Growth and Reproduction of *Dichotomophthora portulacae* and Its Biological Activity on Purslane

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ABSTRACT

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Growth, reproduction, and biological activity of *Dichotomophthora portulacae* on purslane were studied in a controlled environment. Among several nutrient media that supported good growth of the fungus, potato-dextrose agar was selected for uniform growth, stability, and production of conidia by the fungus. Optimum temperatures were 30 C for growth and formation of sclerotia, 24 C for development of conidiophores and conidia, 33 C for germination of conidia, and 27 C for infection. Conidia germinated within 1 hr and infection occurred within 2 hr. Moisture was required on the plant for conidial germination and infection. Infection was also influenced by temperature, time, and inoculum density.

Dichotomophthora portulacae Mehrlich & Fitzpatrick ex M. B. Ellis, a soilborne pathogen, attacks the stems and leaves of purslane (*Portulaca oleracea*) plants (2,3). In irrigated soils, stems attacked by the fungus develop dark discoloration and constriction near the soil line. Plants artificially inoculated and kept in a humid environment develop necrotic leaf spots resulting in leaf drop, dark lesions on the stem, and dieback.

The fungus produces sclerotia and conidia on artificial media and diseased host tissue (2). Some isolates predominantly produce either sclerotia or conidia.

The use of *D. portulacae* to control purslane in Hawaii was suggested by Mehrlich and Fitzpatrick (3). In field trials in New York, the fungus was of little value as a biological control agent under dry summer conditions (4). The lack of documentation regarding the growth, reproduction, and biological activity of *D. portulacae* prompted this study to determine the pathogen's potential as a biological control agent.

MATERIALS AND METHODS

Growth and reproduction in culture. An isolate of *D. portulacae* from purslane was evaluated for growth, stability in culture, and reproduction on cornmeal agar, malt agar, lima bean agar, V-8 juice agar, Czapek's medium, and potato-dextrose agar (PDA). Radial growth was measured from colonies grown on PDA for 96 hr at 15–42 C at three-degree increments.

The effect of temperature on reproduction was determined from the number of conidiophores and sclerotia formed on 6-mm agar disks that were taken from 3- to 4-day-old cultures on PDA and inverted on water agar. Single agar disks were placed in each of five agar plates, which were randomized and incubated for 48 hr at temperatures ranging from 15 to 42 C at three-degree increments.

Germination of conidia and sclerotia. Conidia were taken from 6-, 8-, and 12-day-old cultures of *D. portulacae* growing on PDA, suspended in sterile distilled water (about 1×10^3/ml), and dispensed on water agar in plates. Two plates of 6-day-old conidia were incubated at each of the described temperatures. Two plates each of different aged conidia were incubated at 33 C. Germination was recorded after 1–3 hr and also after 4 hr for 12-day-old conidia. Sclerotia were obtained from cultures of *D. portulacae* grown for 2, 4, 6, and 8 wk in vermiculite saturated with V-8 juice. Vermiculite cultures were air-dried for 1 wk, then the sclerotia were sieved from the vermiculite through a 0.351-mm mesh screen. The sclerotia were surface-sterilized for 1–5 min in 1% sodium hypochlorite and rinsed in sterile distilled water. Twenty sclerotia were placed on water agar in each of three plates per age or treatment and kept at room temperature.

Effect of time, temperature, and inoculum concentration on infection of purslane leaves. Inoculum was prepared from cultures of *D. portulacae* grown on PDA in petri dishes under 13 hr of fluorescent light (4,088 lux) for 6 days at 24 C. Sterile distilled water was added to the cultures, and the mycelium bearing conidiophores and conidia was rubbed and agitated with a transfer needle to dislodge the conidia. Spore concentrations were determined by hemacytometer and adjusted to the desired concentrations from 1×10^4 to 4×10^4 conidia per milliliter.

