Developmental morphology of Caloplaca ulmorum (Fink) Fink, Caloplaca cerina (Ehrh) Th Fr, and Xanthoria elegans (Link) Th Fr

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Developmental morphology of *Caloplaea ulmorum* (Fink) Fink, *Caloplaea cerina* (Ehrh.) Th. Fr., and *Xanthoria elegans* (Link) Th. Fr.

by

Carl Patrick Malone

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Botany and Plant Pathology
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INTRODUCTION

Gross morphology of the lichen thallus traditionally has been important at the generic level. Caloplaca, as an example, is a crustose genus that is similar in every other respect to the foliose genus Xanthoria. Within Caloplaca one can find species that are nearly foliose at the margin, and within Xanthoria one can find species that are nearly crustose at the center. The effects of environmental modification on Caloplaca spp. and Xanthoria spp. and on the taxonomy of the two groups are unknown.

Caloplaca ulmorum (Pink) Fink, C. cerina (Ehrh.) Th. Fr., and Xanthoria elegans (Link) Th. Fr., three common lichens, inhabit tree bark or rock substrates. Caloplaca ulmorum, presently considered to be a crustose species that occurs most commonly on Ulmus spp., was selected for study for several reasons. The type locality is close, having been initially reported from Boone County, Iowa, as a variation of Caloplaca cerina (then Placodium cerinum Naeg. apud Hepp.) by Miller (1904). Some of the original material is still on deposit in the Iowa State University Herbarium. Fink (1910, 1960) distinguished Caloplaca ulmorum from Caloplaca cerina only by the prominence and coloration of the exciple and by the lighter color of the epitheciun.
Caloplaca cerina, first described in 1789 and placed in its present position by Theodor Fries in 1861, was chosen for study because of its relationship to C. ulmorum and because it occurs commonly on both rock and bark substrates. Furthermore, this species has been in numerous genera (Zahlbruckner, 1931).

Xanthoria elegans, described by Link in 1791 and transferred to its present position by Theodor Fries in 1871, was chosen for several reasons. The central portions of the thallus may appear nearly crustose, and, in fact for many years this species was placed in the crustose genus Caloplaca. This species occurs only on rocks and may grow in close proximity to Caloplaca cerina.

This study was conducted to evaluate any differences in fungal morphology of organisms collected over a relatively wide geographical range that would support the separation of Caloplaca ulmorum from Caloplaca cerina and that would support the placement of Xanthoria elegans in the genus Xanthoria rather than the genus Caloplaca.
LITERATURE REVIEW

Tulasne (1852) published one of the earliest microscopic studies of lichens relating to spermatial and pycnidial development. Puistling (1868) reported that the apothecia of Lecidea humosa (Ehrh.) Nyl. formed from the hyphae between the cracks of the crustaceous aerolae on the substrate. The first developmental stages were represented by a complex of slender hyphae which contained a long slender filament composed of short hyphal cells with richer cytoplasmic contents. This special structure disappeared when the hymenium began to form.

Stahl (1877) published some fine developmental studies of some of the Collemaceae and of some species in Physma, Synechoblastus, and Leptogium. He described a septate coiled filament which extended upward toward and through the surface of the thallus of Collema microphyllum Ach. The tip of the trichogyne was swollen and covered with a mucilage to which spermatia would adhere. After dissolution of the wall between the spermatium and the trichogyne, the septa of the trichogyne appeared to swell, and the cytoplasmic contents seemed to deteriorate. A mass of hyphae arose from the ascogonium located in the center of the ascocarp.

Baur (1898) worked on Collema crispum (Huds.) Wig. and essentially confirmed Stahl's work on other members of the Collemaceae. Collema crispum had two forms: one
form was crowded with apothecia and spermogonia, and the other form had very few apothecia and spermogonia. He observed the adherence of spermatia to the tip of the trichogyne and the subsequent deterioration of the trichogyne and assumed the passage of nuclei. He postulated that the difference between the two forms was due to the lack of adequate numbers of spermatia at the time the trichogynes were exposed on the surface. The ascogonial apparatus increased in size by intercalary division, and the ascogenous hyphae arose from the ascogonium. At no time did he observe fusion of nuclei.

