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Studies on Two Members of the Liceales With a Description of the Life Cycle of Licea Biloris

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Echinochloa crus-galli Beauv.
Ellysia nyctelea L.
Elymus villosus Muhl.
Erigeron annuus (L.) Pers.
Erigeron strigosus Muhl.
Eupatorium purpureum L.
Eupatorium rugosum Houtt.
Erythronium albidum Nutt.
Festuca obtusa Biehler
Fragaria virginiana Duchesne
Fraxinus nigra Marsh.
Galium concinnum T. & G.
Geranium maculatum L.
Geum canadense Jacq.
Gleditsia triacanthos L.
Hackelia virginiana (L.) Johnst.
Hedeoma pulegioides (L.) Pers.
Helianthus strumosus L.
Hepatica acutiloba D.C.
Hydrophyllum appendiculatum Michx.
Hydrophyllum virginianum L.
Hystrix patula Moench.
Impatiens pallida Nutt.
Isopyrum biternatum (Raf.) T. & G.
Ribes missouriense Nutt.
Rubus occidentalis L.
Rubus allegheniensis Porter
Sanguinaria canadensis L.
Sanicula canadensis L.
Smilacina racemosa (L.) Desf.
Smilax tamnoides L. var. *hispida* (Muhl.) Fern.
Solidago rugosa Muhl.
Sphenopholis intermedia Rydb.
Sphenopholis obtusata (Michx.) Scribn.
Symphoricarpos orbiculatus Moench.
Thalyctrum dasycarpum Fisch & Ave-Lall.
Tilia americana L.
Triosteum perfoliatum L.
Ulmus rubra Muhl.
Urtica dioica L.
Uvularia grandiflora Sm.
Viola missouriense Greene
Viola papilionacea Pursh
Viola pensylvanica Michx.
Viola sororia Willd.
Zanthoxylum americannum Mill.

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Studies on Two Members of the Liceales With a Description of the Life Cycle of *Licea Biforis*

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Licea biforis was first described in 1893 by B. Morgan (Lister, 1911), and later by Lister (1911), Fullmer (1921), Macbride (1922), Hagelstein (1944), and Martin (1949). Distribution of the species as given by Hagelstein (1944) is: Iowa, Kansas, New York, Ontario, and Pennsylvania. Martin (1949) adds Po-

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land and Eastern Asia, and Macbride (1922) includes Canada. Hagelstein (1944) is of the opinion that *L. biforis* probably has a much wider distribution than that indicated above, but is overlooked because of its small size and its habitat on dead wood.

Describing the sporangium, Lister (1911), Fullmer (1921), Macbride (1922), Hagelstein (1944), and Martin (1949) agree that it has a yellow-brown color, an elongated shape (a sessile plasmodiocarp), and a size of 0.5 mm by 0.1 mm. Lister (1911) and Hagelstein (1944) remark that the mature sporangium resembles a date-stone. Lister (1911), Fullmer (1921), and Hagelstein (1944) describe a papillose sporangium wall; Martin (1949) and Macbride (1922) say the wall is smooth with "minute scattered granules on the inner surface." Lister (1911) and Hagelstein report that the sporangium wall is nearly colorless with superficial deposits of discharged refuse matter. Macbride (1922) considers the sporangia to be gregarious, while Lister (1911), Fullmer (1921), Hagelstein (1944), and Martin (1949) say the sporangia are scattered.

Lister (1911), Macbride (1922), Hagelstein (1944), and Martin (1949) report dehiscence along a central ridge or depression of the peridum. Macbride (1922) and Martin (1949) hold that the two equal parts resulting from such dehiscence remain attached at the base. Fullmer (1921) says the sporangium dehisces into two "lobes".

All descriptions agree that the spores are nearly colorless, the shape of the spores is ovoid to globose, and the size is 9 to 12 μ in diameter. Martin (1949) says the spores are "minutely roughened"; Lister (1911), Fullmer (1921), Masbride (1922), and Hagelstein (1944) say they are smooth. Lister (1911) and Hagelstein (1944) also state that the spore wall is thinner on one side.

