Proceedings of the Iowa Academy of Science

Volume 55 | Annual Issue Article 28

1948

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Lois H. Tiffany
*Iowa Agricultural Experiment Station*

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LOIS H. TIFFANY

Utilizing techniques which make possible the production of perithecia and ascospores of *Claviceps purpurea* (Fr.) Tul. in the laboratory (9), more detailed observations upon various phases of sclerotial and ascospore germination of this fungus have been made. The ergot bodies (sclerotia) used in these studies were collected from forty-one species of Iowa grasses. (Table I). Collections of *C. pusilla* Ces. were also made on three Andropogon species.

The length of time elapsing before stromatal formation occurred varied with the collections from different grass hosts. Some of the sclerotia collected from *Bromus inermis* had begun to form stromata in approximately 3½ months from the time they were tubed and placed at 10°C. Other collections varied widely in the time required, the variations between sclerotia in individual collections being as great as it was between the collections.

McFarland (7) reported that sclerotia over a year old failed to germinate. It has been found, however, that good germination is obtained from two year old sclerotia from *Secale cereale, Bromus inermis, Dactylis glomerata,* and *Bromus marginatus,* as well as from three year old sclerotia from *Bromus inermis.* These sclerotia had been kept in envelopes in a desk drawer in the laboratory.

Not all of the sclerotia that showed evidence of germination formed functional heads. On the sclerotia that did not, there was apparently normal rupturing of the pseudoparenchymatous surface layer of the sclerotium and emergence of a mass of light pinkish to red cells, but there was no further development. In normal germination these cells soon formed a head and stipe. The stipe elongated rapidly,

TABLE I.

Grass Hosts of *Claviceps purpurea* in Iowa.

Agropyron cristatum (Schreb.) Gaertn.
Agropyron inerme (Scribn. & Smith) Rhydb.
Agropyron pauciflorum (Schwein.) Hitchc.
Agropyron repens (L.) Beauv.
Agropyron smithii Rydb.
Agropyron trichophorum (Link) Richt.
Agrostis alba L.
Agrostis canina L.
Alopecurus pratensis L.
Arrhenatherum elatius (L.) Beauv.
Arrhenatherum tuberosum Schultz
Bromus commutatus Schrad.
Bromus inermis Leyss.

Journal Paper No. J-1551 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 884.

Bromus japonicus Thunb.
Bromus marginatus Nees
Bromus polyanthus Scribn.
Bromus purgans L.
Bromus secalinus L.
Calamagrostis canadensis (Michx.) Beauv.
Calamagrostis epigeios (L.) Roth.
Dactylis glomerata L.
Deschampsia caespitosa (L.) Beauv.
Elymus condensatus Presl.
Elymus condensatus Presl. x Mosida wheat
Elymus glaucus Buckley
Elymus giganteus Vahl.
Elymus virginicus L.
Festuca arundinacea Schreb.
Festuca elatior L.
Festuca ovina L.
Festuca rubra L.
Lolium perenne L.
Phalaris arundinacea L.
Phalaris canariensis L.
Phalaris californica Hook. & Arn.
Phalaris caroliniana Walt.
Phleum pratense L.
Poa arachnifera Torr. x Poa pratensis L.
Poa compressa L.
Poa pratensis L.
Stipa viridula Trin.

often to several centimeters in length. The first macroscopic sign of perithecial formation was the apeparance of somewhat round, darker translucent areas scattered over the entire head. As the perithecia approached maturity, the ostioles were very pronounced, extending well beyond the matrix of the head. The heads from a single sclerotium were not always at the same stage of development, though there was a tendency for the stromata from a single sclerotium to be of approximately the same maturity.

There was great variation in the size of head and in the length of stipe formed from the different collections. Ultimate size of stroma seemed to be related to sclerotial size. The largest stromata were observed from the rye sclerotia, these were also the largest of the sclerotia observed.

The sclerotia themselves varied greatly in size. The smallest, collected from *Poa pratensis,* measured 2 mm in length while the largest collected from rye were 2.3 cm. long. Sclerotial size is apparently related to the size of the normal grain of the infected plant.

