A Foliar Disease of Field Bindweed (*Convolvulus arvensis*) Caused by *Phomopsis convolvulus*

J. ORMENO-NUÑEZ, Graduate Student, R. D. REELEDER, Assistant Professor, and A. K. WATSON, Professor, Department of Plant Science, Macdonald College of McGill University, 21111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, Canada H9X 1C0

ABSTRACT

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Phomopsis convolvulus was identified as the causal agent of leaf spots and anthracnose lesions on field bindweed (*Convolvulus arvensis*). Seedlings (10–14 days old) were killed after spray inoculation with alpha-conidia concentrations of 5×10^6 spores/ml or more. Increasing inoculum concentrations from 1×10^5 to 5×10^6 spores/ml resulted in decreases in foliage dry weight. At 20 C, a dew period of more than 3 hr was required before seedling death occurred or before significant reductions in dry matter were evident. The dew period following inoculation could be delayed for up to 48 hr without significantly decreasing disease ratings. Shoot regrowth occurred, however, when the delay was 24 hr or more. Disease severity and mortality following 24-hr dew periods were greater when dew period temperatures were at 20 or 30 C than when at 10 C. When plants were exposed to various temperatures (10–30 C) after a 24-hr dew period, no differences in disease severity were evident. These results suggest that this fungus has potential as a mycoherbicide.

Additional key words: biological control, Convolvulaceae

Field bindweed (*Convolvulus arvensis* L.) is a prostrate or climbing herbaceous perennial weed that has long been considered a serious weed in agricultural areas (26). Traditional methods to control field bindweed normally combine cultivation and crop rotation with the intensive use of postemergence herbicides (8). The use of natural enemies of *C. arvensis*, such as arthropods and pathogens, has been suggested to obtain effective and long-term control of this weed (4,12,14,25). Hasan (10) suggested *Puccinia convolvuli* (Pers.) Cast. and *Erysiphe convolvuli* (DC.) St-Amans as potential biocontrol agents of field bindweed. Other fungal species also have been suggested (14,16), but few specific studies have been conducted on any pathogen, although preliminary data have indicated that *E. convolvuli* possesses sufficient host specificity within the genus *Convolvulus* to merit further evaluation (3). There are no reports of fungi having been evaluated as mycoherbicides for the control of field bindweed.

During the summer and fall of 1984,

several fungal species were isolated from various types of lesions occurring on the foliage of *C. arvensis*. One fungus, *Phomopsis convolvulus* Ormeno (11), was pathogenic to field bindweed plants. This paper reports the occurrence of *P. convolvulus* on field bindweed, its effects on host plant tissues, and the effects of various environmental parameters on disease development.

MATERIALS AND METHODS

Collection of diseased plants. During 1984 and 1985, field bindweed plants with leaf spot symptoms were collected from various areas on the Macdonald College campus at Ste-Anne-de-Bellevue, Quebec, Canada, and from several sites near Montreal, Quebec.

Isolation of pathogens. Isolations from diseased leaves were made immediately upon return to the laboratory. Using a flame-sterilized scalpel, 1-cm² sections of diseased tissue were cut from the leaves and rinsed in distilled water. The sections were then immersed in 1% sodium hypochlorite for 5 min, rinsed quickly with sterile water, and placed on a sterile filter paper to dry. Leaf sections were transferred to 9-cm culture plates containing 20 ml of malt extract agar acidified to pH 4.8 with 88% lactic acid. Advancing edges of fungal colonies were transferred to acidified (pH 4.8) V-8 juice agar (21) and acidified potato-dextrose agar (pH 4.8) (21) and maintained for 1 mo on the laboratory bench (21 ± 1 C). Cultures were irradiated during the second week with near-ultraviolet light (Black Ray UVL 56).