Martin (1949) refers to the plasmodium, describing it as "watery white, then grayish." Martin (1949) implies that the plasmodium of *L. biforis* is of the usual type considered typical of myxomycetes, and Locquin (1949) reported thin, transparent portions in the *L. biforis* plasmodium, in which protoplasm moves in a reversible flow. Wollman and Alexopoulos (1964) also report streaming in the plasmodium of this species.

In a study of material developing on moist chamber bark cultures, McManus (1964) reported that the plasmodium was comparable in size, lack of veins and absence of streaming to the "protoplasmodium" described by Alexopoulus (1960) for *Echinostelium minutum*, but that it differed in that it fruited to form several sporangia. McManus described the plasmodia of *L. biforis* taken from bark as soft brown, nearly spherical masses, about 300 μ across, having no veins, showing no reversible streaming, and remaining rounded up even when migrating. Each plasmodium was reported to have produced from 3 to 5 sessile sporangia.

On bark from apple trees collected in the fall of 1965 on the campus of Mt. Mercy College, Cedar Rapids, Iowa, moist chamber cultures produced in a few days hundreds of fruiting bodies of *Licea biforis*.

Since the successful laboratory cultivation of this species through its complete life cycle had not been reported and we wanted to make further studies of the plasmodial type, cultivation on agar was attempted. Difco corn-meal agar (1.5% in bark extract) plates were inoculated with crushed sporangia. Plasmodia developed on the plates and fruiting bodies appeared in about 9 days. Plasmodia from the bark in moist chamber were also transferred to agar plates, where they completed their development and produced isolated sporangia.

The life cycle of *Licea biforis* was then studied through its stages in culture on agar and on glass slides. Fish food granules and elm bark extract (made by allowing elm bark to stand submerged in tap water for a week, then filtering and sterilizing the supernatant) were supplied to the cultures, which contained bacteria.

This paper reports for the first time the laboratory cultivation of *Licea biforis* from spore to spore and offers evidence that its plasmodial type is similar to the "protoplasmodium" described for *Echinostelium minutum* (Alexopoulos, 1960) and far *Clastoderma debaryanum* (McManus, 1961).

General Morphology

The mature sporangium of *Licea biforis* is a brown plasmodiocarp, usually elongate and averaging 0.47 mm (S. D. = 0.0278 mm) in length by 0.14 mm (S. D. = 0.0015 mm) in width. In shape the sporangia vary: some are straight, others have slight or greater curvature, a few were horse-shoe shaped, (Fig. 1) and some were forked.

The sporangium has been described (Lister, 1911 and Hagelstein, 1944) as resembling a date-stone. It has a central narrow, longitudinal area, lighted in color than the rest, which has been interpreted as the central ridge or depression of dehiscence. Lister (1911) and Hagelstein (1944) describe the sporangium as "dehiscing along a central ridge or depression." Macbride (1922) and Martin (1949) add that "the two equal parts resulting from dehiscence by a longitudinal slit remain attached at the base." Fullmer (1921) says the sporangium dehisces into two "lobes." Our observations, however, do not support any of the above reports regarding dehiscence.

The spore sac of the fruiting structure is an elongate, almost transparent structure that resembles a worm on the substrate. Fruiting bodies that develop in nature arise from plasmodia that have accumulated a good deal of dark debris in their surrounding slime, and the spore sac develops within this dark accumulation. The light strip seen logitudinally on the surface of the

fruiting body is the area of the spore sac not covered over by the materials picked up in the slime. Sporangia that have developed on agar that is not contaminated by mold and debris are very light in color, and consist of the spore sac with only very little outer covering.

No slit of dehiscence or ridge can be detected in any of these, either fresh or dry, when examined at 80 x. The longitudinal clear area is evident in all sporangia as soon as they are fully formed. If a great deal of debris is present in the slime, this clear area probably would be the first place for the sporangium to crack, but it is possible to separate the inner spore sac from the "coating" of debris. The sac can be shelled out, leaving both sac and coating intact. No depression can be seen on the sporangium thus exposed. These observations lead us to believe that a slime coat containing debris from the substrate envelops the fruiting body as it matures and this forms the darker, lateral parts of the mature sporangium.

Lister (1911), Fullmer (1921), and Hagelstein (1944) describe the wall of the sporangium as "minutely papillose," while Martin (1949) says the wall is smooth with "minute granules on the inner surface." Macbride (1922) also described minute granules scattered on the inner surface of the sporangium wall. Our observations by transmitted light of unobscured sporangia developing on agar did show a papillose sporangium wall, but no granules could be detected.

