Water Potential of Ergot Honeydew and Its Influence upon Colonization by Microorganisms

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ABSTRACT

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Water potential of *Claviceps purpurea* honeydew exuded outside the glumes of male sterile barley florets was rarely higher than −100 bar and as low as −750 bar. Within the glumes where the sphacelium was growing the water potential averaged −17 to −35 bar. This range of water potential was favorable for *C. purpurea* mycelial growth and conidial germination (minimum growth −52 bar) as determined on sucrose-amended agar media. Low water potential of honeydew outside the glumes prevented germination of ergot conidia within the sugar-rich droplet. During a simulated rainy period in a mist chamber water potential of honeydew outside the glumes increased to −10 to −30 bar. After the plants were returned to sunny conditions, water potential decreased to less than −100 bar within 4 hours.

Conidia of *Fusarium heterosporum* and other *Fusarium* spp. which colonized honeydew did not germinate at less than −110 to −120 bar on sucrose-amended agar. Mycelial growth ceased at −150 to −160 bar. Therefore, except during conditions of rain or heavy dew, low water potential of honeydew outside the glumes is a major factor preventing colonization by *Fusarium* and most other microorganisms.

The honeydew of *Claviceps* spp. is a unique substrate for microorganisms. It is a concentrated sugary liquid exuded by the mat of hyphae (sphacelium) which colonizes the grass floret (10). The honeydew droplet typically extends outside of the glumes where it is readily accessible to the airborne microflora. There are several reports of microorganisms that colonize ergot honeydew (2, 4, 5, 10, 11, 14) but little information is available about the ecological factors which influence honeydew colonization. Several investigators have observed that honeydew colonization is most prevalent during rainy periods, but virtually absent in dry weather (5, 11, 14). Water potential of a substrate has a major influence upon the microorganisms which colonize it (6). Because environmental changes such as morning dews, rain, or bright sunshine alter the water content of the honeydew droplet, water potential changes of honeydew were investigated as possibly having an effect upon the development of *Claviceps purpurea* on barley and the growth of microorganisms upon honeydew. The effects of osmotic water potential on mycelial growth and conidial germination of *C. purpurea* as well as *Fusarium* spp. which colonize honeydew and inhibit sclerotium maturation were determined. Results of in vitro and in vivo experiments were compared. A portion of this work has been published previously (3).

MATERIALS AND METHODS

Male sterile barley, *Hordeum vulgare* L. 'Paragon' *ms,,av ms,,av* (7), grown in a greenhouse at 22-30 C, was inoculated with *C. purpurea* conidia at anthesis. All water potential readings were made with a Spanner-type thermocouple psychrometer (Wescor, Inc., Logan, Utah). After gaining experience with the general ranges of water potential to be expected under given conditions, two methods of measuring water potential were used. For samples likely to be higher than −40 bar the rapid psychrometer method was used (1). For samples below −40 bar the sample exchange method (1) was used. The only modification was that a cooling time of 5-10 minutes was sufficient to get accurate readings for all except those anticipated to less than −500 bar. For these a 15 minute cooling period was necessary. Accuracy of readings was enhanced by connecting the microvoltmeter to a Heath recording chart. All water potential values were determined from standard curves plotted with NaCl and LiCl standards (1, 13) at 25 C and all readings were corrected to this temperature. Because microvoltmeter readings of thermocouple psychrometers are sensitive to small temperature fluctuations, the sample chamber was kept enclosed in a 2.5 cm thick styrofoam box and temperature was diligently monitored at all times.

Beginning 5-6 days after inoculation, the first day honeydew was visible (day 1), water potential measurements of honeydew and sphacelium inside the glumes and the honeydew exudate outside of the glumes were taken daily for seven days. Additional readings were made on day 9 when the dark rind of the young sclerotia were partially formed, and on day 14 when the sclerotia were mature but had not yet begun to dry out. Day 9 and day 14 readings of samples inside the glumes consisted almost entirely of sclerotial hyphae because honeydew production had ceased by that time. Readings were not made from honeydew outside the glumes on day 14 because the small amount of honeydew which remained had dried at the tips of the sclerotia. Throughout the

experiment the plants were kept at 22-30 C in dry, sunny conditions.

