

## The Influence of Osmotic Water Potential on the Linear Growth of *Endothia* Species

Phil P. Hunter, Gary J. Griffin, and R. Jay Stipes

Graduate Research Assistant and Associate Professors, respectively, Department of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

The authors wish to thank Jean Ratliff for technical assistance and H. B. Couch for the cultures of *Fusarium roseum* and *Rhizoctonia solani*.

Accepted for publication 3 May 1976.

### ABSTRACT

HUNTER, P. P., G. J. GRIFFIN, and R. J. STIPES. 1976. The influence of osmotic water potential on the linear growth of *Endothia* species. *Phytopathology* 66: 1418-1421.

The effect of osmotic water potential on the linear growth of *Endothia gyrosa*, *E. parasitica*, *E. singularis*, *E. tropicalis*, and *Fusarium solani*, together with reference fungi, was investigated. In general, all species of *Endothia* and *F. solani* exhibited decreasing growth with decreasing osmotic water potential. The growth of certain isolates was stimulated on KCl- and sucrose-adjusted media and inhibited slightly on

NaCl- and salt mixture-adjusted media. The *E. gyrosa* isolates were able to grow at -60 bars in vitro. Observations of the changes in water potential in newly pruned branch stubs of pin oak (*Quercus palustris*) and the rate of ingress of *E. gyrosa* into such stubs showed that the fungus typically invaded the pruned stubs before water potentials reached -60 bars.

*Additional key words:* canker, thermocouple psychrometry, moisture stress.

In 1970, Stipes and Phipps (11) reported a new disease of pin oak (*Quercus palustris* Muenchh.) at Hampton, Virginia. The organism inciting the blighting symptoms later was identified as *Endothia gyrosa* (Schw.) Fries (7). The ends of pruned branch stubs served as the infection court. After meteorological records and the results of preliminary experiments were evaluated, an hypothesis was developed that involved moisture stress as a factor predisposing pin oak to the disease. Cook et al. (1) suggested that following plant infection the osmotic component ( $\psi_{\pi}$ ) of water potential ( $\psi$ ) would probably be more important in the growth of soilborne pathogens. Our observations of the pruned branch stub infection court suggested that a similar phenomenon might be occurring in this disease, since these stubs were found to be points of reduced tissue water potential. The technique of simulating decreasing water potential by incorporating various osmotica into an artificial medium has been employed in studies of the influence of soil and plant water potential in the ecology of several plant pathogens (1, 2, 3, 4, 5, 10). As a part of the investigations of our moisture stress-predisposition hypothesis, the studies reported herein were conducted to determine the effects of osmotic potential on the growth of *E. gyrosa* on agar media and to relate these effects to the in vivo growth of the fungus.

### MATERIALS AND METHODS

**Osmotic water potential and linear growth.**—Five isolates of *E. gyrosa* were tested. Also included were three isolates of *E. parasitica* (Murr.) P. J. & H. W. Anderson, one each of *E. tropicalis* Shear & Stevens and *E.*

*singularis* (H. & P. Syd.) Shear & Stevens, and an isolate of *Fusarium solani* (Mart.) Appel. & Wr. emend. Snyd. & Hans. obtained from a canker on tulip poplar (*Liriodendron tulipifera* L.). The additional *Endothia* spp. were included to test the intrageneric responses to controlled osmotic water potential. The *F. solani* isolate was included because, like *E. gyrosa*, it requires a bark wound for infection and produces certain similar symptoms. One isolate each of *Fusarium roseum* (Lk.) Fr. and *Rhizoctonia solani* Kühn, both from turf, and *Aspergillus flavus* (Lk.) Fr. from peanut were included for reference, since information about their growth on such media has been reported by Cook et al. (1), Dubé et al. (2), and Griffin et al. (3), respectively.

The methods used were similar to those of Manandhar and Bruehl (5). Difco cornmeal dextrose agar (CMDA), 19 g/liter, was used as the basal medium. The osmotic potential was adjusted to -10, -20, -30, -60, -90, and -120 bars by adding NaCl, KCl, a salt mixture [NaCl, KCl, and Na<sub>2</sub>SO<sub>4</sub> (5:3:2, w/w)], or sucrose at selected molal concentrations (8, 9). The osmotic potential of the basal medium was assumed to be -1.38 bars as given by Manandhar and Bruehl (5). The required amount of a particular osmoticum was dissolved in 500 ml of distilled deionized water in a 2-liter Erlenmeyer flask which was covered with aluminum foil to prevent evaporation. The CMDA then was added and dissolved. The medium was autoclaved for 20 minutes at 121 C and 20 ml were used per petri plate. Each plate was seeded at the edge with a 7-mm plug cut from the margin of a 7-day-old culture grown on CMDA. Each isolate-salt combination was replicated four times. Plates were incubated in closed plastic containers at 25 C for 10 days, at which time linear growth was measured from the edge of the seed plug to the margin of the colony. The experiment was repeated. Although there were some differences between the

experiments in the absolute values for linear growth, the trends remained the same.

