log_{10}$ plot of the regression analyses of the number of infections was estimated as above. The slopes and R values, respectively, are A = 0.33, 0.99; B = 0.29, 0.61; C = 0.72, 0.97; C_1 = 0.68, 0.94; and C_2 = 0.67, 0.94.

infestation of equipment between use in different fields.

ACKNOWLEDGMENTS

We thank G. J. Griffin for his critical review of this manuscript and K. Hinkelman for assistance with statistical analyses. L. E. Datnoff was supported by a Peace Corps/ACTION graduate assistantship (76-712-99-659) and this research was supported by a grant from the Virginia Agricultural Foundation (VPI & SU 230-11-110-012-848056-1) to J. A. Fox and G. H. Lacy.

LITERATURE CITED

1. Baker, R. 1971. Analyses involving inoculum density of soil-borne plant pathogens in epidemiology. Phytopathology 61:1280-1292.
2. Bochow, H. 1965. The effect of root diffusates of host and non-host plants on the resting spore germination of *Plasmodiophora brassicae*. Pages 296-299 in: Wor. Proc. Symp. Prague. Czech. Acad. Sci.
3. Buczacki, S. T., Toxopeus, H., Mattusch, P., Johnston, T. D., Dixon, G. R., and Hobolth, L. A. 1975. Study of the physiological specialization in *Plasmodiophora brassicae*: Proposals for attempted rationalization through an international approach. Trans. Br. Mycol. Soc. 65:925-303.
4. Colhoun, J. 1957. A technique for examining soil for the presence of *Plasmodiophora brassicae* Woron. Ann. Appl. Biol. 45:559-565.
5. Colhoun, J. 1961. Spore load, light intensity, and plant nutrition as factors influencing the incidence of clubroot of Brassicae. Trans. Br. Mycol. Soc. 44:593-600.
6. Datnoff, L. E. 1981. Detection of *Plasmodiophora brassicae* in and decontamination of irrigation water. M.S. thesis, Virginia Polytechnic Institute and State University, Blacksburg. 75 pp.
7. Datnoff, L. E., Kroll, T. K., and Lacy, G. H. 1981. Detection of *Plasmodiophora brassicae* in the sediment of irrigation water sources. (Abstr.) Phytopathology 71:765.
8. Datnoff, L. E., Lacy, G. H., and Griffin, G. J. 1981. Populations of *Plasmodiophora brassicae* resting spores in irrigation water sediments. (Abstr.) Phytopathology 71:869.
9. Faulkner, L. R., and Bolander, W. J. 1970. Agriculturally polluted irrigation water as source of plant parasitic nematode infestation. J. Nematol. 2:368-371.
10. Fisher, R. A., and Yates, F. 1957. Statistical Tables for Biological, Agricultural and Medical Research. Hafner Publ. Co., New York.
11. Gibbs, J. G. 1931. Clubroot in cruciferous crops. N.Z. J. Agric. 42:1-17.
12. Hutter, M. D. 1978. Integrated control of clubroot of cabbage incited by *Plasmodiophora brassicae* (Wor.). M.S. project and report, Virginia Polytechnic Institute and State University, Blacksburg. 52 pp.
13. Kroll, T. K., Lacy, G. H., and Moore, L. D. 1983. A quantitative description of *Plasmodiophora brassicae* colonization of susceptible and resistant radish plants. Phytopathol. Z. 94:In press.
14. MacFarlane, I. 1970. Germination of resting spores of *Plasmodiophora brassicae*. Trans. Br. Mycol. Soc. 55:97-112.
15. Maklakova, G. F. 1961. O Zhiznesposobnosti spor kily. Zashch. Rast. Moskva 6:54-55. (Reviewed in: Rev. Appl. Mycol. 41:492).
16. Reed, H. S. 1911. Cabbage clubroot in Virginia. Va. Agric. Exp. Stn. Bull. 191:1-11.
17. Seaman, W. L., Walker, J. C., and Larson, R. H. 1963. A new race of *Plasmodiophora brassicae* affecting 'Badger Shipper' cabbage. Phytopathology 53:1426-1429.
18. Shokes, F. M., and McCarter, S. M. 1979. Occurrence, dissemination, and survival of plant pathogens in surface irrigation ponds in southern Georgia. Phytopathology 69:510-516.
19. Steadman, J. R., Bay, R. W., and Hammer, M. J. 1979. Plant pathogen contamination in reused irrigation waste water. Proc. Water Reuse Symp. 3:2038-2045.
20. Vanderplank, J. E. 1975. Principles of Plant Infection. Academic Press, New York. 216 pp.
21. Weber, D. E. 1967. Population behavior of three parasitic nematodes on selected Gramineae and an analysis of the centrifugation-flotation extraction technique. M.S. thesis, Virginia Polytechnic Institute and State University, Blacksburg. 59 pp.
22. Williams, P. H. 1965. A system for the determination of races of *Plasmodiophora brassicae* that infect cabbage and rutabaga. Phytopathology 56:624-626.
23. Wills, W. H. 1964. *Phytophthora parasitica* var. *nicotianae* spread by overhead irrigation. Plant Dis. Rep. 48:35-36.