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E. Robena Luck
*State University of Iowa*

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E. ROBENA LUCK

A foliage disease of *Gleditsia triacanthos* L., caused by a fungus that has been given a variety of names, is of wide distribution in the United States, occurring from the Atlantic seaboard to Nebraska and Texas (1) and will be found to occur wherever its host does. The conidial stage of the fungus has been long known as *Melasmia hypophylla* (B. & R.) Sacc. and appears to be restricted to the one host *Gleditsia triancanthos* L. The fungus was first noted in the vicinity of Iowa City about 1940, and appears to be becoming more common.

The imperfect stage was formerly classified in the family Leptostromataceae of the Fungi Imperfecti and was first described by Léveillé in 1845 as *Sacidium Gleditschiae.* In 1855 Berkley and Ravenel called it *Leptostroma hypophyllum,* but they provided no description. In 1888 specimens of the fungus were sent from Missouri, Louisiana and Kansas to Ellis and Everhart, who named it *Melasmia Gleditschiae.* In 1892 Saccardo concluded that *Melasmia Gleditschiae* and *Leptostroma hypophyllum* referred to the same species but did not recognize *Sacidium Gleditschiae* as a synonym. Saccardo used the binomial *Melasimia hypophylla* (Berk. & Rav.) Sacc. as the name of the tar spot so prevalent on the honey locust.

In 1936 Miller and Wolf (2) reported a leaf spot disease of honey locust occurring within the environs of Durham, North Carolina, and Athens, Georgia. In their study they were concerned with the anatomy of the conidial and perithecial stages with a view toward determining the taxonomic position of the fungus. As a result of an examination of the diseased leaves at intervals throughout the summer and fall, they came to the conclusion that the conidial stage is not a *Melasmia* but a *Gloeosporium.* Prior to their work, the perithecial stage had not been described. They found it to be a *Linospora* and named it *Linospora Gleditsiae.*

They described the conidial stage of the fungus as characterized by the presence of numerous small, flat, black, stroma-like fructifications growing either scattered over the lower leaf surface or localized at a definite lower portion of the leaf. Upon examination, the fructifications exhibited irregular fissures through which conidia are freed. Histological studies of tissues observed in vertical section showed the presence of conidia arising from a thin palisade of conidiophores developing from a stromatic layer seated upon the epidermal cells. The conidia are described as oblong, straight or slightly curved, hyaline and nonseptate, and measuring 3-5 x 1-1.5$\mu$. They concluded that the conidial fructification must be regarded as an acervulus and state that the cover of the fruiting structure consists not of fungus elements but of leaf cuticle.

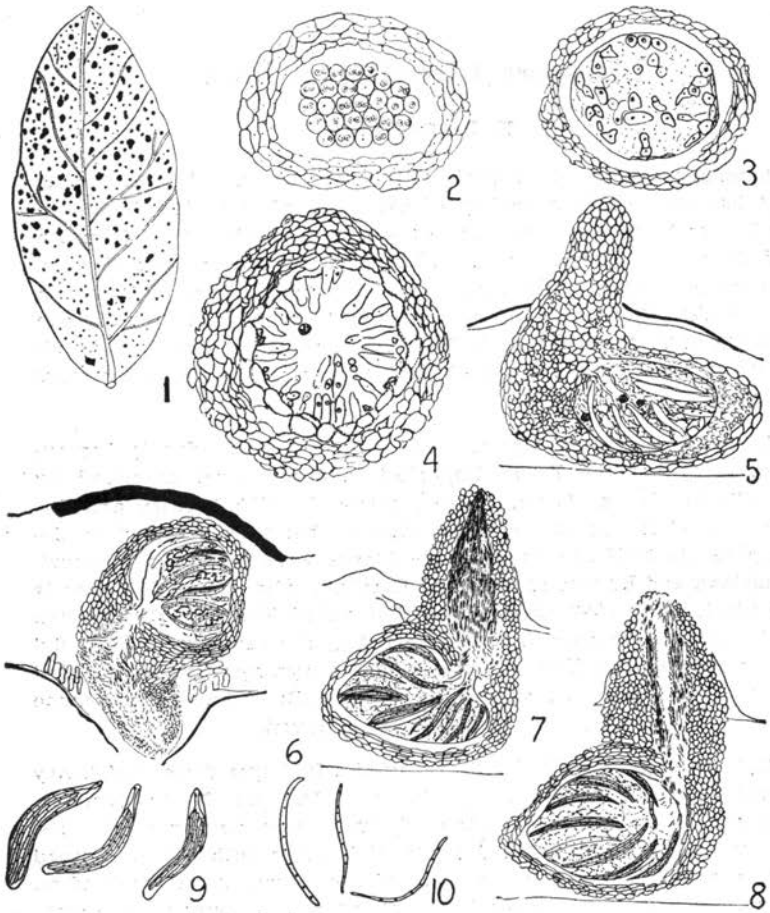


**Plate 1**

*Explanation of Figures*

Figures 2-10 drawn with aid of camera lucida and reproduced at approximately the magnifications indicated.

