Ecology and Epidemiology

Effects of Spore Concentration, Temperature, and Dew Period on Disease of Field Bindweed Caused by *Phoma proboscis*

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This research was supported in part by a grant from CIBA-GEIGY Corporation and by USDA/CSRS Special Grant No. 89-34195-4378. Published with the approval of the Director of the Arkansas Agricultural Experiment Station. Accepted for publication 29 March 1991 (submitted for electronic processing).

ABSTRACT

Heiny, D. K., and Templeton, G. E. 1991. Effects of spore concentration, temperature, and dew period on disease of field bindweed caused by *Phoma proboscis*. Phytopathology 81:905-909.

Spore germination of *Phoma proboscis* in vitro and resulting disease development were evaluated over a range of spore concentrations and after incubation at nine dew periods at each of five temperatures. Reduced percent germination on water agar occurred at spore concentrations above 10^7 spores ml⁻¹. Spore germination on agar was optimal at 24 C. High levels of disease occurred on plants that received 12 h or more of dew

at all temperatures tested, except 32 C. Fresh weight reduction in shoots and roots correlated well with disease ratings. Disease was enhanced relative to constant temperature treatments when plants from dew period temperatures of 16 and 20 C were maintained at a postdew temperature of 24 C. The results of these studies suggest that *P. proboscis* has potential for use as a mycoherbicide.

Additional keywords: biological control, Calystegia sepium, Convolvulus arvensis, epidemiology, mycoherbicides

Field bindweed (Convolvulus arvensis L.), a perennial, herbaceous vine ranked as the twelfth worst weed in the world (6), is distributed throughout most of the United States (2). Heavy infestations cause yield reductions ranging from 33% for winter wheat to 75% for summer-growing crops (15). In 1980, nearly 810,000 ha of crop land in 30 California counties was reported to be infested by field bindweed, resulting in a loss of about \$25 million (11). Although tillage and herbicides are frequently effective in controlling seedling and first-year bindweed plants, destruction of shoots of older plants stimulates vigorous regeneration of shoots from the root buds (16). Complete control of field bindweed sometimes requires up to 5 yr of herbicide applications (16).

Recently, attention has been directed toward the potential of fungi as biocontrol agents of field bindweed (7,8,9,12). Ormeno-Nuñez et al (8) described blighting of field bindweed seedlings and shoots by *Phomopsis convolvulus* Ormeno over a range of

environmental conditions. *Phoma proboscis* Heiny causes symptoms similar to those caused by *P. convolvulus* (5). Here we report an assessment of the impact of disease on field bindweed populations treated with *P. proboscis* under controlled parameters of spore concentration, dew period, and temperature.

MATERIALS AND METHODS

General. Seeds of field bindweed were purchased from Valley Seed Service (Fresno, CA). Seeds were surface sterilized in 0.26% sodium hypochlorite for 1 min, washed repeatedly, presoaked between wet paper towels for 24 h at 22 C, and planted about 1 cm deep in plastic pots 8 cm in diameter, containing vermiculite. After 2 wk in the greenhouse (at 24–32 C), seedlings had one to three true leaves and were thinned to 15 plants per pot unless otherwise stated. Pots were watered daily and fertilized weekly with Peters Professional water soluble fertilizer (N-P-K:20-20-20) at 470 ppm. Excepting spore concentration tests, plants were 2 wk old when inoculated and were held at the inoculation temperature for 1 day before use. Experiments were repeated

once. Because patterns of disease ratings were similar between experiments, fresh and dry weights of roots and shoots were recorded for only one set of experiments.

The isolate of *P. proboscis* was obtained from infected field bindweed collected in Phillips County, CO. Cultures were grown by spreading water suspensions of spores (conidia) on acidified potato-dextrose agar amended with streptomycin sulphate (3 mg ml⁻¹) and incubating at 24 C with a 12-h photoperiod. Inoculum was prepared by scraping spores from 5- to 7-day-old cultures into distilled water, filtering through cheesecloth, and centrifuging to concentrate spores when necessary. Concentrations of spore suspensions were adjusted with distilled water based on hemacytometer counts. Inoculum was applied to seedlings with a hand-operated aerosol applicator that delivered about 1.5 ml per pot. Dew at specified temperatures was provided by unlit dew chambers (Percival Manufacturing Company, Boone, IA). The growth chambers used were Conviron E-7 models (Asheville, NC) with cool-white fluorescent plus incandescent light at 300 μ E·m⁻²·s⁻¹.