Inoculum was misted on leaves (3 ml/plant) of 6- to 8-wk-old purslane plants. Control plants were misted with sterile distilled water. Three plants were inoculated and one left uninoculated for each increment of time, temperature, and inoculum concentration. To maintain high humidity, plants were covered with moistened plastic bags that were fastened over the pots. Bags were removed after the plants were incubated at different temperatures for various periods. The plants were then placed in random order under lights (13-hr photoperiod, 4,088 lux) and observed for symptom development and disease progression. Infection was assessed on the basis of necrotic spot development on three primary leaves on the central stem 48–72 hr after inoculation.

RESULTS

Growth and reproduction in culture. Optimum temperatures for growth of *D. portulacae* ranged from 27 to 33 C (Table 1). The fungus grew on each medium; however, PDA was the best medium for sporulation, uniform growth, and stability in culture. White-tufted mycelia occasionally produced in cultures did not produce conidiophores. Maintenance of such cultures usually led to loss of typical growth and conidial production. To avoid such losses, isolates were kept in autoclaved soil containing wheat bran in glass tubes stored in a refrigerator at 6 C.

Conidiophores were produced on all media but were most abundant on PDA, malt agar, and cornmeal agar. Production of conidiophores and conidia was observed within 24 hr in PDA cultures incubated at 21, 24, and 27 C and within 48 hr at 15 and 18 C. Optimum temperature was 24 C. Conidiophores were not produced at 30 C or higher, even with extended incubation periods. Diurnal formation of conidiophores in concentric rings occurred in cultures kept under 13 hr of light (4,088 lux). Sporulation did not occur in cultures kept in constant light and was sporadic in cultures kept in constant darkness.

Sclerotia developed in cultures incubated at 27, 30, and 33 C for 48 hr (Table 1). However, a few sclerotia also formed

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Table 1. Growth, spore germination, and reproduction of *Dichotomophthora portulacae* on potato-dextrose agar

Temperature (C)	Radial growth (mm) after 96 hr[a]	No. of conidiophores formed/48 hr[b]	No. of sclerotia formed/48 hr[b]	Percent germination 100 conidia	
				1 hr	2 hr
15	17	33	···	2	80
18	25	94	···	4	86
21	29	260	···	48	90
24	35	354	···	61	94
27	47	64	12	71	96
30	50	···	41	85	96
33	49	···	34	88	96
36	37	···	···	85	96
39	18	···	···	82	92
42	···	···	···	70	90

[a] Means of five colonies.
[b] Mean of number of structures formed on each of five 6-mm agar disks.

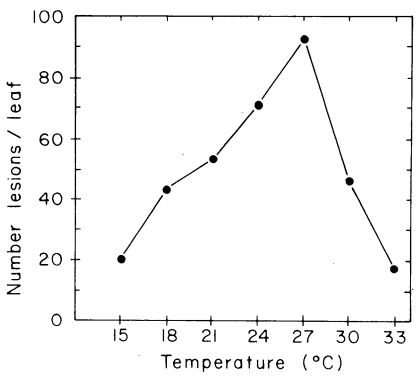

Fig. 1. Effect of temperature on infection of purslane by *Dichotomophthora portulacae*. Three milliliters of 1×10^4 conidia per milliliter were applied per plant. Plants were held at indicated temperatures for 18 hr. Values are means of lesion numbers from nine leaves.

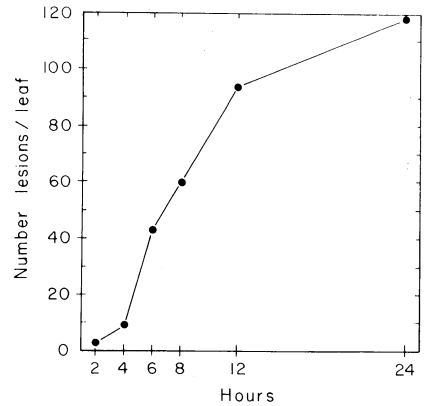

Fig. 2. Effect of time on infection of purslane by *Dichotomophthora portulacae* at 27 C. Three milliliters of 1×10^4 conidia per milliliter were applied per plant. Values are means of lesion numbers from nine leaves.

at 15–24 and 36 C within 7 days.

