Colonization of Ergot Honeydew by Fusarium heterosporum

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ABSTRACT

The colonization of ergot (caused by *Claviceps purpurea*) honeydew by an isolate of *Fusarium heterosporum* is described. *Fusarium heterosporum* did not prevent ergot infection, but it prevented sclerotium formation when inoculated into rye and male-sterile barley florets from 3 days prior to ergot inoculation until 3 days after honeydew appeared. *Fusarium heterosporum* grew upon honeydew when inoculated 7 days after honeydew appeared but mature sclerotia formed. *Fusarium heterosporum* uses ergot honeydew as a food base, but does not penetrate ergot conidia or sphacelial hyphae. There was only a tenfold reduction in the number of ergot conidia within *Fusarium*-colonized sphacelia and the conidia that remained infected rye and barley. The possible use of *F. heterosporum* as a biological control is discussed.

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The use of male sterility for the development of hybrid cereals has been accompanied by high incidence of ergot in many areas of the United States (6, 10). Male-sterile cereals are highly susceptible to ergot because the pathogen infects only unfertilized ovaries or fertilized ones within a short time after fertilization (2, 6). In addition, the glumes of florets with unfertilized ovaries remain open thereby permitting inoculum to reach the infection court easily. Effective control measures have not been found.

Claviceps purpurea (Fr.) Tul. is not found in the southeastern United States, but *C. paspali* Stev. & Hall, the cause of ergot on *Paspalum* spp., is quite common (11). *Fusarium* spp. frequently have been reported to colonize the honeydew of these and other *Claviceps* spp. (3, 7, 11). The purposes of this investigation were to study aspects of the biology of *Fusarium heterosporum* Nees ex Fr. [= *F. roseum* (Lk.) Snyd. and Hans.] on ergot honeydew and its possible role in the absence of *C. purpurea* in the Southeast. The potential of *F. heterosporum* as a biological control for ergot was also assessed. Recently, Mower et al. (9) have reported on the control of ergot on rye with *Fusarium*. The work reported here provides additional information about *F. heterosporum* on ergot. A portion of this work has been reported previously in abstract form (4).

MATERIALS AND METHODS.—*Fusarium heterosporum* was isolated from *Claviceps paspali* honeydew on *Paspalum distichum* L. at Experiment, Ga. The *C. purpurea* isolate used was from a sclerotium on rye grown at Aberdeen, Idaho. Ergot inoculum was prepared from honeydew on infected rye (*Secale cereale* L. 'Vitagraze') or male sterile barley (*Hordeum vulgare* L. 'Paragon' *msg,,av msg,,av*). *Fusarium heterosporum* was grown on fresh potato-dextrose agar at 20-25 C in light supplied by fluorescent tubes. Under these conditions *F. heterosporum* sporulated profusely in 2-3 days. Rye and male sterile barley were grown in Cecil clay loam in the greenhouse at 20-25 C.

For routine inoculations spore suspensions of each fungus containing about 10^6 conidia per ml were used. Unless otherwise stated, all inoculations were made by injecting spore suspensions into florets with a hypodermic syringe. Male-sterile barley florets were inoculated at the time of emergence of the spike from the boot. Rye florets were inoculated just prior to anthesis when anthers were still green. It was not necessary to keep plants in a mist chamber to obtain a high level of infection.

The terms sphacelium and sphacelial tissue used in this paper refer to the mass of hyphae and conidiophores formed by *C. purpurea* in the parasitized grass floret. The spacelium produces and is surrounded by the sugary exudate, honeydew, in which conidia are borne.

RESULTS.—*Colonization of ergot honeydew.—Fusarium heterosporum* colonization of ergot became visible shortly after the onset of honeydew formation. Colonization was abundant at 20-30 C, but slight at 15 C. At 20-28 C in the greenhouse, *F. heterosporum* first appeared 1 - 2 days after honeydew as a pinkish cottony growth on the honeydew surface. Within an additional 2 days the entire young sphacelium was engulfed by mycelium.