Measurement of disease impact. Disease severity on each plant was assessed with a 0-7 disease rating scale: 0 = no disease; 1 = dieback of bud or youngest leaf and petiole only; 2 = rating 1 plus lesion at juncture of young shoot; 3 = rating 2 plus young shoot death encompassing adjacent true leaf; 4 = rating 3 plus death of additional young leaves; 5 = rating 4 plus one or both cotyledons killed; 6 = rating 5 plus lesion below cotyledonary node; 7 = plant death.

For biomass measurements, all plant tissue above the vermiculite surface (shoots) was clipped and weighed. Roots were washed free of vermiculite, blotted with paper towels, and surfacedried at room temperature with circulating air for about 10 min. For dry weight measurements tissues were placed in an oven at 85 C for at least 15 h.

Determination of percent spore germination. Aliquots (0.1 ml) of various concentrations (indicated below) of spores were spread onto surfaces of 1.5% water agar and held at the temperatures and intervals used for plant inoculations (indicated below). Germination was arrested with lactophenol cotton blue at various time intervals, as described below. More than 300 spores were counted for each treatment unless otherwise indicated.

Effect of spore concentration and temperature on disease development. Eight pots with 10 seedlings each (3-wk-old seedlings with three to four true leaves) were inoculated with 0, 10⁴, 10⁵,

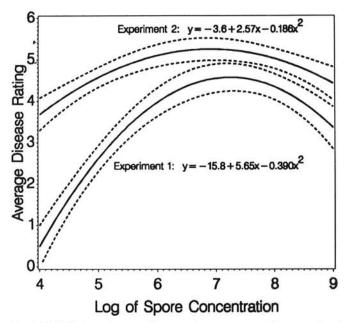


Fig. 1. Field bindweed average disease rating response to *Phoma proboscis* spore concentration for two similar experiments (40 plants per treatment). Seedlings were incubated in dew for 12 h at 20 or 24 C following inoculation and rated after 2 wk. Results at 20 and 24 C were averaged together for each experiment. Broken curves are 95% confidence bands.

10⁶, 10⁷, 10⁸, or 10⁹ spores ml⁻¹. Spores from each concentration were also spread on water agar plates. The pots and plates were placed in dew for 12 h at 20 or 24 C (simultaneous experiments, 28 pots per temperature). Pots were transferred to a 24-C/21-C, 14-h photoperiod growth chamber with approximately 60% RH. Germination on agar plates was stopped after 12 h, and at least 200 spores per treatment were counted. After 2 wk, disease was rated and fresh and dry weights of shoots and roots were recorded. The effect of spore concentration at the two temperatures on disease ratings was analyzed by regression.

Effect of dew period and temperature on disease ratings. Seedlings in 27 pots inoculated with 4×10^7 spores ml⁻¹, 27 pots of noninoculated seedlings, and spore germination plates were placed in dark dew chambers at 16, 20, 24, 28, or 32 C. At 3-h intervals up to 24 h, three inoculated pots, three noninoculated pots, and germination plates were removed. Pots were placed in a growth chamber at the same temperature as the dew temperature. Spore germination on plates was stopped with lactophenol cotton blue. An additional six pots and germination plates were transferred from dew after 48 h. Growth chambers were programmed for 14-h days and 10-h nights with temperature (C) settings of 16/13, 20/17, 24/21, 28/25, or 32/29. Disease ratings were recorded 1 and 2 wk after inoculation. Nonlinear regression analysis was used to characterize the effect of temperature and dew period on disease ratings averaged over two replications of the experiment. The model used can be written as follows:

$$Y = A(1 - B^X) \tag{1}$$

in which Y represents disease rating, X equals the dew period in hours, and A and B are constants to be estimated; A is asymptotic disease rating and B is inversely related to the rate of disease development. Fresh and dry weights of shoots and roots were measured at 2 wk after inoculation.