Inoculum production. Alpha-conidia

of *P. convolvulus* were obtained by culturing the fungus on a PDA or PDA12 (12 g/L of potato-dextrose broth, 15 g/L of Difco Bacto agar, and 100 ml/L of bindweed decoction, prepared by boiling 200 g of fresh bindweed leaves in 1 L of water for 30–40 min, followed by filtering and autoclaving for 15 min). Cultures were kept on the laboratory bench at 21 ± 1 C with 12–14 hr of cool-white fluorescent light at 21 ± 1 C. Using a 1-ml sterile syringe, conidial droplets oozing from pycnidia were retrieved from 1-mo-old cultures and transferred to 10-ml vials containing 3 ml of distilled, deionized water. Spore concentrations were determined with a hemacytometer and adjusted to $3-5 \times 10^5$ spores/ml with sterile water or to 5×10^6 spores/ml with 0.1% gelatin. For most inoculations, a single-conidium isolate (8412) was utilized.

Plant production. Initial pathogenicity tests were performed on clonal material obtained from a field bindweed plant collected locally. This plant was multiplied by cutting 3-cm sections from root-bud-bearing roots, each of which was subsequently planted in moist potting mix at a depth of 3–4 cm in 13-cm diameter plastic pots. Two or three shoots were produced in each pot.

A commercial sample of bindweed seeds was used for production of seedlings. Seeds were scarified with concentrated sulfuric acid for 30 min, rinsed with distilled water, dried at 21 C, and stored in the dark for approximately 2 mo at 4 C. Five germinated seeds were sown in 10-cm plastic pots in moist potting soil at a depth of about 1 cm. Pots were placed in growth cabinets (Conviron, model E-15, Controlled Environments, Winnipeg, Man.) adjusted to a day/night temperature regime of 25/20 C, with a photoperiod of 15 hr (300 μE·m⁻²·s⁻¹) (cool-white fluorescent plus incandescent light). Plants were later thinned to three seedlings per pot. Relative humidity was maintained at 50–55%.

Pathogenicity tests. One or two droplets (0.1 ml) of a conidial suspension of $3-5 \times 10^5$ spores/ml were placed on the center of the lamina of a leaf of a 2- to 3-wk-old clonal shoot (four or five leaves). Droplets were spread evenly over the leaf surface with a glass rod. In a second experiment, 12 seedlings at the three- to five-leaf stage (10–14 days old,

7–10 cm tall) were sprayed to wetness (noticeable coalescing of droplets) using a suspension of 5×10^6 spores/ml in 0.1% gelatin.

Inoculated plants were placed in a darkened dew chamber (Percival, model DC 20, Boone, IA) for 24 hr at 20 C, then transferred to a growth cabinet as described previously. Reisolations from diseased plants were done using the procedures described previously.

Effects of environment on disease development. *General inoculation procedures.* Plants (three- to five-leaf stage) were sprayed to wetness with conidia suspended in 0.1% (w/v) gelatin, using an atomizer mounted on a 250-ml screw-cap Erlenmeyer flask. Four or five replicate pots (12–15 plants) were used for each treatment in each of the experiments described below, and identical numbers of plants sprayed with 0.1% gelatin alone served as controls. Plants were sprayed from the sides and from above, to ensure complete coverage of the foliage. A suspension of alpha-conidia, adjusted to 5×10^6 spores/ml, was used in all experiments unless otherwise indicated. Approximately 1.0–1.5 ml of suspension was used for each pot. Except where indicated, plants were permitted to air-dry for approximately 10 min before incubation in a darkened dew chamber for 24 hr at 20 C. Plants were subsequently transferred to growth chambers adjusted as described previously. In all experiments described below, disease severity was assessed 15 days after inoculation.

Effect of inoculum concentration on disease severity. Alpha-conidia suspensions of 1×10^5, 5×10^5, 1×10^6, 5×10^6, and 1×10^7 spores/ml in 0.1% gelatin were applied to five replicate pots of bindweed seedlings. Control treatments and dew chamber and postinoculation conditions were as described.

Effect of dew period duration on disease severity. Field bindweed seedlings inoculated with a suspension of 5×10^6 spores/ml in 0.1% gelatin were subjected to dew periods of 0, 1.5, 3, 6, 12, 24, or 48 hr at 20 C. Control treatments and postinoculation conditions were as described.