Plants with honeydew were inoculated in the afternoon and kept in a mist chamber overnight at 25-30 C. By the next afternoon the honeydew was completely overgrown by *F. heterosporum*. Pink to red-orange masses of *F. heterosporum* macroconidia appeared on the surface of colonized tissue. In culture and on honeydew *F. heterosporum* sporulated only where it was exposed to direct light.

Three to 4 days after honeydew was colonized no more growth of *F. heterosporum* was seen in some florets. The colonized tissue became shrivelled and flattened. In other cases the colonized sphacelium continued to enlarge for several additional days indicating that ergot continued to draw nutrients from the host.

During *F. heterosporum* inoculation the syringe needle was often inserted through the lemma. *F. heterosporum* grew upon the wounded area, but did not further colonize the lemma. Also, after the florets had matured *F. heterosporum* did not grow upon the dead glumes.

Time of Fusarium heterosporum inoculation.—Fusarium heterosporum was inoculated into rye or male sterile barley florets 1 or 3 days prior to ergot inoculation, simultaneously with ergot, or 3, 5, 7, or 12 days after ergot. In this experiment and the one which follows, counts were made of the number of ergot-infected florets and ergot-infected florets colonized by *F. heterosporum*.

TABLE 1. Colonization of *Claviceps purpurea* honeydew on barley and rye by *Fusarium heterosporum* in relation to time of inoculation of the fungi

Inoculation treatment	*Fusarium* colonization of ergot-infected florets (%)[a]	
	Barley	Rye
F. heterosporum only	0	0
C. purpurea only	0	0
F. heterosporum; C. purpurea 3 days later	65	...
F. heterosporum; C. purpurea 1 day later	61	90
C. purpurea and *F. heterosporum* simultaneously	62	82
C. purpurea; F. heterosporum 3 days later	54	72
C. purpurea; F. heterosporum 7 days later	63	47
C. purpurea; F. heterosporum 12 days later	41	18

[a]At least 250 florets were inoculated for each treatment.

Honeydew became visible 5 days after inoculation with ergot and 45-55% of all florets became infected. At inoculation dates from 3 days prior to ergot inoculation until 7 days after ergot inoculation, *F. heterosporum* colonized 54-65% of the ergot-infected barley florets (Table 1). Ninety percent of the ergot-infected florets of rye were colonized when *F. heterosporum* was inoculated 1 day before inoculation with ergot, but colonization declined to 47% when *F. heterosporum* was inoculated 7 days after ergot. On all inoculation dates from 3 days prior to ergot inoculation to 7 days after ergot, *F. heterosporum* prevented formation of mature sclerotia. *Fusarium heterosporum* grew well upon honeydew when inoculated 12 days after ergot inoculation, but the sphacelium was quite dense by this time and mature sclerotia formed. No evidence was found which would indicate that *F. heterosporum* inoculated prior to or at the same time as ergot reduced ergot infection.

Fusarium heterosporum concentration.—Tenfold dilutions of *F. heterosporum* conidia from 4 to 4×10^7 conidia per milliliter were prepared. Conidial concentrations were calibrated with a Sedgwich-Rafter counting chamber. Individual rye florets were inoculated with the appropriate dilutions. Plants were inoculated with *C. purpurea* one day later.

At concentrations of 4×10^5 conidia per ml or greater, over 90% of the ergot-infected rye florets were colonized by *Fusarium heterosporum* (Table 2). With as few as 40 conidia per ml, 51% of the ergot-infected florets were colonized. The percentage infection of florets by ergot varied from 51-58% for all treatments, except that at the highest (4×10^7) *F. heterosporum* concentration, only 27% of the florets were infected. It appeared as though this high level of *F. heterosporum* prevented ergot infection. However, subsequent experiments showed that *F. heterosporum* had colonized and killed some of the ovaries. *Fusarium heterosporum* is reported to be a weak parasite on the spikes of grasses and it may be responsible for extensive seed rotting in hot, humid climates (1).

