GERMINATION REQUIREMENTS OF *CLAVICEPS PASPALI* SCLEROTIA

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SUMMARY

Germination of *Claviceps paspali* sclerotia occurred over a wide range of storage and incubation temperatures. Cold, moist storage conditions greatly enhanced germination but were not obligatory. Highest percentage germination and most rapid germination occurred after storage in moist sand at 5 and 10 C for 2 mo followed by incubation at 20 C for 41–44 da. Preconditioning periods at 20 and 25 C, incubation at 30 C, and dry storage of sclerotia were not conducive to germination.

Claviceps paspali Stev. et Hall, causing ergot of *Paspalum* spp., is an endemic plant pathogen throughout the southern USA. Dallisgrass, *P. dilatatum* Poir., a species used for forage, is heavily infected each year. Toxic compounds in sclerotia always present a potential for cattle poisoning as occurred in Louisiana in 1976 (R. Cole, personal communication).

Several workers have reported sclerotium germination in the field and laboratory. Brown (1) gathered *Claviceps paspali* sclerotia during the winter in Mississippi. These sclerotia germinated in 20–30 da. Sclerotia were found germinating in the field in mid to late May. Wells and Burton (6) made similar field observations in south Georgia. Lambert and McIllveen (3) stored sclerotia on moist sand for 3 mo at 4 C followed by incubation at 20 C. Fertile stromata were produced after 1 mo. Luttrell (4) found that storage in moist sand for 10 wk at 5 C followed by incubation near 20 C was conducive to germination although percentage germination was often erratic. Cunfer and Seckinger (2) found that optimal germination occurred after 6–9 mo when *C. paspali* sclerotia were placed in the field in October.

The purpose of this research was to delineate more accurately the effects of dryness and moisture and various temperature regimes upon germination of *C. paspali* sclerotia. Knowledge of optimal germination requirements provides a better understanding of the life cycle of *C. paspali* and facilitates research on the ascigerous stage.

MATERIALS AND METHODS

Claviceps paspali sclerotia were collected from dallisgrass at the same site at Experiment, Ga., in August of 2 yr. Approximately 180 sclerotia were buried in sand in each of eight 400-ml beakers. Sand in two beakers was kept dry. The sand in six beakers was moistened as needed with sterile distilled water to just below saturation to prevent anerobic conditions.

The first yr half of the beakers of each group were stored at 5 C and half at 10 C. One beaker with moist sand of each storage temperature was placed at −25 C for 24 h every 2 wk during the storage period.

In the second yr, additional storage temperatures (15, 20, 25 C) and one additional incubation temperature (30 C) was included. Identical sets of dry-stored and moist-stored sclerotia were prepared as in the first experiment. One set of sclerotia stored moist at 5 C was frozen for 24 h at 0 C once each month.

Each month, 20 sclerotia were removed from the beakers. Five sclerotia were placed in each of four Petri dish moist chambers. The sclerotia were then incubated at 15, 20, 25 and 30 C in the dark for 8 wk. Sclerotia stored at 20 and 25 C were incubated only at temperatures equal to or greater than the storage temperatures. The plates were stored in sealed polyethylene bags and sterile distilled water was added to the filter papers to maintain moisture.

In an additional experiment, newly collected sclerotia and sclerotia stored dry in the laboratory for 1 yr were placed in moist chambers and incubated at 10, 15, 20, 25 and 30 C for 8 wk. There were 15 sclerotia at each temperature.

RESULTS

Germination of *Claviceps paspali* sclerotia occurred over a wide range of storage and incubation temperatures. A cold moist period was essential for rapid germination of sclerotia in high numbers. Sclerotia germinated following all combinations of 5 and 10 C moist storage and incubation at 15, 20 and 25 C (TABLE I). A few sclerotia germinated after 1 mo cold storage and some germinated after 15 mo storage. However, optimal germination followed moist storage at 5 and 10 C for at least 2 mo and incubation at 20 C for 41–44 da. Percentage germination of sclerotia stored at 5, 10 or 15 C ranged from 19 to 40% when sclerotia were subsequently incubated at 20 and 25 C.