The immature sporangium which has developed on clean agar is light yellow-brown in color, sometimes nearly colorless and opaque. As they mature, the sporangia darken and if debris has been present in their surrounding slime, they become dark brown laterally. The light, central longitudinal strip remains as the sporangium dries.

Spores become evident as the sporangium matures. They are globose or ovoid in shape and almost colorless. The contents of the spore are visible through the spore wall. Martin (1949) says the spores are "minutely roughened," but Lister (1911), Fullmer (1921), Macbride (1922) and Hagelstein (1944) say they are smooth. In our material, the spores were quite smooth. Lister (1911) and Hagelsten (1944) state that the spore wall is thinner on one side; this could not be detected in our material. All descriptions agree on size of the spores: 9 to 12 μ in diameter. We found the spore size to be quite uniform, averaging 11.44 (S.D. = 0.0364) μ in diameter.

Laboratory Culture of Licea biforis

Bark gathered from apple trees was placed in plastic containers lined with brown paper toweling and flooded with water. After a few days, these moist chamber cultures showed fruiting bodies identified as *Licea biforis*, fruiting bodies of two other species of myxomycetes, and several unidentified grey phanero-

plasmodia. Various pieces of bark were then isolated in petri dishes on moist paper in an attempt to separate the various species for further study. Material which warranted further investigation was transferred to slides for microscopic examination, or to agar plates for culture. The myxomycetes fed on bacteria growing in the cultures; granules of fish food and oatmeal were added as nutrients. The plates were kept moist with bark extract.

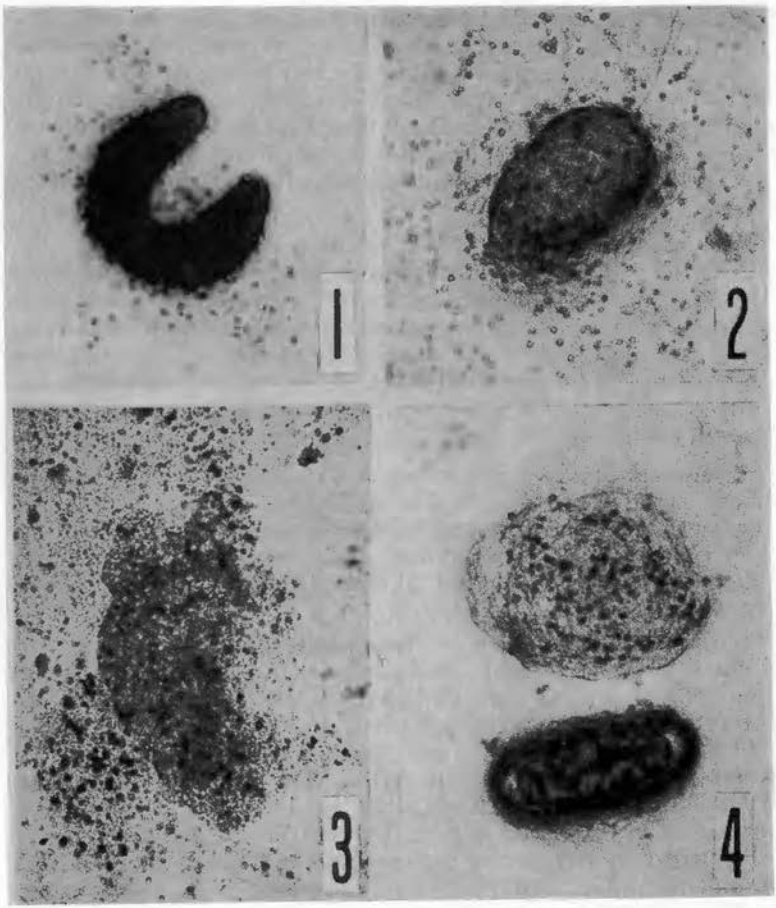

Fig. 1. Horseshoe-shaped sporangium of *Licea biforis* on surface of agar plate. Photographed by transmitted light. X 50.

Fig. 2. Plasmodium of *Licea biforis* on surface of agar plate. Photographed by transmitted light. X 50.