**Water potential measurements.**—Thermocouple psychrometers were used to determine the effect of pruning on the water potential of stems of pin oak. A single branch (approximately 3 m above the ground) on each of 16 6-year-old trees of similar height and vigor was pruned on 1 June 1975. At 30-day intervals thereafter, the water potential of the stubs on four trees was measured at various distances from the pruned end (see Table 5) using temperature-compensated thermocouple psychrometers. The methods used were similar to those described by Wiebe et al. (12). The psychrometers were placed in holes  $0.71 \times 1$  cm. The holes first were drilled to less than 1 cm with a pointed bit using a hand drill, then extended to 1 cm with a flat-tipped bit. The implanted psychrometers were sealed with grafting compound and covered with 2.5 cm of foam rubber. The psychrometers previously had been calibrated using 0.2, 0.5, 1.0, and 1.5 molal KCl solutions at 10, 20, 30, and 40 C. An equation for predicting water potential from microvolt output and temperature was developed from the calibration data as

described by Meyn and White (6).

**Artificial inoculation studies.**—The rate of ingress of *E. gyrosa* into pruned branch stubs of pin oak was determined by artificially inoculating six trees on 1 July 1975. These trees were located in the same plot as those used in the water potential studies. The inoculation procedure consisted of pruning branches 30 cm from the main stem and applying a 7-mm diameter plug of mycelium cut from the edge of a 7-day-old culture of *E. gyrosa* grown on glucose-yeast extract medium. A plug of the culture medium served as control inoculum. The plugs were covered with a slightly moist piece of cheesecloth, then the ends of the pruned branches were wrapped with polyethylene and sealed with tape. After 2-3 weeks, the cheesecloth and polyethylene covers were removed and *E. gyrosa* ingress measured.

## RESULTS AND DISCUSSION

The linear growth of each of the *Endothia* isolates and the *F. solani* isolate, expressed as a percentage of that on the basal medium, is presented in Tables 1-4. All of the

TABLE 1. Linear growth (%)<sup>a</sup> of selected *Endothia* spp. isolates and the *Fusarium solani* isolate at 25 C on Difco cornmeal dextrose agar medium with the osmotic water potential adjusted by varying NaCl concentration

Fungal species	Isolate no.	Osmotic water potential (-bars)					
		10	20	30	60	90	120
<i>E. parasitica</i>	1	52	18	2	0	0	0
	43	79	36	21	3	0	0
	46	49	5	3	0	0	0
<i>E. gyrosa</i>	29	33	23	10	15	0	0
	35	29	17	6	10	0	0
	38	35	21	9	9	0	0
	47	88	43	11	13	0	0
	55	40	20	5	7	0	0
<i>E. tropicalis</i>	57	15	5	0	0	0	0
<i>E. singularis</i>	58	85	26	8	8	0	0
<i>F. solani</i>		99	81	66	41	15	0

<sup>a</sup>Linear growth expressed as a percentage of that on the basal medium.

TABLE 2. Linear growth (%)<sup>a</sup> of *Endothia* spp. isolates and a *Fusarium solani* isolate at 25 C on Difco cornmeal dextrose agar medium with osmotic water potential adjusted by varying KCl concentration

Fungal species	Isolate no.	Osmotic water potential (-bars)					
		10	20	30	60	90	120
<i>E. parasitica</i>	1	101	45	15	0	0	0
	43	123	67	48	4	0	0
	46	84	28	9	0	0	0
<i>E. gyrosa</i>	29	64	10	10	10	0	0
	35	49	103	91	9	0	0
	38	53	53	71	18	0	0
	47	80	99	96	19	5	0
	55	52	69	56	13	0	0
<i>E. tropicalis</i>	57	48	5	5	0	0	0
<i>E. singularis</i>	58	133	47	42	7	3	0
<i>F. solani</i>		119	81	79	32	13	7

<sup>a</sup>Linear growth expressed as a percentage of that observed on the basal medium.