Four pots inoculated at 4×10^7 spores ml⁻¹ and four non-inoculated pots were placed in dew for 12 h at each of five temperatures: 16, 20, 24, 28, or 32 C. All pots were placed at a postdew incubation temperature of 24/21 C with a 14-h photoperiod. After 2 wk, plants were rated for disease development, and fresh and dry shoot and root weights were recorded. These 24 C postdew treatments (Group A) were compared to the 12-h dew treatments at each of the five temperatures, in which postdew temperature was the same as the dew temperature (Group B).

TABLE 1. The effect of spore concentration on fresh and dry biomass of field bindweed inoculated with *Phoma proboscis*^{a,b}

Inoculum concentration (spores/ml)	Fresh shoots (g/pot)	Fresh roots (g/pot)	Dry shoots (g/pot)	Dry roots (g/pot)
0	2.44	1.05	0.27	0.13
104	2.12	0.98	0.25	0.13
105	2.03	0.93	0.23	0.11
106	1.23	0.71	0.19	0.07
107	0.77	0.52	0.14	0.06
108	1.03	0.79	0.17	0.09
109	1.70	0.91	0.22	0.09
SE	0.122	0.072	0.016	0.011
Significance of to	rend:c			
Linear	P < 0.01	P = 0.13	P = 0.01	P = 0.01
Quadratic	P < 0.01	P < 0.01	P < 0.01	P < 0.01
Cubic	P < 0.01	P = 0.17	P = 0.04	P = 0.97

^a Plants were incubated 12 h in dark dew at 20 or 24 C, transferred to a growth chamber (24 C, 14 h light/21 C, 10 h darkness) and harvested after 2 wk.

^b Data are means of eight pots: four pots from 20 C and four pots from 24 C. Each pot contained 10 plants.

Orthogonal polynomials (linear, quadratic, and cubic) were fit to the data, excluding controls. There was no lack of fit nor was there interaction with temperature.

RESULTS

Effect of spore concentration on percent germination in vitro. Optimum germination (96%) of spores on water agar at 20 C after 12 h occurred at 10⁵ spores ml⁻¹, and was slightly less (94%) at 10⁴ and 10⁶ spores ml⁻¹. Percent germination dropped to 86%, 43%, and 7% at 10⁷, 10⁸, and 10⁹ spores ml⁻¹, respectively. Percent germination in 12 h at 24 C was nearly 100% for concentrations up to 10⁷ spores ml⁻¹ but was greatly reduced at 10⁸ (54%) and 10⁹ spores ml⁻¹ (10%).

Effect of spore concentration and temperature on disease development. Average disease ratings after 2 wk increased with each 10-fold increase in spore concentration up to 10^7 spores ml⁻¹ at both 20 and 24 C (Fig. 1). Disease ratings decreased at higher concentrations. Disease at 20 C was slightly higher but not statistically different from disease at 24 C (P = 0.085) at all concentrations except 10^8 and 10^9 spores ml⁻¹, at which ratings from 20 C treatments were slightly less than from 24 C treatments. Ratings at the two temperatures were combined for analysis. The replications of the experiment were significantly different, but the resulting disease patterns were similar, with a peak of disease ratings at 1.7×10^7 spores ml⁻¹ in the first experiment, and at 8.1×10^6 spores ml⁻¹ in the second experiment (Fig. 1). The residual error from regression was sufficiently small to discount higher polynomial factors. No lack of fit was found in either regression equation (Fig. 1).

No differences in fresh weights of shoots or roots were found between 20 and 24 C at any spore concentration, and data were combined. With temperatures pooled, inoculum concentrations affected the amount of bindweed biomass produced (Table 1). Lowest yields occurred in the pots treated with 10⁷ spores ml⁻¹ (Table 1).