Bachmann (1912, 1913) worked extensively on Collema pulposum (Bernh.) Ach. and reported that spermatia originated as groups of cells budded from vegetative hyphae in scattered regions of the thallus. The trichogynes of this organism were different from those reported for other members of the Collemaceae; the trichogynes traveled laterally through the thallus until they contacted an area where spermatia occurred. Subsequently, the cross walls were observed to swell, and the trichogynes were observed to deteriorate. Ascocarp development was similar to what Stahl and Baur reported after dikaryon establishment. Bachmann stressed the similarity of Collema pulposum to Pyronema spp. and to some of the Laboulbeniales.

Lindau (1888) established the presence of ascogonia and trichogynes in some species of Anaptychia, Physcia,
Ranallina, Lecanora, and Lecidea. He frequently observed spermatia in contact with trichogynes but was unable to demonstrate any degenerative changes. In Parmelia tiliacea (Hoffm.) Ach. and in Xanthoria parietina (L.) Th. Fr. he was able to demonstrate ascogonia but never any trichogynes. The course of events in apothecial development was similar to that which Stahl (1877) described for Collema.

Baur (1904) further examined Anaptychia and essentially confirmed the series of events that Lindau had already reported. However, he reported that ascogonia occurred in clusters, and that apparently several ascogonia could enter into the formation of a single hymenium. Vegetative hyphae below the ascogonia grew vigorously upward and around the ascogonia; algae in the same region also grew vigorously along with the rapidly growing hyphae and eventually formed the thalline margin. As the young ascocarp grew upward and enlarged, the ascocarp was exposed when the fibrous upper cortex weathered away. In Xanthoria the rapidly growing hyphae pushed their way between the cells of the cortex exposing the ascocarp. In Lecanora subfusca (L.) Ach. there was an extended period of sterile growth followed by the abundant production of both spermogonia and ascogonia. Ascogonia were a tangled clump of cells sometimes showing distinct spirals. The surrounding hyphae grew towards the cortex
before ascogonial production and were visible on the surface of the thallus before the trichogynes were.

Borzi (1878) also established the presence of ascogonia and trichogynes in several species of Parmelia, Anaptychia, Sticta, Picasola, and Lecanora. Darbishire (1900) established for Physcia pulverulenta (Schreb.) Hampe the same developmental pattern that Lindau had demonstrated for other Physcia species.

Hue (1906) considered the emergence of the lichen ascocarp. The vegetative hyphae surrounding the ascogonium in the medulla grew rapidly and branched vertically carrying the young fruiting body upward to a superficial position. In the lecideine series the encircling hyphae rose upward through the algal layer to the surface and there expanded laterally and then turned upward to form the exciple. Algae were lacking from the hypothecium and the exciple. In the lecanorine series the algae were carried upward by the expanding vegetative hyphae which gave rise to the algal layer in the hypothecium. The algal layer of the thallus and the cortex were also associated with the medullary column and grew along with it providing a thalline margin.

Erbisch (1969a, b) studied ascus and ascospore development in five species of the lichen Pertusaria and ascocarp development in one species of the lichen Melonaria. He reported that the nuclei of vegetative
hyphae were very small (0.5 to 0.8 microns), the nuclei of ascogenous hyphae were of moderate size (0.8 to 1.0 microns), and the fusion nuclei of the young asci were the largest (2 to 3 microns). Nuclear divisions were hard to find, and only one undoubted meiotic division was seen. He reported three patterns of ascus development: asci arose directly by the enlargement of an upright, uninucleate terminal cell of an ascogenous hypha, asci arose in a similar manner but from binucleate hyphae, or the asci arose from what looked like a crozier except all cells were binucleate.