1. Stromata bearing acerculi on the lower surface of leaf, x 3.
2. Immature perithecium showing a number of binucleate cells inside the perithecial cavity, x 350.
3. Independent ascogenous cells resulting from fragmentation of ascogonium, x 350.
4. Cross section through an immature perithecium showing radially disposed columns of ascogenous cells, x 350.
5. Immature asci inside cavity; beak protruding through upper epidermis; ostiole not formed, x 190.
6. Beak penetrating upper epidermis; note stroma (above in drawing), x 190.
7. Paraphyses disintegrating, periphyses filling the ostiolar cavity, x 190.
8. Periphyses projecting into the cavity of the ostiole, x 190.
9. Typical asci, x 350.
10. Typical ascospores, x 350.

The perithecial stage was first observed in 1933. Maturation of perithecia occurs from the middle of May until mid-August. Miller and Wolf record as many as twenty cylindrical perithecial beaks arising individually or in pairs on the upper surface of each infected leaflet. A vertical section through such an infected leaf shows that the large perithecia are imbedded within the leaf mesophyll and that the axes of these perithecia are parallel with the leaf surface.

Perithecial initials were first observed within leaf sections of material collected in December and at that time they consisted of spherical masses of fine hyphae inclosing larger elements in a rather loose center.

The long neck of the ostiole originates as a cone at the tip of the perithecium and is directed toward the center of the leaf. Fully developed beaks, resulting from a proliferation inwardly of the cell walls, measure from 0.5-1.5 mm. in length and appear on the side of the leaf opposite that in contact with the soil.

Upon examining the early stages of the perithecial centrum, Miller and Wolf found that it consisted of true paraphyses with their ends directed toward the "upper center" of the cavity. There seems to be a continuation of these structures in the beak as the wall apex grows upward; however, when the beak matures, these threads later form the periphyses of the ostiolar cavity.

They further observed the presence of ascogenous hyphae lying at the base and sides of the perithecial wall. Their penultimate cells become the asci which grow upward among the paraphyses. Mature asci are cylindrical, curved, or straight, tapering to a narrow base though thickened or rounded at the apex. They measure 80-110$\mu$ x 10-15$\mu$ and are directed toward the opening of the neck. Within each ascus there are eight filiform, hyaline ascospores which measure 70-90 x 3$\mu$.

Miller and Wolf maintain that there is no fungous structure within the leaf that one could call a stroma. The dark threads within the epidermal cells form a pseudoclypeus through which the beaks protrude.

In 1939, G. E. Thompson (3) reported a related foliage disease on *Populus tacamahaca* Mill. The disease, caused by *Linospora tetraspora,* was first observed by Thompson occurring in the Claude River Valley, Gaspé County, Quebec, in September, 1928, and in the Temagami Forest Reserve, Ontario, during 1930-31. Since that time, specimens of the disease have been collected by other interested investigators so that it is now known to exist from British Columbia to Quebec. Because a *Linospora* affecting poplars has not been reported from North America, the fungus is regarded as a new species.

Thompson found no true imperfect stage connected with *Linospora tetraspora.* Instead, he found in the lesions developed during late July, August, and September what he called a spermatial stage.

The perithecial stage is characterized by the presence of a pseudostroma 400-750$\mu$ x 200-250$\mu$ developing within the tissue of the leaf beneath the pseudoclypeus. He was able to illustrate the pathogen-

icity of the fungus by inoculating the healthy leaves with a suspension of ascospores.

*Methods*

Leaves of *Gleditsia triacanthos* used in the investigation were obtained within the city limits of Iowa City. Leaves with the imperfect stage of the disease were collected at various intervals and placed in a moist chamber. Observations were made at intervals between February 13, 1942, and June 12, 1942. Petri dishes containing infected leaves were placed on a shelf and oriented toward the light in order to ascertain whether the perithecial beaks exhibited phototropism.

Permanent slides of the early and later stages of development were made. The structures developed in the moist chamber were killed either in Nawashin's solution or in Formalin-Acetic Alcohol at intervals of three or four days.

