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BOTANY

Sporulation of Helminthosporium dictyoides *on Filter Paper* [1]

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Helminthosporium dictyoides Drechs. and *H. sativum* Pam. King and Bakke were the fungi most commonly isolated from plants of *Poa pratensis* L. infected with leaf spot in Minnesota during 1960-1962. *H. dictyoides* has not been implicated as a pathogen of *P. pratensis* (it is pathogenic on *Festuca* spp.) and so studies on its physiology and pathogenicity seemed necessary. Such work usually requires a large quantity of spores but *H. dictyoides* sporulated sparingly on the acid potato – dextrose agar used for isolating it from the plant tissue. We decided to study methods for inducing abundant sporulation as preliminary work for the more basic studies to follow.

Methods and Results: H. dictyoides grew profusely on water agar, water agar plus sterilized plant material (1), V-8 juice agar (5), Sachs agar (6) and Czapeks agar (5) largely as submerged mycelium, but sporulation was very poor at room temperature.

Lukens (4) found that *H. vagans* Drechs. sporulated on filter paper and so this method was evaluated and found satisfactory for *H. dictyoides*. The fungus was grown aseptically in a synthetic medium (3) for several days. Then the mycelium was fragmented for 2 minutes in a blendorizer. The fragmented hyphae were filtered out of the medium and suspended in a 0.02 M phosphate buffer (pH 6.4). Two ml. of this suspension were poured over dry Whatman No. 1 filter papers in Petri dishes.

H. sativum and *H. dictyoides* sporulated abundantly on the filter papers within 2 days but H. vagans did not sporulate, even with the method developed by Lukens with this fungus. Apparently this method will not be satisfactory for every isolate of *Helminthosporium*.

When the experiment was repeated, the dishes containing the filter papers were placed at 7°, 19°, and 27°C. At intervals the filter papers were examined for spores with a dissecting microscope. *H. sativum* sporulated abundantly at all three temperatures. Sporulation by *H. dictyoides* varied with the temperature at which the cultures were incubated (Table 1). Sporulation began within 24 hours at 19° and reached a maximum after 72 hours. Sporulation began after 48 hours at 7°C and after 72 hours at 27°C. Mycelial growth of the fungus was not apparent after 24 hours at 7° and 19°C, but it was sparse at 27°C. It was sparse after 48 hours at 7° and 19°C., and abundant at 27°C.

After 72 hours the cultures at 7° and 27°C were placed at 19°C. to determine if these cultures would sporulate. Those which had been kept at 27°C, where growth was largely mycelial, did not increase sporulation. The isolates, kept initially at 7° C where growth began slowly, began to sporulate rapidly when placed at 19°C and within 48 hours appeared to sporulate as profusely as did cultures kept continuously at 19°C.

The size of the conidia of *H. dictyoides* varied with temperature. At 19°C the size of 100 conidia was as follows: range –: 45.5 – 101.5 x 14.0 – 24.5 microns, (av. 83.6 x 18.9 microns); septa: 2 – 7 (av. 5.3). At 7° C the size was as follows: range –: 91.0 – 220.5 x 14.0 – 24.4 microns, (av. 142.1 x 19.9 microns), septa: range: 4 – 12, (av. 8.3). These differences are shown in Figure 1. At 27°C so few conidia were formed that it was not possible to determine the size accurately.

Light is often reported to influence sporulation of fungi (2). Cultures were prepared on filter paper. Some of the dishes were wrapped in aluminum foil and all were placed on the laboratory table. The cultures in continuous darkness sporulated as profusely as those which were continuously illuminated (natural plus fluorescent).

One experiment also was made to determine whether

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spores and *H. sativum* on filter paper might be stored for a long time. The cultures were kept at 0°C for one year.

TABLE 1. The relative amounts [a] of sporulation by *H. dictyoides* when growing on filter paper at three different temperatures.

Age of Culture (hours)	7°C	19°C	27°C
24	0	X	0
48	0	XX	0
72	X	XXX	0
96	XX	XXX	X

[a] X = average of 1 conidium per conidiophore
XX = two or more conidia on 1% of the conidiophores
XXX = two or more conidia on 50% of the conidiophores

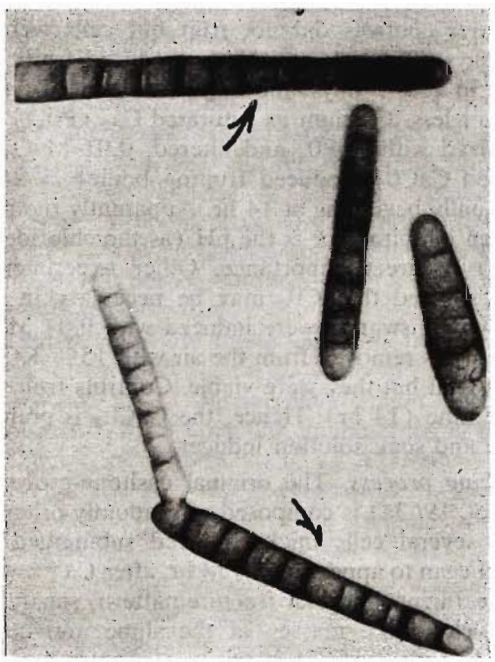

FIGURE 1. Conidia of *H. dictyoides* produced at 7° and 19°C. Larger conidia produced at 7°C.

When the cultures were removed from storage and placed at 19°C they grew, and within 96 hours sporulation was apparent.

Conclusion: H. dictyoides sporulated on filter paper. The temperature at which the cultures are placed after inoculation of the filter papers is critical. For *H. dictyoides* 19°C was optimum for rapid and abundant sporulation. At 27°C growth is primarily mycelial, whereas at 7°C there is sporulation, but at a slower rate than at 19°C. Transfer of the cultures from 27°C to 19°C did not increase sporulation, however, transfer of culture from 7° to 19°C resulted in a rapid increase in sporulation. Temperature also influenced the size of conidia of *H. dictyoides*. The conidia produced at 7°C were much larger than those produced at 19°C. Light did not affect sporulation. When *H. sativum* was stored at 0°C for 1 year it grew and sporulated when placed at 19°C. Thus the filter paper method is useful in producing conidia in the classroom, in collecting inoculum, and in storing cultures conveniently.

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