Effect of temperature on disease severity. Three 24-hr dew period temperatures (10, 20, and 30 C) were compared with respect to their effects on disease severity. Control treatments and postinoculation conditions were as described. In a separate experiment, the effect of temperature during the incubation period following the 24-hr dew period (20 C) was determined. Day/night temperature regimes of 20/10, 20/20, and 30/20 C were evaluated. Other conditions were as described.

Effect of delay of onset of dew period on disease severity. Field bindweed seedlings produced and inoculated as described were immediately transferred to a growth cabinet under conditions as described. Plants were kept in the illuminated cabinet for a period of 0, 1.5, 3, 6, or 12 hr, then transferred to a dew chamber for 24 hr at 20 C. Subsequently, plants were returned to the growth cabinet and rated for disease severity after 15 days. In a similar experiment, plants were air-dried after inoculation for periods of 0, 12, 24, 36, or 48 hr before placement in the dew chamber. An additional treatment consisted of plants inoculated as described but never subjected to a dew period.

Assessment of disease severity and analysis of data. Foliar (leaf and stem) necrosis was assessed after 15 days on individual bindweed plants using a 0–4 rating system, with 0 = no visible symptoms, 1 = less than 25% necrosis, 2 = 25–50% necrosis, 3 = 51–75% necrosis, and 4 = greater than 75% necrosis. Seedling mortality was determined 15 days after inoculation by determining the number of individual plants that did not have new shoots growing from cotyledonary, stem, and underground hypocotyledonary axillary buds. Preliminary results indicated that such plants never showed regrowth and that inoculated shoots eventually became completely necrotic, collapsed, and died. The sampling unit for disease ratings and plant mortality was the individual plant in each pot, and individual ratings subsequently were pooled and averaged for each pot. Dry matter production was determined by severing the plants at ground level and drying them at 35 C for 6–7 days. Dry weights were recorded as grams per pot rather than per plant, since intertwining of bindweed vines made separation of individual plants difficult.

All experiments were repeated once, using a completely randomized design with four or five replications per treatment. Experimental results for the two trials of each experiment were pooled after testing for homogeneity of the variances using Bartlett's test (17). Differences of treatment means (dry matter) were established with Tukey's W test ($P = 0.05$). Linear regressions were performed on log-transformed dry matter data and the associated independent variable (17). Disease ratings were compared with the Friedman two-way analysis of variance by ranks test, and an ad hoc multiple-comparison procedure was subsequently used to test differences between treatment means (6).

RESULTS

Collection of plant material and disease symptoms. Lesions of various type and size were observed on leaves of field bindweed plants. Light brown lesions were rounded to irregular in outline, and in many cases this irregularity in appearance was due to veins at the edge of the lesion. The margins were quite distinct and often were surrounded by a yellowish green zone that varied in width but was usually narrow in comparison with the lesion. The central portion of the lesion became lighter in color than the outer portion as the lesion expanded (Fig. 1). *P. convolvulus* (11) was consistently isolated from tissue with these symptoms.