No consistent differences in color were found among the stromata, all of them being a white to pale pink when the outer layer of the sclerotium was broken, and becoming dark red to rerdish-purple as they approached maturity. Varying numbers of stromata were pro-

 

duced from a single sclerotium. The largest number per sclerotia were observed from the rye collection.

Free hand sections were made from the mature heads and asci and ascospore measurements were made from them. The mature asci do not remain intact long when they are mounted in water, but soon disintegrate, releasing the eight ascospores. The immature asci are transparent and are narrowed at the base and aper. Accompanying the asci are paraphyses which closely resemble them. They are shorter than the asci and approximately the same width, but can be distinguished by their granular contents that contrast with the filamentous spores of the asci. They are more stable in a water mount, disintegrating very slowly.

The asci themselves are hyaline, thread-like, and often slightly curved. Ascospore measurements, based on measurements of seventy-five spores, show differences in the average length of the spores from the different host collections. The ranges in length of all the collections overlap however. All are somewhat larger than the range of 50 to 76 microns which Saccardo (8) lists as characteristic of the ascospores of *Claviceps purpurea.*

Relatively few observations have been made on the ascospores themselves, and these reports are conflicting. Kühn's (6) drawings of *Claviceps purpurea* ascospores are non-septate, and Tulasne (10) described the ascospores as continuous. Güssow (5), examining ascospores from sclerotia from barley, observed three distinct septa, one central and one each at equal distances from the center septum with the end cells longer than those in the center. Freeman (3) in a general discussion of ergot of grasses stated that the ascospores are divided into sixty-four cells which separate readily, each cell then being capable of growing into a mycelium. Fyles (4), working with Claviceps sclerotia from wild rice confirmed Güssow's report of the tri-septate condition. She believed that it constituted a generic character, as the species she was studying was not *C. purpurea.*

In the present studies, young ascospores from all of the sclerotial collections which had formed capitula were found to be continuous and to contain minute oil droplets. Slightly more mature spores were found to have three septa, one in the center of the spore with another septum on either side at equal distances from the center septum. The end cells of the spore were much longer than the central ones. This is evidently the stage of development observed by Güssow when he reported ascospores from barley ergot to be tri-septate. As the spores became more mature, eight, then sixteen to twenty septa were observed. This was not true of the spores from all collections, however. Ascospores from sclerotia of *Bromus purgans* remained continuous throughout their development, apparently no septa occurring even in the fully mature spores. No spores with the large numbers of cells reported by Freeman were observed.

Kühn (6) in his studies observed ascospore germination and figured typical germination, but this brief account is apparently the only reference to this important part of the life cycle of *Claviceps*

*purpurea*. In order to obtain a more complete concept of this phenomenon, mature ascospores from all sclerotial collections which had formed stroma were mounted in water in deep well slides and germination observed. All the spores from the various collections germinated in the same general way, requiring only a few hours in water for the formation of germ tubes. The first evidence of germination was the appearance in one and a half to two hours of small lateral swellings irregularly arranged the entire length of the spore. Ordinarily these did not occur directly opposite each other. At the end of five hours, the germ tubes were well developed, growing as delicate hyaline tubes from the basal swollen areas. They were often ten to fifteen microns in length at the end of this period. It seemed to be quite usual for the germ tubes to branch into a mycelial type of growth when they were less than ten microns long.

After a forty-eight hour incubation period on two per cent water agar at room temperature, each ascospore had formed several well-developed germ tubes which were 150 to 200 microns in length. There did not seem to be any set pattern in germ tube formation, varying numbers of germ tubes being formed from different areas on the individual spores.

No fragmentation of spores during germination was observed. It would be difficult to determine whether each individual cell of the mature ascospore germinated. It is certain that all of them did not in most of the spores studied, for spores were very frequently noted which had scattered areas ranging from ten to twenty microns in length which showed no visible signs of germ tube formation. In others, however, the germ tubes were scattered regularly throughout the length of the spore. Formation of germ tubes from the ends of the spore was seldom seen. The germ tubes from a single spore did not develop at the same rate, growth being highly variable. Well developed germ tubes could be found on spores that also had tubes just emerging from the basal swollen areas.

BOTANY AND PLANT PATHOLOGY SECTION,
IOWA AGRICULTURAL EXPERIMENT STATION,
AMES, IOWA.

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