Percent germination of spores at various temperatures. Germination rate was slowest at 16, 28, and 32 C and maximal at 24 C (Fig. 2). Percent germination was reduced through 18 h for both 16 and 28 C treatments relative to 20 and 24 C treatments and was lowest throughout at 32 C.

Effect of dew period and temperature on disease ratings. Disease did not develop on noninoculated plants. Disease severity in inoculated treatments increased during the interval from 1 to 2 wk after inoculation at all temperatures. At 32 C, low disease severity increased only slightly (Fig. 3). At 2 wk, high levels of disease were achieved in treatments that received at least 12 h of dew, and the lowest amount of disease occurred at 32 C. A nonlinear regression model (eq. 1) adequately approximated the effects of temperature and dew period on disease severity (Fig. 4). There was no systematic departure from the fitted model. Disease severity at 20 C was not significantly different from disease severity at 24 C. However, 16 and 28 C had overlapping disease

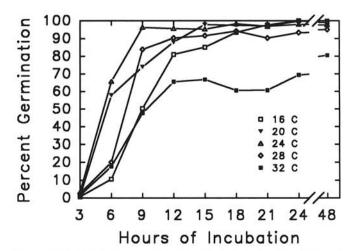


Fig. 2. Percent germination on water agar of *Phoma proboscis* conidia $(4 \times 10^7 \text{ spores ml}^{-1})$ incubated for various times at five temperatures. More than 300 spores were counted for each treatment per experiment.

rating curves that differed significantly from the 20 and 24 C curves as dew periods increased beyond 12 h.

Plants from the 16 and 20 C dew temperature treatments subjected to a postdew period temperature of 24 C (Group A) developed significantly greater disease than plants given 12-h dew treatments at the corresponding temperatures followed by incubation at the corresponding temperatures (Group B). A quadratic regression model for disease rating on dew temperature was fit to the means (average across pots) for each replication and postdew group (Fig. 5). Slight disease developed in the 32-C dew, 24-C postdew treatment, but the variance was much less than at the other temperatures, so this factor was left out of the model. The two models were restricted to have a common value for the same treatment that occurred in both groups (24-C dew, 24-C postdew). There was no lack of fit in either regression model.

Effect of temperature, dew period, and disease on biomass. Because P. proboscis infects young shoots and buds of field

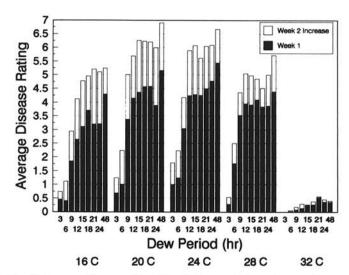


Fig. 3. Average disease ratings (45 plants per treatment averaged over two replications) of field bindweed seedlings inoculated with *Phoma proboscis* and given nine dew-period treatments at each of five temperatures. The ratings after 1 and 2 wk are presented for comparison.

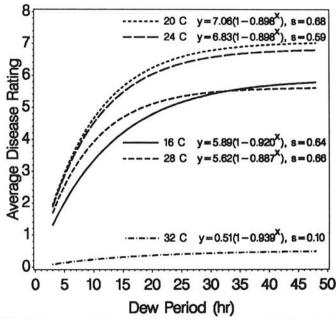


Fig. 4. Response of disease rating to increasing dew periods in each of five temperatures, for which s = the root mean square error from fitting the nonlinear regression model (eq. 1). SE of A, B: 16 C, 0.646, 0.020; 20 C, 0.557, 0.021; 24 C, 0.483, 0.019; 28 C, 0.503, 0.028; 32 C, 0.132, 0.031.

bindweed, growth suppression was an obvious effect of disease. However, minor infections often allowed subsequent rebudding from the cotyledonary node or new bud formation in the main root below the vermiculite surface. Increasing dew periods resulted in progressively less shoot tissue available for harvest in inoculated treatments.