Pathogenicity tests. Small, pinpoint, foliar lesions were the first symptoms to appear on clonal shoots, usually within the first week. In immature tissue, these lesions expanded to form spots on leaves, petioles, and stems. In some cases, anthracnose-like symptoms appeared in areas where spores accumulated during inoculation, such as leaf margins and primary and secondary foliar veins. Infection also resulted in dieback symptoms of bindweed apices, usually affecting the last two unfolded leaves and the youngest folded leaf (which corresponds to the tip of the vine). In mature, fully expanded foliage of field bindweed,
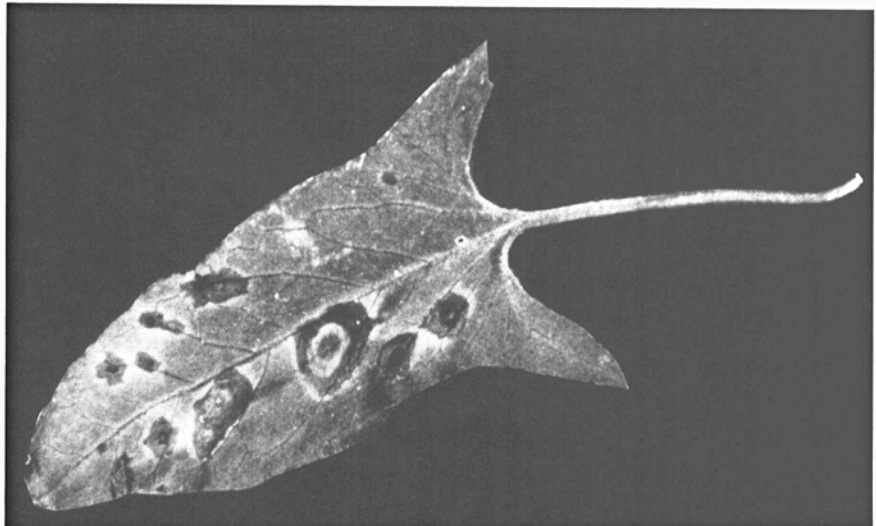

Fig. 1. Leaf of field bindweed collected from the field showing damage produced by *Phomopsis convolvulus*.

disease did not progress beyond the formation of leaf spots (1 mm diameter).

When seedlings were sprayed with spore concentrations of 5×10^6 spores/ml, scattered pale-brown flecks were the only visible symptoms 24 hr after inoculation. After 48 hr, leaves of inoculated plants were in an upright position and curved to the inside, stems and petioles were slightly twisted, and plant growth had ceased (Fig. 2A). By 72 hr, the vine tips, leaves, and cotyledons were showing symptoms of desiccation (Fig. 2B). Subsequently, the entire foliage acquired a light dull green color, and blighting was evident (Figs. 2C and D). Within 8–10 days, the entire plant, including the hypocotyl, was completely blighted. In leaves and stems of blighted plants, lighter pinpoint areas were observed, corresponding to the flecks observed at early stages of disease development.

Pycnidia were seen only on lower sections of tissue that were close to or in direct contact with the soil. Placing dead bindweed foliage on moistened filter papers always resulted in formation of pycnidia, usually within 10 days. Conidial droplets, which occurred readily on pycnidia produced in pure culture, were rarely observed on pycnidia formed on stems and leaves.

Effect of inoculum concentration. The degree of growth reduction in bindweed seedlings after inoculation with *P. convolvulus* was dose-dependent (Table 1, Fig. 3A). Inoculation with concentrations of less than 1×10^6 spores/ml failed to cause mortality of all seedling shoots. However, inoculum levels equal to or greater than 5×10^6 spores/ml resulted in

complete necrosis of the foliage with no regrowth. At intermediate inoculum concentrations (1×10^6 spores/ml), most field bindweed seedlings were killed, but shoot regrowth occurred from cotyledonary axillary buds. Regrowth was not vigorous, however, as illustrated by the low dry matter values.

Effect of dew period duration. Inoculated bindweed seedlings subjected to a dew period of less than 3 hr showed minimal disease development (Table 2). Disease development was restricted to localized necrotic spots in unfolded apical leaves, young internodes, or laminar veins.