Fig. 3. Elongate, migrating plasmodium of *Licea biforis* on surface of agar plate Note that debris from the agar surface has been picked up by the plasmodium, leaving a clear track behind it. X 50.

Fig. 4. Mature plasmodium and differentiated fruiting body of *Licea biforis* on agar. Note that the slime coating surrounding the plasmodium contains dark spores that it has picked up from the contaminants on the agar surface. The inner spore case can be seen in the fruiting body, distinct from the outer dark mass surrounding it. Photographed by transmitted light. X 50.

Sporangia of *L. biforis* were picked off the bark with forceps, crushed, and put in a drop of elm bark extract and sealed under cover slips on glass slides. These preparations were examined by transmitted light and by phase contract. Many spores germinated within 24 hours.

Observations on the Life Cycle

Spore germination.—Spores in slide preparations germinated in one day, but the actual germination process was not observed. Examination of the empty spore cases at high magnification by phase contrast illumination revealed the presence of small openings or breaks in the spore walls. From this evidence, we assume the method of germination is by means of a pore in the spore wall.

Swarm cells.—Observations with phase contrast of sealed cover slip preparation of germinating spores revealed pear-shaped protoplasts which appeared to have a single anterior flagellum. Eventually these swarm cells changed into myxamoebae.

Myxamoebae.—Myxamoebae appeared two days after inoculation of the agar plates with spores. These amoeboid protoplasts moved slowly, without forming large pseudopods. No fusion of myxamoebae was ever observed. Some of the myxamoebae encysted, in dry or moist conditions, even when additional food was given.

Plasmodium.—The "typical" myxomycete plasmodium is a large fan-shaped structure that forms a system of veins in which protoplasm streams with regular reversal of direction of flow. The plasmodium of *L. biforis* does not conform to this description. It is microscopic, resembling in most respects the plasmodia of *Clastoderma debaryanum* (McManus, 1961) and first described for *Echinostelium minutum* by Alexopoulos (1960) and designated a "protoplasmodium" (Alexopoulos, 1960 [1961]). It It does not grow beyond microscopic size and each plasmodium gives rise to but a single sporangium. It migrates very slowly, extending only very short pseudopods, and remains always roughly spherical. (Fig. 2) Streaming is sluggish and without a discernible pattern. Never in its plasmodial stage does it differentiate into veins or form an advancing fan, but always resembles the very early stages of the plasmodium of other types of myxomycetes. This plasmodium retains juvenile characteristics throughout its existence. We have found that the plasmodium of *Licea biforis* generally conforms to the characteristics of the "protoplasmodium." Its general shape is roughly spherical, often somewhat elongate as it becomes more mature. In the young stages it feeds and grows, engulfing bacteria, spores, and other small encysted plasmodia. The plasmodium of *L. biforis* does not actively migrate in the early stages, and it never forms a network of veins or fans and never shows reversible streaming. Protoplasmic movement was observed in *L. biforis* only rarely. It was

slow and irregular. A slime layer at the margin is usually evident. The mature plasmodium of this species shows only slight changes from its original form except in size and quantity of ingested material. Just before it fruits, it begins to migrate actively, eroding the surface of the agar in its path (Fig. 3). Long "migration tracks" can be followed in the agar as the plasmodium travels across the plate. McManus (1961) reported similar erosion of agar by migrating plasmodia of *Clastoderma debaryanum*. McManus (1961) also described the myxomycete's avoidance of areas contaminated by mold in a culture, and its seeming inhibitory effect on mold growth. A similar observation was made in our cultures of *L. biforis*. Mold and myxomycetes did not grow in the same location.

In a previous study of plasmodia of *L. biforis* which developed on bark and were removed to agar for study when they were nearly mature, McManus (1964) described them as soft brown masses. These masses migrated for some distance on the agar and then fruited. The plasmodia which developed in our agar cultures in the present study were colorless. The previously reported brown color of the plasmodia was probably due to ingested material from the bark, and debris accumulated in the slime of the plasmodia that have developed and traveled on bark.