In Melonaria the earliest stage of ascocarp development was the appearance of a mass of binucleate hyphae in the lower part of the thallus. Larger irregular hyphal cells developed in this mass to form a compact hyphal aggregation. The apothecial ball began to flatten out, a lacuna began to form, and asci arose from the enlarged irregular binucleate hyphae. In the early stages of development all the asci in the hymenium were the same size. No croziers were observed.

Letrouit-Gallinou (1968) studied apothecial development in a number of lichens: Buellia canescens (Dicks.) De Not., Lecidea elaeochroma (Ach.) Ach., Lecanora subfuscata Mang., Phlyctis agelaea (Ach.) Plot., Roccella montagnei Bel., Thelotrema lepadinum (Ach.) Ach., Pertusaria pertusa (L.) Tuck., Cladonia floerkeana (Fr.)
Somm., Baeomyces rufus (Huds.) Rabent., Graphis elegans (Borr. ex Sm.) Ach., Opegrapha viridis (Pers. ex Ach.) Nyl., and Graphis scripta (L.) Ach. She described a number of structures which entered into the formation of an ascocarp (Cf. Table 1). The primordial plexus was a mass of hyphae that enclosed the ascogonial apparatus. The primary corpus was derived from the primordial plexus and consisted of a carpocentrum and a pericentrum. The carpocentrum was eventually divided into two parts: the subhymenial net which consisted of \((n+n)\) hyphae and \(n\) hyphae and the paraphysidal net was attached below to the subhymenial net and above to the pericentral envelope. Epicentral filaments grew upward from the subhymenial net and became the primary paraphyses of the ascocarp. Elements of the paraphysidal net were not true paraphyses.

The pericentral envelope or pericentrum consisted of two layers: the textile part (roof) and the lower part (floor). The lateral annular wall of the pericentrum was the pericentral muff; it joined the roof and the floor and completely encircled the carpocentrum.

The young primordium was surrounded by a circum-central plexus. New hyphae formed on the external face of this structure while filaments on the internal face differentiated into either roof, floor, subhymenial net, or paraphysoidal net. The floor of the pericen-
trum was enlarged by the proparathecium of the peribase. The subhyphal net was enlarged by a region called the circum-central link.

Letrouit-Gallinou distinguished six apothecial types on the basis of the presence and absence of certain of the regions listed above. She noted that in *Lecanora subfuscata* and in *Lecidea elaeochroma* the primordial plexus forms well before the ascogonial apparatus was visible.

Bellemere (1967a, b) studied apothecial development in some inoperculate non-lichenized Discomycetes. He distinguished two basic forms: the discostromatic form in which the apothecium was devoid of a typical parathecium and amphitheclium and the discopodecial form in which there was a more or less complete apothecium with a well developed parathecium and amphitheclium. The discostromatic forms were further subdivided into three groups: the lenticular group, the non-lenticular group, and the pseudodiscopoid group. The lenticular group was most similar to the lichens and was characterized by the method of enlarging their developing ascocarps. Hyphae at the margins of the young ascocarp extended it laterally; the carpocenter likewise grew marginally. The young ascocarps developed rapidly in the form of a biconvex lens. The lenticular group was further subdivided on the basis of the kinds of tissue present
in the conceptacle. Bellemere suggested that some of the apothecial forms studied may be ascostromatic in nature and not Euascomycetes since they originated from mycelium or from stromata before ascogonia were visible.

Corner (1929a) studied ascocarp development in some non-lichenized Discomycetes (Cf. Table 1). With apothecia that demonstrated angiocarpic development, sterile hyphae arose from the stalk of the ascogonium. These enveloping hyphae branched profusely, suggesting a sympodium, so that a large ball of hyphae with an ascogonium at its center resulted. Cells in the outer layer enlarged and became pseudoparenchymatous. A cavity formed on the inside of the ball of hyphae mostly by lysigenous action. Hyphae grew from the floor and sides of the cavity to form a palisade layer. The first asci form at nearly the same time that the cortical tissue was ruptured. In the gymnocarpic developmental pattern, the enveloping hyphae do not form a closed ball but grow upward to form a palisade layer; the thickness of the apothecium depended on the amount of upward growth at this time. Paraphyses matured centrifugally, and cortex matured centrifugally and acropetally. Hemian-giocarpous development was intermediate between angiocarpic and gymnocarpic development.