After placement at the incubation temperature the time until germination varied from 13 to 101 da (TABLE I) but most sclerotia germi-

TABLE I

GERMINATION OF *Claviceps paspali* SCLEROTIA AT VARIOUS MOIST STORAGE AND INCUBATION TEMPERATURES

Storage temperature C	Incubation temperature C	Number of germinating sclerotia	% germinating sclerotia	Average number clavae per sclerotium	Average number da until germination after start of incubation	Range of da for germination after start of incubation
5[a]	15	19	11.9	1.2	74	46–98
	20	61	38.1	1.8	44	16–78
	25	34	21.3	2.3	42	13–81
	30	0	0	0	0	0
10	15	33	20.6	1.5	54	23–98
	20	63	39.4	1.8	41	16–101
	25	41	25.6	2.0	42	16–81
	30	2	2.9	2.0	35	29–42
15[b]	15	0	0	0	0	0
	20	19	27.1	2.0	29	16–44
	25	13	18.6	1.8	35	29–55
	30	1	1.4	2.0	23	23
20	20	0	0	0	0	0
	25	3	4.3	2.7	47	33–54
	30	0	0	0	0	0
25	25	2	2.8	1.0	22	16–29
	30	0	0	0	0	0

[a] 5 and 10 C storage results, except 30 C incubation, sum of 2 yr data. 30 C incubation data from 1 yr only. 160 sclerotia per incubation temperature.

[b] 15, 20 and 25 C storage results based on 1 yr data. 70 sclerotia per incubation temperature.

nated after 20–50 da. Following storage at 5 and 10 C, the number of days until germination occurred declined from the 15 to 25 C incubation temperature. These results are consistent with the germination rates of sclerotia stored outdoors (2).

Germination percentages were quite low for all other treatments. Prolonged incubation at 30 C inhibited germination. No sclerotia germinated at the 15 C moist storage and incubation treatment. This was unexpected because other treatments near these temperature combinations caused sclerotial germination. During both years, sclerotia stored moist at 5 C and −25 C for 24 h failed to germinate.

Dry storage was unfavorable for sclerotial germination. In the first experiment, sclerotia stored dry at 10 C germinated at rates of 30% and 12% when incubated at 20 and 25 C, respectively. In the second year, none of the sclerotia stored dry germinated. Very often, dry-stored sclerotia were quickly overgrown with *Penicillium*, *Aspergillus* and *Fusarium* spp. when incubated in moist chambers. In a related study (2), the senior author suspended sclerotia 1 m above ground and placed other sclerotia in or on soil. Germination of the above-ground

sclerotia which were dry for long periods was much reduced, 2.5% compared with 40% for the soil-stored sclerotia which remained moist much of the time.

The average number of clavae per sclerotium (1.2–2.3) increased from the 15–25 C incubation temperature after 5 and 10 C storage. One to two clavae per sclerotium were commonly noted and three or four clavae were occasionally found.

DISCUSSION

As in *Claviceps purpurea* (Fr.) Tul. (**5**), a minimum cold moist period of about 2 mo is the most favorable preconditioning treatment to induce *C. paspali* sclerotia to germinate. A cold moist period is not obligatory, however. Sclerotia stored dry at 5 and 10 C germinated erratically and in low numbers. Also, a few sclerotia stored moist at 20 and 25 C germinated. Optimal germination of *C. paspali* occurs after exposure to storage and incubation temperatures which are about 5 C higher than for *C. purpurea* (**5**). This temperature requirement reflects the distribution of *C. paspali* in the southern USA whereas *C. purpurea* is most common farther north.

Mitchell and Cooke (**5**) reported *C. purpurea* sclerotia did not germinate after exposure to periods at −5 C. *Claviceps paspali* sclerotia did not germinate in our tests after exposure to 0 C or −25 C. Because temperatures below 0 and −5 C are common in regions where these fungi are found, sclerotial survival may be due to protection in suitable microenvironments.

Metabolic activity of *C. paspali* with regard to initiation of the ascigerous stage is linked to the moderate temperatures of spring and early summer. The wide range of conditions favorable for germination insures the presence of ascospore inoculum during the flowering period of *Paspalum* spp.

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