Fruiting.—When the plasmodium of *L. biforis* is ready to fruit, it becomes more elongated and sausage-shaped. The newly formed fruiting bodies are yellowish tan in color and appear to have a slime coat around them. As the sporangia mature and dry, any debris that has accumulated laterally dries adherent to the sporangium wall. Thus, the fully mature sporangium is darker laterally with a central lighter strip, giving the appearance of a date-stone. Figure 4 shows a plasmodium beside a freshly formed fruiting body on agar.

In the study reported in 1964, McManus noted that several sporangia appeared at the site of fruiting of the dark brown masses that had been removed from bark cultures to agar. Since the masses had migrated as a unit, the units were assumed to be individual plasmodia.

Observations here, on plasmodia that have developed on agar and have accumulated a minimum of debris, indicate that each plasmodium fruits to produce a single sporangium. The previously reported brown masses may have contained several plasmodia, or the contained single plasmodium may have fragmented into several before fruiting.

Fragmentation of mature plasmodia has not been observed in our cultures of *L. biforis*, but it has often been observed in *Clastoderma debaryanum* and in *Cribraria violacea*.

Cribraria violacea Rex

Plasmodia of *Cribraria violacea*, another member of the order Liceales, appeared in moist chamber cultures of elm bark col-

lected in the fall of 1965. These plasmodia were transferred to agar plates and to glass slides for study of protoplasmic streaming.

The plasmodium of *C. violacea* can be seen with the naked eye as a black speck when it is on a light substrate. In a previous paper, McManus (1963) reported that the black color of this species is due to the pigmented granules of the plasmodium rather than to ingested materials. McManus (1964) also reported that, unlike the typical protoplasmodium, the plasmodium of *C. violacea* stretches out into a small net and displays a fan-like anterior portion when it is actively migrating. In migrating plasmodia, protoplasmic flow was observed to be unidirectional, there being no discernible regular reversal of streaming.

In our cultures of *C. violacea,* the observed protoplasmic movement and locomtoion of small plasmodia were similar to the migratory activity of an amoeba. Protoplasmic streaming was unidirectional–in the direction of movement.

A plasmodium of *C. violacea,* transferred from culture in a petri dish to a sealed preparation on a glass slide, showed a peculiar "tail" formation with a contractile vacuole posteriorly. This was later withdrawn into a small black lump on the edge of the plasmodium. Subsequent itermittent observations of other plasmodia with "tails" revealed no clue to the reason for their formation. To determine this, further study of them will be necessary.

Conclusion

The plasmodia of both *L. biforis* and *C. violacea* conform in several respects to the original description of the protoplasmodium in remaining microscopic until fruiting, in forming no veins, in showing no reversible streaming, and in producing one sporangium from each plasmodium. However, the plasmodium of *C. violacea* differs from that of the protoplasmodium in displaying active protoplasmic streaming and in the formation of a small system of veins. As further studies of minute myxomycetes are undertaken, other differences may be discovered which would warrant modifying the concept of the protoplasmodium to include such a deviation.

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The Remnant Prairie Flora in Northeast Iowa[1]

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Abstract. A list of 360 species of vascular plants inhabiting prairie remnants in northeast Iowa is presented. Descriptions and locations of the prairie remnants are also included.

Recently, the writer completed a survey of the vascular plants of an area of northeastern Iowa which was covered by the Iowan lobe of the Wisconsin glaciation (1). The original vegetation of this region was, in large part, tall grass prairie, interrupted by strips of woodland along the streams and along the adjacent highlands. All that remains of this prairie biome are a few remnants in state or county parks or preserves, along unsprayed railroads or highways, and on private land unsuitable for cultivation. This paper lists the species of vascular plants collected and observed in these prairie remnants during the floristic survey, and indicates the native prairie species which still persist and also the introduced species which have become naturalized.

Prairies are generally defined as open regions dominated by grasses; yet within prairie regions there is a vegetational continuum from the dry upland grass-dominated knolls to the moist low sedge meadows, or swales. In this report I have included all species inhabiting this prairie continuum, excluding only plants inhabiting disturbed areas and the marsh and aquatic plants.

The list of species was compiled from collections and observations made in the field during the growing seasons of 1962 and 1963, and collections in the herbaria of the University of Iowa at Iowa City, Iowa State University at Ames, and the State College of Iowa at Cedar Falls. No literature citations are included in this list unless substantiating specimens were seen.

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