Foliar damage occurred on plants subjected to a 6-hr period, but necrotic areas were restricted generally to vine tips and/or scattered necrotic spots. Although shoot mortality was low, reductions in dry matter were significant. A dew period of 12 hr did not result in plant mortality, but shoot regrowth was less vigorous than on plants subjected to fewer hours of dew (Table 2, Fig. 3B). This weak shoot regrowth, derived from cotyledonary axillary buds, contributed only a small amount of dry matter, and no differences were found in dry matter accumulation between plants subjected to dew periods of 12, 24, or 48 hr. Plants
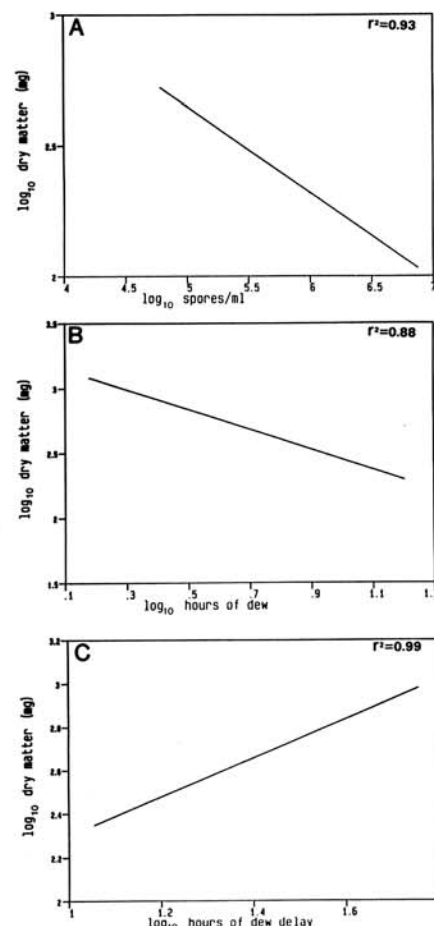
Table 1. Effect of inoculum concentration of *Phomopsis convolvulus* on severity of leaf spot of field bindweed

Inoculum concentration (spores/ml)	Disease rating[x]	Mortality (%)	Dry matter (g/pot)
1.0×10^5	0.9 a[y]	0	0.47 d[z]
5.0×10^5	2.0 ab	0	0.33 c
1.0×10^6	3.9 b	66.7	0.17 b
5.0×10^6	4.0 b	100.0	0.13 a
1.0×10^7	4.0 b	100.0	0.11 a

[x] 0 = No visible symptoms, 1 = less than 25% necrosis, 2 = 25–50% necrosis, 3 = 51–75% necrosis, and 4 = greater than 75% necrosis.
[y] Means followed by the same letter in the same column are not significantly different at $P = 0.05$, according to the Friedman test.
[z] Means followed by the same letter in the same column are not significantly different at $P = 0.05$, according to Tukey's W test.

Table 2. Effect of dew period duration on severity of leaf spot of field bindweed caused by *Phomopsis convolvulus*

Dew hours	Disease rating[w]	Mortality (%)	Dry matter (g/pot)
Control[x]	0 a[y]	0	1.02 c[z]
0	0 a	0	0.85 c
1.5	0 a	0	1.00 c
3.0	1.3 a	0	0.98 c
6.0	2.8 ab	8.3	0.56 b
12.0	4.0 b	66.7	0.15 a
24.0	4.0 b	100.0	0.12 a
48.0	4.0 b	100.0	0.12 a

[w] 0 = No visible symptoms, 1 = less than 25% necrosis, 2 = 25–50% necrosis, 3 = 51–75% necrosis, and 4 = greater than 75% necrosis.
[x] Mean value for inoculated control plants for all treatments.
[y] Means followed by the same letter in the same column are not significantly different at $P = 0.05$, according to the Friedman test.
[z] Means followed by the same letter in the same column are not significantly different at $P = 0.05$, according to Tukey's W test.
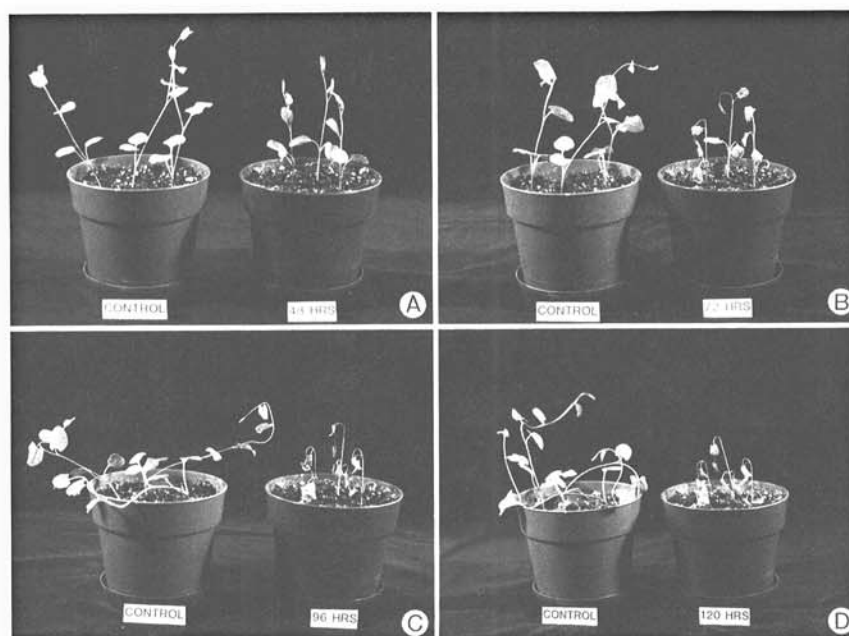
Fig. 2. Seedlings (10–14 days old) of field bindweed inoculated with a conidial suspension of *Phomopsis convolvulus* (5×10^6 spores/ml) and subjected to a 24-hr dew period. Disease development at **(A)** 48 hr, **(B)** 72 hr, **(C)** 96 hr, and **(D)** 120 hr.