Corner (1929b), while studying apothecial growth, also found that where the hypothecium and the cortex
meet there was a group of sympodially branching hyphae which he called the marginal sheaf. Cortical cells were formed on the external face, and paraphyses were formed on the internal face. He noted that in the young apothecium what appear to be paraphyses were actually apically growing, monopodially branching hyphae in contrast to the secondary paraphyses formed by the marginal sheaf.

Werner (1930) reported that spores of several lichens including Xanthoria parietina (L.) Th. Fr. and Caloplaca vitellinula (Nyl.) Oliv. matured in the spring and were discharged then. Germ tubes appeared at the ends of the polarilocular spores. The hyphae ramified considerably and formed a light colored colony with the spores still visible at the center.

Rudolph and Geisy (1966) found that the young ascus of Physcia aipolia (Ehrh.) Hampe was thin-walled and initially lacked a terminal pad. Later in development the ascus wall thickened, and the apical pad became prominent; there was an irregular boundary between the terminal pad and the epiplasm. At maturity there were three layers to the ascus: the outer relatively electron dense layer, the inner relatively electron transparent layer, and the apical pad.

Brown and Wilson (1968) reported that the lower cortex of Physcia aipolia had a rather striking microfibrillar nature. The microfibrils appeared to origi-
nate in the outer region of the hyphal wall and were generally oriented perpendicular to it. Numerous bacteria were also visible growing on the undersurface of the thallus. Bednar and Juniper (1964) reported similar microfibrils in the cortex of *Xanthoria parietina*.

Jacobs and Ahmadjian (1969) and Ahmadjian and Jacobs (1970) also reported bacteria within the thallus of *Anaptychia palmatula* (Michx.) Vain. and *Endocarpon pusillum* Hedw. respectively.

Moore (1962) in his treatment of the non-lichenized Discomycete *Ascodesmis* reported that the ascus was uniseriate even though there was some darkening over the outer surface of the ascus wall. Reeves (1967) reported that the ascus of *Pyronema domesticum* (Sow. ex Fr.) Sacc. was uniformly electron transparent. He also reported that ascospore formation was associated with an infolding of the ascus membrane. Greenhalgh and Griffiths (1970) while reporting on the ascus vesicle showed that the ascus of *Hypoxylon rubiginosum* (Pers. ex Fr.) Fr. was uniformly electron transparent with a slight amount of darkening over the surface.

Reznick, Peveling, and Vahl (1968) examined the rhizines and cilia of several lichens with both the transmission and scanning electron microscopes. They also reported microfibrillar material around the cell walls of the hyphae in the cilia and rhizines. They demon-
strated that the rhizines, cilia, and undersurface of the thallus were remarkably uniform and show very few intercellular spaces.

Luttrell (1951) proposed that there were two kinds of asci that were of taxonomic importance in the Euascomycetes and Loculoascomycetes. Lichen asci have not been thoroughly studied, and their relationship to unitunicate or bitunicate asci is not well understood. Dennis (1968) indicated that some members of the Lecanorales have bitunicate asci, while others have thick-walled asci that stain blue with iodine. Chadefaud (1942 and 1960) believed that all asci consisted of two layers which may or may not separate from each other.

Ziegenspeck (1926) reported that in the lichens Nephroma, Cetraria, Ramalina, and Imbricaria the ascus wall separated into two layers: a thicker outer wall that blued with iodine and a thinner slime wall.