isolates of *Endothia* and the *F. solani* isolate exhibited a pattern of decreasing linear growth with decreasing osmotic water potential, with the following exceptions. *Endothia parasitica* isolates 43 and 1 and *E. singularis* isolate 58 showed slight increases over the basal medium at -10 bars with KCl and/or the salt mixture. At -20 and -30 bars most of the *E. gyrosa* isolates showed a slight stimulation of growth on the KCl-adjusted medium over -10 bars (Table 2). The results were similar to those of Sommers et al. (10) who reported increased linear growth of three *Phytophthora* spp. on a KCl-adjusted medium. When sucrose was used, all the isolates of *Endothia* exhibited increases in growth over the other osmotica. Also, all of these isolates except *E. parasitica* isolate 1 grew at -90 bars, which most of them did not do on the other media. Perhaps the additional carbon source provided by the sucrose stimulated linear growth. Again, this result is similar to those reported by other workers (10). When NaCl was used there was a rapid decrease in growth of the *E. gyrosa* isolates between -10 and -30 bars (Table 1). This pattern also was evident, although to a

lesser degree, with the salt mixture in which the predominant ion was Na<sup>+</sup>. This response also occurred with the other *Endothia* species. These results indicate that the Na<sup>+</sup> ion may have been somewhat toxic.

The *F. solani* isolate exhibited a stimulation of growth at -10 bars with KCl and at -10 and -20 bars with the salt mixture. Patterns similar to those observed with *E. gyrosa* between -10 and -30 bars on the NaCl- and salt mixture-adjusted media also occurred with *F. solani*. However, the rate of decrease in growth was not as rapid as with *E. gyrosa*. In contrast to most of the *Endothia* isolates, *F. solani* grew at -90 bars on the KCl-, NaCl-, and salt mixture-adjusted media. The level of stimulation on the sucrose-adjusted medium that was observed with the *Endothia* isolates did not occur with *F. solani*.

Three other fungi were included as internal checks to ensure that the methods used were yielding conditions similar to those of other workers. In each case, the results were comparable to those of Dubé et al. (2) for *R. solani*, Cook et al. (1) for *F. roseum*, and Griffin et al. (3) for *A. flavus*.

TABLE 3. Linear growth (%)<sup>a</sup> of *Endothia* spp. isolates and a *Fusarium solani* isolate at 25 C on Difco cornmeal dextrose medium with osmotic water potential adjusted using a salt mixture<sup>b</sup>

Fungal species	Isolate no.	Osmotic water potential (-bars)					
		10	20	30	60	90	120
<i>E. parasitica</i>	1	87	48	9	0	0	0
	43	110	89	41	11	3	0
	46	90	30	9	0	0	0
<i>E. gyrosa</i>	29	62	36	10	0	0	0
	35	49	23	10	10	5	0
	38	52	29	9	7	4	0
	47	71	48	24	31	0	0
	55	38	26	15	9	0	0
<i>E. tropicalis</i>	57	39	10	5	0	0	0
<i>E. singularis</i>	58	117	64	20	5	0	0
<i>F. solani</i>		121	114	88	58	24	8

<sup>a</sup>Linear growth expressed as a percentage of that on the basal medium.

<sup>b</sup>Salt mixture consisted of NaCl, KCl, and Na<sub>2</sub>SO<sub>4</sub> (5:3:2, w/w).

TABLE 4. Linear growth (%)<sup>a</sup> of *Endothia* spp. isolates and a *Fusarium solani* isolate at 25 C on Difco cornmeal dextrose medium with osmotic water potential adjusted by varying sucrose concentration

Fungal species	Isolate no.	Osmotic water potential (-bars)					
		10	20	30	60	90	120
<i>E. parasitica</i>	1	117	145	93	44	0	0
	43	129	170	95	52	31	0
	46	130	166	93	42	20	0
<i>E. gyrosa</i>	29	92	605	302	67	23	0
	35	101	280	139	52	20	0
	38	111	259	151	54	13	0
	47	198	345	188	59	22	0
	55	90	210	94	42	15	0
<i>E. tropicalis</i>	57	148	204	134	60	22	0
<i>E. singularis</i>	58	131	142	94	36	5	0
<i>F. solani</i>		101	117	84	35	11	0

<sup>a</sup>Linear growth expressed as a percentage of that on the basal medium.

TABLE 5. Length of cankers produced by *Endothia gyrosa* and the water potential changes in pruned branch stubs of pin oak (*Quercus palustris*)

Time after pruning (days)	Thermocouple psychrometer placement (cm) <sup>a</sup>	Water potential (-bars) <sup>b</sup>	Length of canker (cm) <sup>a,c</sup>
30	2.5	2.16	2.6
60	2.5	34.79	5.3
90	5.0	67.75	8.1
120	10.0	60.34	10.6

<sup>a</sup>Placement distance and length of canker measured from the end of the pruned branch stub toward the main stem.