Readings were also taken from honeydew droplets following conditions simulating a rain or heavy dew. Measurements were made outside the glumes following exposure of the plants to 12 hours of 100% relative humidity in a mist chamber at 23-28 C. All samples were taken from honeydew on day 4.

Drops of honeydew or portions of sphacelium were collected from florets on dry 5-mm diameter filter paper disks and immediately inserted into the sample chamber. Nine to 12 determinations from three separate sampling dates were made for each treatment.

RESULTS

Honeydew water potential.—Honeydew production was quite copious during the first 4 days after honeydew became visible. Then, as the sphacelial tissues continued to enlarge, honeydew droplets became smaller and more viscous. These observations are reflected in the relatively high water potentials (−158 to −173 bar) of honeydew droplets outside the glumes observed on days 1 and 2 (Table 1). On day 3 the values decreased to −352 bar. Less honeydew was exuded from day 4 onward and average values remained below −500 bar. Relatively wide fluctuations in water potential were noted among individual samples (Table 1). Much of the variation encountered was associated with relative humidity fluctuations in the greenhouse. Relative humidity ranged from 50-70% and was recorded each time a sample was taken. Generally, low relative humidities were associated with low (drier) water potentials and vice versa. However, as will be discussed later, water potential of honeydew outside the glumes rarely was high enough to permit growth of fungi upon the honeydew.

Within the glumes water potential was vastly different (Table 1). Water potential averaged −17 to −35 bar throughout the 14-day period. During the first 4 days of honeydew production water potential varied from −27 to −35 bar. On day 5 it increased substantially to −17 bar and decreased only to −21 bar by day 6. After day 6 water potential again returned to the −29 to −34 bar range. Numerous additional readings were made on days 5 and 6, but the results remained consistent. These changes may reflect a change in the metabolism of the sphacelium from conidia production to formation of the sclerotium.

Vast differences in water potential existed between honeydew inside and outside the glumes under conditions of full sunlight and 22-30 C. In individual florets this difference ranged from −40 to −730 bar during the 9-day period.

Honeydew water potential after simulated rain.—After 12 hours in a mist chamber honeydew droplets on diseased barley florets enlarged 2-3 times their normal size of 0.2 − 0.3 cc. The decrease in honeydew viscosity was noted by the observation that most of the ergot conidia had settled to the bottom of the droplet. Within a few hours after the plants were returned to about 50% relative humidity in the greenhouse, much of the water in the droplets had evaporated and conidia were dispersed throughout the honeydew. Honeydew water potential decreased from −26 bar during the first 30 minutes out of the mist to less than −50 bar after 3 hours (Table 2). After 4 hours honeydew water potential decreased to less than −100 bar and most droplets had attained their viscosity prior to the moist period.

TABLE 1. Comparison of water potential of *Claviceps purpurea* honeydew within glumes and outside the glumes throughout the development of ergot on barley

Day of honeydew production	Honeydew water potential (−bar)	
	Within glumes	Outside glumes
1	35	173 (104-257)[b]
2	27	158 (78-250)
3	27	352 (170-560)
4	34	505 (330-620)
5	17	505 (375-660)
6	21	636 (520-738)
7	29	606 (440-770)
9	34[a]	501 (420-599)
14	32[a]	...[c]

[a]Very little honeydew was present within the glumes at these dates. Measurements represent water potential of hyphae of the maturing sclerotium.

[b]Numbers in parentheses represent the range of values recorded.

[c]Sclerotia were mature and the remaining honeydew had dried.