Fig. 3. Linear relationships between bindweed tissue dry matter and **(A)** inoculum concentration, **(B)** length of dew period, and **(C)** delay of dew period.

inoculated with the same spore concentration and kept for 24 hr or longer in the dew cabinet always were killed.

Effect of dew period temperature. Disease severity was greater in plants incubated at 20 C than in those incubated at 10 C (Table 3). There were no differences in dry matter accumulation or disease severity between plants incubated at 20 and 30 C. Formation of dew droplets on plant surfaces was restricted at 10 C. Although this was partly compensated for by spraying plants in the dew chamber with a fine mist of distilled water, the total amount of free water formed on the bindweed seedlings was visibly lower than that at 20 and 30 C.

Effect of temperature during the incubation period. There were no significant differences among inoculated plants for any of the temperature combinations tested. However, inoculated plants exposed to day/night incubation temperatures of 30/20 C showed more tissue dehydration than those incubated at lower temperatures.

Effect of length of delay of onset of dew period on disease severity. In the experiment in which the air-drying periods were from 0 to 12 hr (with continuous illumination), no differences were observed between treatments for any parameter measured. Similarly, disease ratings were unchanged by delaying the 24-hr dew period for up to 48 hr after inoculation, as compared with the plants that were immediately transferred (Table 4). However, plant mortality decreased as the period of delay increased. Shoot regrowth was commonly observed among plants in which the delay was 24 hr or more, and this was reflected in the higher dry matter production in these plants than in those subjected to fewer hours (Fig. 3C). Nevertheless, regardless of the time between inoculation and placement in the dew chamber, all inoculated plants produced significantly lower amounts of dry matter than untreated seedlings at the time of harvesting.

DISCUSSION

Despite its importance elsewhere, field bindweed has never been considered an important weed in Quebec, although it probably has been present in the province since the arrival of the early settlers (15). The weed prefers warm, dry, sunny habitats (22), and diseased bindweed was rarely found in such locations. Leaf spots usually were observed on plants located in moist, shaded habitats.

The number of fungi reported associated with field bindweed is limited, and most of these reports refer to isolations from dead plant material, thus leaving pathogenicity open to doubt (1,5). Most of the fungal species recovered from *Convolvulus* have been reported from Europe (9,16), and only five species were reported from North America: *Septoria*

Table 3. Effect of temperature during a 24-hr dew period on severity of leaf spot of field bindweed caused by *Phomopsis convolvulus*

Dew period temperature (C)	Disease rating[w]	Mortality (%)	Dry matter (g/pot)
Control[x]	0 a[y]	0	0.89 c[z]
10	2.3 a	0	0.45 b
20	4.0 b	100.0	0.11 a
30	3.7 b	82.3	0.17 a