Germination of conidia and sclerotia. Conidia germinated within 1 hr regardless of incubation temperature, with the highest percentage germinating above 24 C. Germination was greater than 90% within 2 hr at 21 C and higher (Table 1). Germination of conidia from 6-, 8-, and 12-day-old cultures was, respectively, 90, 80, and 12% within 1 hr and 99, 93, and 26% within 3 hr. The percentage of germinating conidia from 12-day-old cultures did not increase when the incubation period was extended to 4 hr.

Sclerotia that were surface-sterilized for 1 and 3 min germinated at 70 and 73%, respectively. Germination was reduced to 3% when sclerotia were surface-sterilized for 5 min. There was no difference in percent germination between 2- and 8-wk-old sclerotia.

Effect of temperature, time, and inoculum density on infection of purslane leaves. *D. portulacae* infected leaves and stems of purslane at 15–33 C during an 18-hr incubation period (Fig. 1). Infection was most extensive at 27 C. Forty-eight hours after inoculation, necrotic sunken lesions were distinguishable on leaves, which turned light green, then yellow, and then dropped off. Ninety to 100% of the leaves of plants held at postinoculation temperatures of 18–30 C dropped off within 4–7 days. Subsequently, branches developed black lesions and terminal dieback. Progression of the disease within lateral branches and stems led to collapse and death of the plant.

Infection of leaves increased with longer incubation periods (Fig. 2). Infections were sparse but occurred within 2 hr at 27 C and in a parallel experiment at 21 and 24 C. Incubation periods of 2–4 hr were required for infection at 21, 30, and 33 C; 4–6 hr at 18 C; and 6–8 hr at 15 C. No infection occurred when inoculated plants were not enclosed in moistened plastic bags. Disease development and severity were more rapid and pronounced on plants with the longest incubation periods.

Moderate levels of infection occurred from inoculations with 1×10^4 conidia per milliliter; however, as inoculum increased from 1×10^4 to 4×10^4, the number of lesions increased from 53 to 118 per leaf. Subsequent development of disease and collapse of plants were greater when higher levels of inoculum were applied.

DISCUSSION

Although *D. portulacae* was reported as a pathogen on *P. oleraceae* in 1935 (3), there is little documentation of subsequent investigations regarding its potential for biological activity on purslane. *D. portulacae* was highly pathogenic to the foliage and stems of purslane plants over a range of temperatures. Moisture is essential for the fungus to initiate infection; however, after infection, disease development progresses regardless of environmental conditions. Longer periods of humid conditions favor extensive infection and severe disease. Thus, the pathogen is not expected to infect leaves and stems in nature in the absence of rain or dew. Application of the fungus as a bioherbicide in nature supplemented with sprinkler irrigation should be investigated in geographical areas with dry summer conditions.

Overall, *D. portulacae* has attributes favorable for use as a biological control agent. It is easily cultured and sporulates readily by producing large quantities of conidia that germinate rapidly over a range of temperatures. Infection of purslane occurs within as little as 2 hr at optimum temperatures. Sclerotia produced in host tissue presumably serve as survival structures in the soil and as inoculum for basal stem infection. Because of its devastating action on the plant in controlled experiments, the pathogen appears to be more efficient when applied as a foliar inoculum than when the stem is infected by soilborne inoculum. Infection and constriction of the stem at the soil line is more likely to be lethal if purslane plants are infected at a young age (2). Disease caused from stem infection by soilborne *D. portulacae* may be enhanced by the biological activity of sawfly (*Schizocerella pilicornis* Holmgren) and weevil (*Hypurus bertrandii* Perris) larvae and weevil adults (1) on the plants.

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