The taxonomic position of lichens has long been in a state of flux (Smith, 1921). Pink (1915) made a strong argument for placing the lichens with the fungi. Pink argued that the five orders of lichens be placed co-ordinately with the free-living Ascomycete orders. Many years later the International Code of Botanical Nomenclature (Lanjouw et al., 1966) declared that a name based on two or more discordant elements was invalid unless one of those elements could be selected for
a satisfactory type. Dennis (1968) has made an attempt to integrate the lichens into the Ascomycetes.

Nannfeldt (1932) separated the Lecanorales from the Ostropales and the Helotiales on the basis of the duration of the ascocarp, the texture and wall thickness of asci, the intensity of the iodine reaction, and texture and branching pattern of paraphyses.

Richardson (1970) reviewed the generalities of ascus and ascocarp structure as known in the lichens and stated that the characteristics of the exciple and other characteristics of the ascocarp typically used by mycologists might be more successfully applied to lichen taxonomy. Families in the Helotiales are presently delimited in part by the texture of the exciple (Dennis, 1968). Korf (1951) has suggested a revised listing of excipular characteristics which has become accepted.
Table 1. Equivalency of some terms used in developmental asccocarp morphology

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MATERIALS AND METHODS

Collection Sites for Specimens

Specimens of *Caloplaca ulmorum* were collected at two sites in Franklin Township (T-76N, R-35W) and at one site in Cass Township (T-75N, R-37W) in Cass County, Iowa, at two sites in Milford Township (T-84N, R-23W) in Story County, Iowa, and at two sites in Todd Township (T-140N, R-35W) in Hubbard County, Minnesota. Specimens were collected on several dates in March, April, May, July, August, and October during 1968 to 1971.

Specimens of *Caloplaca cerina* were collected at two sites in Cass Township (T-75N, R-37W) in Cass County, Iowa, at one site in Fairfield Township (T-92N, R-7W) in Fayette County, Iowa, and at three sites in Todd Township (T-140N, R-35W) in Hubbard County, Minnesota. Specimens were collected on several dates in April, August, and October during 1970 and 1971.

Specimens of *Xanthoria elegans* were collected at two sites in Lakeville Township (T-99N, R-37W) in Dickinson County, Iowa, and at one site in Milville Township (T-92N, R-1W) in Clayton County, Iowa. Specimens were collected on several dates in February, May, June, August, September, October, and December during 1968 to 1971.
Light Microscopy

Recently collected lichen thalli were fixed either in the dry field condition or after hydrating for 24 hours. For paraffin sections thalli were fixed either in Craf IV (Nawaschin) or in Bouin's fluid according to Sass (1958). After fixation, dehydration was accomplished either in ethyl alcohol or in tertiary butyl alcohol; with ethyl alcohol either xylene or chloroform was used as a solvent for the paraffin. Most specimens were embedded in 52° Tissuemat, and the remainder were embedded in 56° Tissuemat.

For thin plastic sections 10% acrolein in distilled water or 2% gluteraldehyde buffered to pH 7.5 in 0.1M phosphate buffer was used as a fixative; specimens remained in the fixative in the refrigerator at 4°C for 24 hours. Specimens were then dehydrated in ethyl alcohol, were transferred to propylene oxide, and were finally embedded either in Araldite/Epon embedment according to Anderson and Ellis (1965) or Epon embedment according to Luft (1961).

Paraffin sections were cut eight to 12 microns thick on a rotary microtome; the temperature of the microtome room was varied between 24°C and 2°C. Specimens that sectioned poorly were removed from the microtome and soaked in water for 24 to 48 hours before sectioning again. Sections were mounted serially on glass slides
using 2% gelatin adhesive.

One micron thick plastic sections were cut using glass knives and an LKB Ultratome III; the block face was less than one millimeter square. A thin layer of 1% gelatin in distilled water was placed on the glass slide and allowed to dry. The thin plastic sections were picked up with a hair loop and transferred to a drop of distilled water on the gelatin coated slide. Sections were flattened, and the water was evaporated by placing the slide on a 60°C slide warmer. A light stain of 1% safranin in distilled water was applied to aid in locating the sections on the slide.