TABLE 2. Water potential of *Claviceps purpurea* honeydew outside the glumes on barley following a 12-hour period at 100% relative humidity in a mist chamber

Time interval after mist (hours)	Honeydew water potential (−bar)[a]
0-0.5	26
0.5-1.0	31
1.0-1.5	47
1.5-2.0	51
2.0-2.5	45
2.5-3.0	52
3.0-3.5	68
3.5-4.0	110

[a]All data were obtained from infected barley florets on the fourth day of honeydew exudation.

TABLE 3. Germination of *Fusarium heterosporum* and *Claviceps purpurea* conidia at 25 C on media of different osmotic water potentials[a]

Water potential of medium[a] (−bar)	Percentage germination	
	F. heterosporum	*C. purpurea*
1	98 (12 hr)	17 (24 hr)
5	87 (12 hr)	23 (24 hr)
13	97 (12 hr)	32 (24 hr)
52	85 (12 hr)	31 (48 hr)
84	61 (24 hr)	0 (120 hr)
115	6 (120 hr)	
136	0 (120 hr)	
157	0 (120 hr)	

[a]Basal medium was Difco corn meal agar amended with sucrose to attain the desired water potential.

In vitro growth and spore germination of Claviceps purpurea and Fusarium heterosporum.—*Claviceps purpurea* and *Fusarium heterosporum* were grown on corn meal agar media with water potentials adjusted with sucrose according to the tables of Robinson and Stokes (13). Mycelial growth and conidial germination were measured at 15, 20, and 25 C. An additional isolate of *C. purpurea* and three additional isolates of *Fusarium* spp. which colonized honeydew were tested at 25 C only. Mycelial growth was measured after 6 days of growth on five replicate plates. Percentage germination of 100 conidia per treatment were counted. All experiments were conducted two times. As has been observed for other fungi (6), mycelial growth of both species was optimum at −1 to −5 bar (Fig. 1). Growth of the *C. purpurea* isolates decreased rapidly below −5 bar; they grew very slowly at −52 bar and no growth was detected at −84 bar.

Fusarium heterosporum on the other hand, grew well over the range −1 to −13 bar and still exhibited slight growth at −157 bar (Fig. 1). At −52 and −84 bar, where *C. purpurea* ceased growth, *F. heterosporum* grew prolifically.

In the conidial germination study, the minimum limit for *C. purpurea* was −52 bar, the same as for mycelial growth (Table 3). The minimum limit was −115 bar for *F. heterosporum,* about −40 bar greater than the minimum for mycelial growth. At 15 C and 20 C growth and germination responses were similar but not as rapid as at 25 C. The other *Fusarium* isolates tested at 25 C responded similarly to *F. heterosporum*.

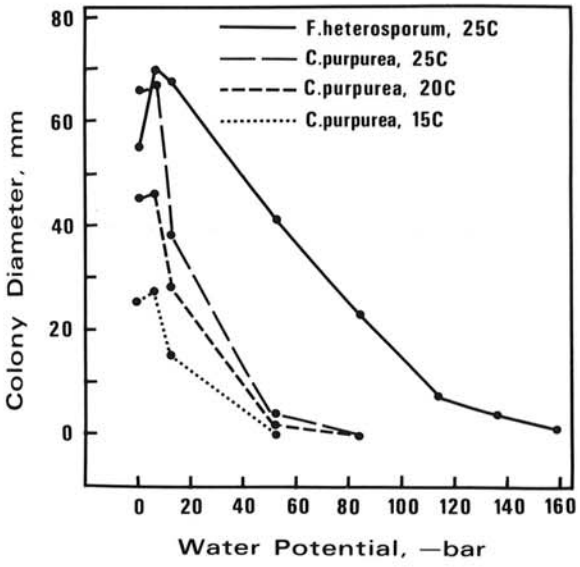

Fig. 1. Mycelial growth of *Claviceps purpurea* and *Fusarium heterosporum* after 6 days on corn meal agar amended with sucrose to obtain different osmotic water potentials.