Fresh weights of shoots correlated well with fresh weights of roots (r = 0.72, P < 0.000l), indicating that root development was impaired by shoot infection. The dry shoot to dry root weight correlation was not as high (r = 0.63, P < 0.000l). However, fresh shoot weight to dry shoot weight had a high correlation (r = 0.94, P < 0.000l). Therefore, only the results of fresh shoot weight analysis are presented here (Fig. 6).

Fresh weight reduction in shoots, determined by subtracting mean inoculated biomass from mean noninoculated biomass for each dew-temperature treatment, correlated well with disease ratings (r = 0.84, P < 0.0001). High disease ratings were coincident with large fresh weight differences. The maximum fresh weight reductions (growth suppression due to disease) resulted from treatments of 18-h dew at 16 C, 18-h dew at 20 C, 48-h dew at 24 C, and 48-h dew at 28 C (Fig. 6). The differences in fresh biomass per dew period at 32 C, at which little disease occurred, ranged from 0.29 g to -0.34 g per pot. The highest mean weight difference (2.60 g per pot) occurred in the 24-C, 48-h dew period treatment. The smallest difference, other than in the 32-C treatment, was 0.22 g per pot in the 20-C, 6-h dew period treatment. It was also noted that noninoculated plants given a 48-h dew treatment were slightly smaller than the other control plants, probably because of the extended dark period to which they were subjected. Although plant growth in infected and noninoculated pots given the same dew treatment may be proportional, it is possible that weight reduction values for the 48-h treatments were artificially small because of the darkness effect on controls.

Growth of inoculated field bindweed seedlings (Group A) in the first replication of the experiment was significantly reduced when incubated at 24 C after any dew temperature except 32 C (Table 2).

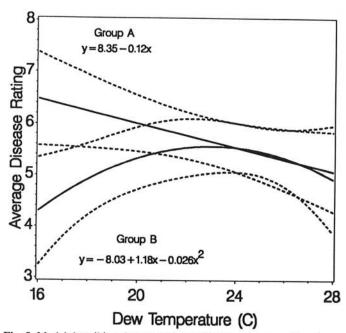


Fig. 5. Model describing the comparison of average disease ratings from field bindweed plants given 12 h of dew at one of five temperatures followed by postdew incubation at 24 C (Group A) to ratings from plants placed at a constant temperature during and after a 12-h dew treatment (Group B). The fit for Group A did not significantly (P=0.10) depart from linearity. Broken curves are 95% confidence bands. Plants were inoculated with *Phoma proboscis* conidia at 4×10^7 spores ml⁻¹ before dew treatments.

DISCUSSION

The optimum conditions for adequate infection of field bindweed seedlings with P. proboscis are an inoculum concentration of about 10^7 spores ml⁻¹, a temperature of 20–24 C, and a dew period of at least 12 h. Postdew incubation at 24 C enhances disease development when dew period temperatures are less than 24 C. This confirms the optimum condition of 24 C. At a field site in northwest Arkansas from June through September of 1988, the dew period exceeded 12 h on 38 days when temperatures ranged from 12 to 31 C (Heiny, unpublished). From June through August of 1989 the dew period exceeded 12 h on 68 days during which temperature minimums were at or below 24 C. More than 12 h of dew corresponded to temperature means at 24 C or below on 35 days (Heiny, unpublished). Thus, natural environmental conditions should be favorable for field bindweed disease caused by P. proboscis. Obviously, extending the dew period to 24 or 48 h would be desirable if P. proboscis were used as a mycoherbicide. The problem of the lack of optimum dew periods is common to a number of potential mycoherbicides but might be overcome by formulation techniques (1,10,14).

Other prerequisites to mycoherbicide development include determination of storability and host range limitations. Preliminary work indicates that spores of *P. proboscis* can withstand drying with only a slight drop in viability. After 5 mo of dry storage at room temperature, germination after 24 h at 24 C was still 70-80% (Heiny, unpublished). Preliminary host-range tests determined that hedge bindweed (Calystegia sepium (L.) R. Br.) is susceptible to infection but less severely damaged than *C. arvensis*. None of the crops tested so far are susceptible. More complete host-range testing with *P. proboscis* will be reported elsewhere.