[w] 0 = No visible symptoms, 1 = less than 25% necrosis, 2 = 25–50% necrosis, 3 = 51–75% necrosis, and 4 = greater than 75% necrosis.
[x] Mean value for noninoculated control plants for all treatments.
[y] Means followed by the same letter in the same column are not significantly different at $P = 0.05$, according to the Friedman test.
[z] Means followed by the same letter in the same column are not significantly different at $P = 0.05$, according to Tukey's W test.

convolvuli Desm., *S. flagellaria* El. & Ev., *S. septulata* Beach, *Stagonospora convolvuli* Dearn & House (1,3), and *Rhabdospora* sp. (2). Only *Rhabdospora* has been proved pathogenic to field bindweed seedlings (2). In a recent report of natural enemies of field bindweed in California, the presence of leaf spots was reported, but no attempts were made to isolate fungi from diseased plants (13).

P. convolvulus produces distinctive foliar damage on inoculated field bindweed plants, primarily expressed in the form of leaf spots or anthracnose lesions. Inoculations at concentrations of 5×10^6 spores/ml produced severe damage and usually resulted in a rapid blighting of bindweed seedlings and shoots. This rather rapid blighting of the foliage, although obtained in a controlled environment, indicated that *P. convolvulus* was as virulent as other fungi being evaluated as mycoherbicides (7,20,23,24).

The results from the experiment in which the onset of the dew period was delayed indicate that inoculum potential is maintained on dry leaves for at least 24 hr following inoculation. This would be a useful property for a mycoherbicide, although field evaluation of this characteristic is required. The length of the dew period proved to be of prime importance for symptom development and disease expression. Cumulative effects of short dew periods, interrupted by dry periods, on infection of bindweed by *P. convolvulus* are as yet undetermined. Decreasing temperatures from 20 to 10 C during the dew period resulted in lower disease ratings and decreased plant mortality. However, these two parameters were not affected when the temperature in the dew chamber was increased to 30 C. A similar temperature effect was observed in related studies on germination of conidia in vitro (Ormeno, *unpublished*). It was apparent in those studies that at 10 C, spore germination and germ tube growth were delayed such that little or no

Table 4. Effect of length of delay of onset of dew period on severity of leaf spot of field bindweed caused by *Phomopsis convolvulus*

Number of dry hours[v]	Disease rating[w]	Mortality (%)	Dry matter (g/pot)
No dew[x]	0 a[y]	0	1.64 d[z]
48	3.9 b	0	0.79 c
36	3.8 b	33.3	0.60 b
24	3.9 b	58.3	0.47 b
12	4.0 b	83.3	0.23 a
0	4.0 b	100.0	0.21 a

[v] Number of hours in which inoculated plants were kept in growth cabinet before transfer to dew cabinet.
[w] 0 = No visible symptoms, 1 = less than 25% necrosis, 2 = 25–50% necrosis, 3 = 51–75% necrosis, and 4 = greater than 75% necrosis.
[x] Inoculated plants not subjected to dew period.
[y] Means followed by the same letter in the same column are not significantly different at $P = 0.05$, according to the Friedman test.
[z] Means followed by the same letter in the same column are not significantly different at $P = 0.05$, according to Tukey's W test.

appressorium formation occurred during the dew period.

A major obstacle for obtaining satisfactory control of field bindweed is its regeneration ability (19). Shoot regrowth occurs from damaged established plants and older seedlings (18). This regenerative capacity also was observed in this study. In some instances, new shoots emerged within 10 days from plants with as much as 75% necrosis 5 days after inoculation. Shoot regrowth was observed to originate from the two cotyledonary axillary buds and/or the lower portion of the hypocotyl. This was of particular importance, since lower sections usually were the last part of the seedling to collapse. High weed mortality was achieved when all dormant axillary buds became necrotic and the hypocotyl was completely destroyed. Two or more applications of *P. convolvulus* may be required for complete control of established plants.

These results indicate that *P. convolvulus* was effective in reducing growth of bindweed seedlings over a wide range of environmental conditions. This pathogen, therefore, merits further investigation with respect to its potential in the biological control of field bindweed.

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