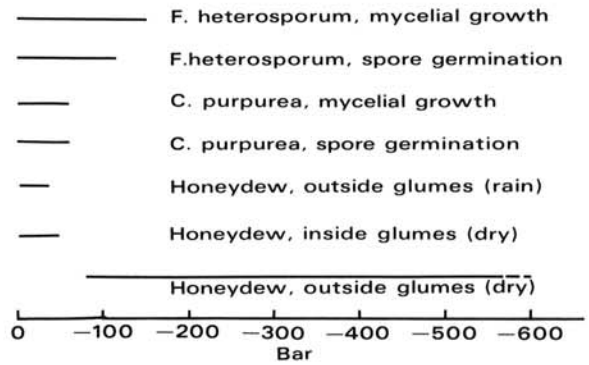

Fig. 2. The ranges of ergot honeydew water potential under various conditions in relation to water potential ranges favorable for mycelial growth and conidial germination of *Fusarium heterosporum* and *Claviceps purpurea*. For honeydew outside glumes (dry) the dashed line indicates that droplets gradually dried out and water potential decreased far beyond −600 bar.

DISCUSSION

Water potential changes in ergot honeydew have a major influence on growth of microorganisms alighting upon it and upon growth of *C. purpurea*. These relationships, summarized graphically in Fig. 2, are discussed below. Recent studies (Cunfer, *unpublished*) indicate that conidia-free honeydew exudate also contains an inhibitory factor(s) which suppresses spore germination of fungi which are unable to colonize honeydew. However, this factor does not suppress germination of honeydew colonizers.

Honeydew is rich in sugars, a readily available source of nutrients for microorganisms. However, during warm, sunny weather the water potential of the honeydew droplet outside the glumes is rarely above −100 bar. The *Fusarium* isolates studied are capable of only very limited growth below −100 bar. Most other fungi and bacteria are similarly restricted (6). Unless honeydew water potential is increased during periods of dew or rain *Fusarium* colonization is restricted or entirely prevented on honeydew outside the glumes. However, if *Fusarium* conidia dispersed during rain reach the honeydew within the glumes they should be able to germinate and grow even during dry weather (Fig. 2). This possibility will require further field tests.

Futtrell and Webster (5) reported that ergot honeydew on male sterile sorghum in Nigeria was colonized by *Fusarium* spp. during the rainy season and that sclerotia failed to form. During the dry season sclerotia matured without any evidence of colonization. Simpson and West (14) reported identical observations on ergot of *Paspalum* spp. in Florida. Overhead irrigation was required to obtain a high level of *Fusarium* colonization of honeydew of rye ergot in artificial inoculation experiments conducted by Mower et al. (11). Therefore, the experimental results reported here concur with field observations.

Water stress below optimum for mycelial growth within the glumes may be beneficial for sporulation. Sporulation of *C. purpurea* is limited on common laboratory media which have water potentials of about −0.5 to −2.0 bar. *Claviceps purpurea* sporulated profusely on the medium of Kybal et al. (8) with the modifications described by Puranik and Mathre (12). This medium contains 200 g of sucrose per liter. Although the solute content of both media is essentially the same,

Kybal's formulation contains less water. Both media were prepared and the respective water potentials were measured. The values obtained for Kybal's and Puranik and Mathre's media were −27 and −23.5 bar, respectively. These values are within the range of water potential values recorded for sphacelium and honeydew within the glumes (Table 1).

Lewis (9) found that *C. purpurea* conidia remained viable and did not germinate for 5 days in solutions containing 34 to 66% sucrose. I determined that sucrose solutions at these two concentrations have water potentials of −42.5 and −209 bar, respectively. The minimum limit at which ergot conidia will germinate is about −52 bar (Fig. 2). Therefore, Lewis' observations fit well with the spore germination data of the present study.

In summary, low water potential of ergot honeydew outside the glumes affects the fungus in two ways. It prevents germination of most ergot conidia until they are disseminated and prevents growth of most potential colonizers upon the exposed honeydew surface. Within the glumes water potential is adequate for sphacelial growth and abundant sporulation. Efforts aimed at biological control of ergot with honeydew-colonizing microorganisms must take these factors into account.

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