One of the difficulties encountered in attempting to kill field bindweed is the capacity of seedlings to regenerate (13). Following injury, buds develop in the cotyledonary nodes or below the soil surface at the base of the hypocotyl or from the main root. However, if the shoot is severely infected below the cotyledons, the seedling dies before regeneration is possible. Ormeno-Nuñez et al (8) also observed this phenomenon in seedlings inoculated with P. convolvulus. Studies of the effect of light on field bindweed suggested that plants under a crop canopy may be more susceptible to control because of reduced vigor (3). A foliar mycoherbicide for bindweed might be applied early in the season to reduce the population and favor crop canopy establishment, then reapplied when bindweed plants are weakened by crop shading. The effect of light on disease caused by P. proboscis and the potential of P. proboscis for controlling older bindweed plants remain to be investigated.

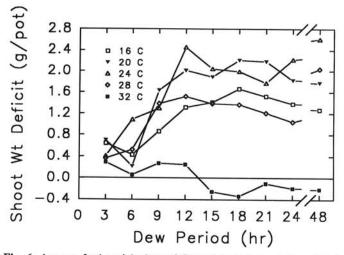


Fig. 6. Average fresh weight loss of field bindweed shoots inoculated with *Phoma proboscis* at five temperatures and nine dew periods. Fresh weight loss is the difference between the means of three noninoculated and three inoculated pots 2 wk after inoculation.

TABLE 2. Fresh and dry biomass of field bindweed noninoculated or inoculated with *Phoma proboscis* and incubated at selected dew temperatures followed by incubation at 24 C

Tissue type	Dew period temp. ^a (C)	Fresh tissue			Dry tissue ^d		
		Noninoculated (g/pot) ^b	Inoculated (g/pot) ^b	Significance ^c	Noninoculated (g/pot) ^b	Inoculated (g/pot) ^b	Significance
Shoots	16	2.54	0.40	***	0.31	0.13	***
	20	3.05	0.35	***	0.36	0.10	***
	24	2.82	0.70	***	0.36	0.14	***
	28	2.90	1.10	***	0.32	0.19	***
	32	2.70	2.62	NS	0.32	0.28	NS
Roots	16	1.50	0.73	***	0.17	0.05	***
	20	1.61	1.10	*	0.20	0.05	***
	24	1.30	0.50	***	0.21	0.05	***
	28	1.38	0.93	*	0.15	0.11	*
	32	1.53	1.81	NS	0.21	0.17	NS

^a Plants were incubated 12 h in dew, then transferred to a lighted growth chamber at 24 C, 14 h/21 C, 10 h day/night environment.

d Tissue dried at least 15 h at 85 C.

Disease occurring in plants treated with 10⁸ and 10⁹ spores ml⁻¹ was not reduced as much as expected considering germination inhibition on water agar. Runoff and distribution over plant surfaces may partially dilute the spore suspensions. Contact with plant surfaces may also compensate in some way for unknown factors responsible for germination inhibition in vitro.

The low level of disease in inoculated plants incubated at 32 C has interesting physiological implications. Since more than 50% of spores do germinate on water agar at 32 C, the reduced ability to cause disease may be related to factors in the host. Resistance mechanisms in the host and penetration processes of the fungus under varying conditions of moisture and temperature need further study.

The fungal pathogen, *P. proboscis*, has satisfied some of the requirements of a biological weed control agent as outlined by Daniel et al (4): it produced abundant and durable inoculum in artificial culture, and infection of field bindweed was rapid enough to greatly reduce weed stands within a reasonable time after treatment. Host range specificity and performance under field conditions are additional criteria that must be assessed to determine the mycoherbicide potential of this pathogen.

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^b Data are means of four replicates (pots), each containing 15 plants. Biomass was measured 2 wk after inoculation with 4 × 10⁷ spores ml⁻¹.

Degree of significance by linear contrast of noninoculated to inoculated biomass (P = 0.05, *; 0.01, **; 0.001, ***; NS = not significant).