<sup>b</sup>Values are means of four replications.

<sup>c</sup>Values are means of three replications.

All of the *E. gyrosa* isolates grew at -60 bars on all the media, with the exception of *E. gyrosa* isolate 29 on the salt mixture-adjusted medium. Even on the NaCl- and salt mixture-adjusted media, where the possible toxic effects were observed, the *E. gyrosa* isolates had linear growth values at -60 bars comparable to those observed in cultures on the KCl-adjusted medium. Although the growth at -60 bars was slow, it was similar to that observed in the artificially inoculated pruned branch stubs. The mean linear growth per day of the *E. gyrosa* isolates on the four media was 1.26 mm and 0.64 mm per day at -30 and -60 bars, respectively. In the field studies, *E. gyrosa* had a 0.88 mm/day rate of ingress into the pruned branch stub. In addition, as shown in Table 5, the margins of the developing cankers occurred in sections of pruned branches where the measurements indicated that the water potentials probably were -60 bars or greater. Although the thermocouple psychrometer methodology dictated the use of different branches for measurement of ingress and water potential, we feel that these data can be compared.

The results reported here show that the linear growth of the *E. gyrosa* isolates tested was not comparable to that reported for other fungi on similar media. However, the *E. gyrosa* isolates were able to grow in the range of the lowest water potential (about -60 bars) observed in the pruned branch stubs of pin oak. The growth rates of the isolates in vitro were comparable to the growth rates of the pathogen in artificially inoculated pruned branch stubs. We concluded that, while the growth of *E. gyrosa* is

reduced at lower osmotic potentials (-20 to -60 bars), its growth rate is sufficient to allow the fungus to invade pruned branches of pin oak in advance of water potentials that would be limiting. Further research is being conducted to determine other possible direct or indirect roles of low water potential in canker development.

#### LITERATURE CITED

1. COOK, R. J., R. I. PAPENDICK, and D. M. GRIFFIN. 1972. Growth of two root-rot fungi as affected by osmotic and matric water potentials. *Soil Sci. Soc. Am. Proc.* 36:78-82.
2. DUBE, A. J., R. L. DODMAN, and N. T. FLENTJE. 1971. The influence of water activity on the growth of *Rhizoctonia solani*. *Aust. J. Biol. Sci.* 24:57-65.
3. GRIFFIN, G. J., R. H. FORD, and K. H. GARREN. 1975. Relation of *Aspergillus flavus* colony growth on three selective media to recovery from naturally infested soil. *Phytopathology* 65:704-707.
4. GROGAN, R. G., and G. S. ABAWI. 1975. Influence of water potential on growth and survival of *Whetzelinia sclerotiorum*. *Phytopathology* 65:122-128.
5. MANANDHAR, J. B., and G. W. BRUEHL. 1973. In vitro interactions of *Fusarium* and *Verticillium* wilt fungi with water, pH, and temperature. *Phytopathology* 63:413-419.
6. MEYN, R. L., and R. S. WHITE. 1972. Calibration of thermocouple psychrometers: A suggested procedure for development of a reliable predictive model. Pages 56-63 in R. W. Brown and B. P. Van Haveren, eds. *Psychrometry in water relations research*. Utah State Agric. Exp. Stn., Logan. 342 p.
7. ROANE, M. K., R. J. STIPES, P. M. PHIPPS, and O. K. MILLER, JR. 1974. *Endothia gyrosa*, causal pathogen of pin oak blight. *Mycologia* 66:1042-1047.
8. ROBINSON, R. A., and R. H. STOKES. 1965. *Electrolyte solutions*, 2nd ed. Butterworths, London. 571 p.
9. SCOTT, W. J. 1953. Water relations of *Staphylococcus aureus* at 30°C. *Aust. J. Biol. Sci.* 6:549-564.
10. SOMMERS, L. E., R. R. HARRIS, F. N. DALTON, and W. R. GARDNER. 1970. Water potential relations of three root-infecting *Phytophthora* species. *Phytopathology* 60:932-934.
11. STIPES, R. J., and P. M. PHIPPS. 1971. A species of *Endothia* associated with a canker disease of pin oak (*Quercus palustris*) in Virginia. *Plant Dis. Rep.* 55:467-469.
12. WIEBE, H. H., G. S. CAMPBELL, W. H. GARDNER, S. L. RAWLINGS, J. W. CARY, and R. W. BROWN. 1971. Measurement of plant and soil water status. *Utah Agric. Exp. Stn. Bull.* 484. 71 p.