After fixation, dehydration and infiltration, the leaf tissues were embedded in Fisher's Tissuemat. Microtoming was at 10$\mu$. Both young and mature stages of the perithecia were stained in rapid safranin with a counterstain of fast green.

The living organism was further studied by placing the material in a drop of water. Lesions from the leaves were cut out under binocular observation by means of two well-sharpened dissecting needles. The material was dissected and a small portion placed on a slide which had been prepared with a drop of 95% alcohol. Then 3% KOH was added, followed by a drop of 2% Phloxine. A coverslip was placed over this material and slightly crushed. In studying the development of asci, an oil immersion lens was used.

**Conidial Stage**

As noted by Miller and Wolf, black lesions, ranging in size from very minute spots to large irregularly-shaped fructifications about 1-2 mm. in diameter, were found to occur in indefinite arrangement dispersed over the entire area or sometimes aggregated in patches over a portion of the lower surface of the leaf (Fig. 1). When these fructifications are examined, fissures may be seen through which the conidia are liberated. The conidial stroma forms a clypeus upon later development. Miller and Wolf are quite justified in claiming that the fructification is an acervulus. Conidia are characteristically curved or straight, hyaline and unseptate. They were found to measure 3-6$\mu$ x 1-2$\mu$, which is in close agreement with the dimensions given by Miller and Wolf.

**Perithecial Stage**

The first external indication of the presence of the perithecial stage was the appearance of yellow, cylindrical beaks projecting from not only the upper surface of the leaf but from the lower surface as well. The perithecial stage is produced from the middle of May until the middle of August on leaves that have over-wintered, as

was stated by Miller and Wolf. In a moist chamber perithecia became fully developed within two weeks on leaves collected in late winter or early spring. The beaks are more numerous on the upper surface, numbering from as few as ten to over a hundred. Daily observations showed that the yellow beaks were frequently oriented in a parallel direction.

**Early Development**

Miller and Wolf state that early development takes place by the formation of coiled hyphae located in the center of a pseudostroma. Until Andrus and Harter's investigations on *Ceratostomella,* relatively little had been reported of cell behavior amongst species in which asci develop within a perithecium. Andrus and Harter (4) maintain that in certain species of *Ceratostomella* such as *C. multiannulata* and *C. fimbriata,* "successive divisions of the binucleate fragments of the ascogonium" end in the formation of a number of tiny unwalled independent cells which subsequently form asci. It is believed that the same phenomenon takes place in the young perithecia of *Linospora Gleditsiae.* Figure 3 compares somewhat favorably, in this respect, with Andrus' and Harter's (5) reference to development in *Ceratostomella multiannulata.*

**Multiplication of Ascogenous Cells**

The early phase of cell proliferation is believed to begin with a fragmentation of the multinucleate ascogonial coil. Each fragment of the divided ascogonium continues dividing until eventually numerous binucleate cells occupy the cavity of the perithecium (Fig. 2). Immediately before formation of the beak the perithecium consists of an outer pseudoparenchymatous region and an inner zone of thin-walled cells that seem isolated and free from the wall of the perithecium. At a later phase in the proliferation of ascogenous cells, the uninucleate elements stop developing and become disorganized so that the asci which will soon develop will come to rest upon the parenchyma of thin-walled cells at the base of the cavity. About the same time, the immature perithecium contains a number of radially disposed columns of ascogenous cells (Fig. 4). The smallest and most recently formed cells are usually found next to the lining, though they may be found at times in the center.

The free cells with one and two nuclei seen intermingled in the cavity, seemed to be without hypal connection with the wall of the perithecium. In cases where the cells cling together, they seem to do so by slender filaments.

The earlier stages in cell multiplication may be differentiated from later stages by the larger size of individual cells and nuclei and by the absence of the crozier type of cell division. It seems that uninucleate cells of the ascogonium undergo what Andrus and Harter refer to as independent proliferation in the cavity. The small, scattered, uninucleate cells seemed to have a weak affinity for stain in

all early stages of the perithecial development. Nuclear vesicles are quite distinct in *Linospora Gleditsiae,* so much so that at certain stages, the prefusion nucleus seems to be floating in a sort of hyalosphere. A well-defined nuclear membrane was not seen.