Ergot conidia in colonized sphacelial tissue.—Because ergot conidia were commonly found in *Fusarium*-colonized sphacelial tissue, an experiment was performed to compare the concentration of ergot conidia in normal sphacelial tissue and *Fusarium*-colonized sphacelial tissue. Rye florets were inoculated with *C. purpurea* alone or in a mixed inoculum with *F. heterosporum*. Ten days after inoculation, the spikes were harvested, the sphacelial tissue air-dried at 25 C and weighed. Sphacelial tissue from individual florets was macerated in sterile distilled water and ergot conidia concentrations determined.

TABLE 2. Effect of *Fusarium heterosporum* inoculum density on colonization of ergot-infected florets on rye

F. heterosporum inoculum level (conidia/ml)	Florets with ergot (%)[a]	Ergot-infected florets colonized by *F. heterosporum* (%)
0	51	0
4	56	5
40	53	51
4×10^2	51	55
4×10^3	56	62
4×10^4	57	87
4×10^5	58	98
4×10^6	52	91
4×10^7	27	90

[a]At least 300 florets were inoculated for each treatment.

Florets inoculated with *C. purpurea* alone had an average of 7.4×10^7 conidia per mg of air-dry sphacelial tissue whereas the concentration in *Fusarium*-colonized sphacelial tissue averaged 5.7×10^6 conidia per mg. This represents only slightly more than a tenfold reduction in the number of ergot conidia.

Microscopic examinations of colonized honeydew revealed that *F. heterosporum* did not invade ergot hyphae or conidia. These ergot conidia germinated readily on agar. Inoculum was prepared from colonized sphacelial tissue and inoculated onto rye; typical ergot honeydew developed which subsequently became colonized by *F. heterosporum*.

DISCUSSION.—*Fusarium heterosporum* prevented formation of ergot sclerotia when sufficient inoculum reached ergot honeydew within 12 days after ergot inoculation in the greenhouse. *Fusarium heterosporum* did not reduce the number of ergot-infected florets except when it killed ovaries at very high inoculum levels. Because of this limitation *F. heterosporum* can only partially account for the absence of *C. purpurea* in the Southeast.

Fusarium heterosporum apparently thrives upon the abundant quantities of sugar (8) and other metabolites in honeydew. Other workers (3, 7) who observed colonization of honeydew in the field concluded that *Fusarium* grows as a saprophyte. Mantle (8) observed that the saprophytic bacterium *Leuconostoc mesenteroides* converted honeydew sugar to dextran which encapsulated the spacelial hyphae at the point of attachment to the host, thereby detaching it and preventing further movement of nutrients into the sphacelium. In honeydew colonized by *F. heterosporum* the sphacelium remained attached to the host. Like *L. mesenteroides, F. heterosporum* did not prevent ergot infection. The mechanism by which *F. heterosporum* inhibits *C. purpurea* remains unknown.

Several attributes of *F. heterosporum* favor it as an organism for biological control of ergot. Relatively low inoculum loads are needed to initiate honeydew colonization under favorable conditions and it is only weakly pathogenic on rye and barley. Mower et al. (9) have reported control of ergot to be possible in field experiments on rye. However, two problems are encountered. Prolonged periods of moisture provided by rain or sprinkler irrigation are necessary for effective control of ergot by *Fusarium* spp. (7, 9, 11). This may invite damage from foliar and seed-infecting pathogens as well as increasing ergot infection. I (5) have found that during prolonged periods of high moisture, honeydew water potential is sufficiently high to permit rapid colonization by *F. heterosporum,* but under sunny conditions the water potential drops below the minimum required for spore germination. Secondly, since *F. heterosporum* does not prevent ergot infection, reduced seed set can occur before *F. heterosporum* becomes effective. These problems must be overcome before control of ergot on male-sterile cereals by *F. heterosporum* is possible.

Ergot lends itself well to control by biological methods. The host is susceptible for only a brief period during the entire growing season. An optimum biological control would be one that would parasitize germinating ergot ascospores or conidia or prevent their infection of the unfertilized ovary. It would only need to remain effective until shortly after host fertilization.

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