About the time that the beak forms, the first immature asci may be seen within the cavity (Fig. 5). From then on, ascogenous cells in all stages of development are seen in the same perithecium. Individual cells may be seen clinging together in groups. Nuclear behavior within asci was not followed. The presence of a compact group of cells suggests the possibility that the cell divisions have occurred simultaneously in three dimensions. As in *Ceratostomella fimbriata,* there is believed to be an endogenous wall surrounding the spore-producing region. In general, the position of the wall corresponds to the periphery of the original nuclear vesicle. The wall of the fusion nucleus becomes the wall of the ascus.

**Later Stage**

Numerous perithecial beaks were observed penetrating the lower epidermis, although penetration of the upper epidermis is more common. Miller and Wolf believe that there is a pseudoclypeus present through which the beaks project. It must be emphasized that the black fungous structure is hardly a pseudoclypeus because the perithecia project from the lower surface as well as the upper (Figs. 5 & 6). It seems rather to be merely a black stroma through which the beaks penetrate incidentally. Again, the beaks do not necessarily appear on the side opposite that in contact with the soil as stated by Miller and Wolf, but on either the upper or lower side. The perithecia are situated laterally within the leaf mesophyll while the beaks, the largest ones measuring 227$\mu$ wide x 170$\mu$ long and the smallest 150$\mu$ long by 140$\mu$ wide, are bent in such a fashion as to be perpendicular to the leaf surface.

Upon microscopic examination, the perithecial wall is found to consist of 2-4 layers of thin-walled, narrow or somewhat broadened irregularly shaped pseudo-parenchymatous cells. The colorless wall is formed from one or more layers of hyphae. There is a definite ostiole present through which the spores are discharged.

No paraphyses were observed in mature perithecia, but periphyses were abundant. They project into the cavity of the ostiole or else fill the entire cavity (Figs. 7 & 8). At maturity, the asci are long, cylindrical, curved or straight, and taper to a narrow base though somewhat thickened or rounded at the apex (Fig. 9). They arise from the floor aand sides of the perithecial wall and are directed toward the opening. The asci seem to reach maturity at different ages within the perithecium. As stated by Miller and Wolf, the bases of the asci are soluble in water. The largest measure 88-89$\mu$ x 10-11$\mu$.

The filiform, hyaline, one-celled ascospores (Fig. 10) are borne eight in an ascus. They were found to measure 44-79$\mu$ x 3-4$\mu$, slightly shorter and thicker and somewhat more variable than reported by Miller and Wolf.

 

*Summary*

1. The conidial stage of a fungus present on the lower surface of leaves of the honey locust has been designated as *Melasmia hypophylla.*

2. The fructification of the imperfect state is an acervulus—a feature which places it in the form genus *Gloeosporium.*

3. The perithecial stage is known as *Linospora Gleditsiae* and is characterized by the presence of yellow cylindrical beaks projecting equally from the upper and lower surfaces of diseased leaves kept in a moist chamber.

4. At an early stage of perithecial development there is a continuous fragmentation and division of the ascogonial coil until eventually a number of binucleate cells occupy the center of the perithecium—a feature simulating the condition described by Andrus and Harter in their work on species of *Ceratostomella.*

5. Later, in the proliferation of the ascogenous cells, the uninucleate elements cease their development and become disorganized so that the asci which develop will rest upon the parenchyma cells at the base of the cavity.

This study was suggested by Dr. G. W. Martin and carried out under his supervision in the Mycological Laboratory.

DEPARTMENT OF BOTANY,
STATE UNIVERSITY OF IOWA.

*Literature Cited*

1. Weiss, F., 1941. Check list revision. Plant Disease Reporter, 25: 224.
2. Miller, J. H., and Wolf, F. A., 1936. A leaf-spot disease of honey locust caused by a new species of *Linospora.* Mycologia, 28: 171-178.
3. Thompson, G. E., 1939. A leaf blight of *Populus Tacamahaca* Mill. caused by an undescribed species of *Linospora.* Canadian Journal of Research, C, 17: 232-238.
4. Andrus, C. F., and Harter, L. L., 1937. Organization of the unwalled ascus in two species of *Ceratostomella.* Journal of Agricultural Research, 54: 19-46.
5. Andrus, C. F., 1936. Cell relations in the Perithecium of *Ceratostomella multiannulta.* Mycologia, 28: 133-153.
6. Andrus, C. F., and Harter, L. L., 1933. Morphology of reproduction in *Ceratostomella fimbriata.* Journal of Agricultural Research, 46: 1059-1078.
7. Gäumann, E., 1940. Neuere Erfahrungen über die Entwicklungsgeschichte der Ascomyceten. Zeitschrift fur Botanik, 35: 461-464.