Paraffin sections were stained according to Sass (1958) using iodine-crystal violet-iodine or Heidenhain's iron hematoxylin. Either a two or four hour schedule for iron hematoxylin was satisfactory, and destaining was accomplished in two to three minutes either in iron-alum mordant diluted to half strength with distilled water or in a saturated aqueous solution of picric acid. Either 1% fast green in 95% ethyl alcohol or 1% orange G in clove oil was used as a counterstain for the iron hematoxylin.

For propio-carmine stain, thin free hand sections were placed in 60°C ethyl alcohol:acetic acid (3:1) for 20 minutes and were then transferred to 2N HCL at 60°C for 20 minutes. Sections were finally rinsed three times
in distilled water and placed in a drop of propiocarmine made according to Sass (1958). Specimens were covered with a cover slip, squashed, and placed in the refrigerator overnight.

Observations were recorded on a Leitz Ortholux microscope using either bright field or phase contrast optics and Kodak Panatomic X film.

Transmission Electron Microscopy

Single ascocarps were removed from freshly collected thalli, were hydrated for 24 hours, and then were placed either in cold 10% acrolein in distilled water or in cold 2% gluteraldehyde in 0.1M phosphate buffer at pH 7.5. Specimens were kept in the refrigerator at 4°C for one to 24 hours. Specimens were post-fixed in 1% osmic acid buffered to pH 7.5 in 0.1M phosphate buffer. A graded ethyl alcohol series was used for dehydration, and propylene oxide was used as a solvent for the embedment. Either Araldite/Epon embedment according to Anderson and Ellis (1965) or Epon embedment according to Luft (1961) was used. Infiltration time of the propylene oxide:resin (1:3) mixture was increased to 24 hours, and specimens remained in the pure embedment for five days prior to polymerization.

Sections were cut 500 to 700 Angstroms thick on
an LKB Ultratome III using glass knives and a block face no larger than 300 microns on a side. Sections were collected on 100, 150, or 200 mesh Formvar coated grids. Lead citrate according to Reynolds (1963) and uranyl acetate according to Stempak and Ward (1964) were used as stains either individually or in combination.

Observations were made either on an RCA EMU-3F or on an Hitachi HS-8 electron microscope and were recorded on DuPont Cronar film.

Scanning Electron Microscopy

Thalli in the dry field condition and thalli treated with 100% acetone for 24 hours were freeze-dried and then coated with palladium-gold while being rotated in a vacuum evaporator. Observations were made on a Jeol SEM-1 scanning electron microscope and were recorded on Kodak Ektapan film.

Spore Harvesting and Germination

Recently collected thalli were hydrated for 24 hours and then placed right-side-up on a small block of wood so that the epithecium was approximately one millimeter below the surface of standard 2% water agar in the bottom of an inverted petri dish which was also
supported by wooden blocks. Thalli remained in this position overnight in a transfer chamber. Evaporation of water from the thallus and spore dispersal were speeded up by placing dishes of NaOH pellets near the inverted petri dishes. After spore germination, small bits of uncontaminated agar and spores were transferred to standard potato dextrose agar.

If spores could not be collected by the above method, thalli were hydrated for 24 hours, placed in 15% calcium hypochlorite for ten minutes, and then rinsed in sterile distilled water. Apothecia were removed from the thallus and placed in a drop of sterile distilled water on a sterile microscope slide. The apothecia were thoroughly crushed, and a drop of the crushed ascocarp in water was transferred to standard 2% water agar. After spore germination, small bits of uncontaminated agar with spores were transferred to potato dextrose agar.
RESULTS

**Caloplaea ulmorum**

The thallus of *Caloplaea ulmorum* is composed of closely fitting ellipsoidal masses of interwoven hyphae and algae 100 to 150 microns thick and is appressed to the substrate (Figures 2, 3); hyphae from the thallus can be seen in the dead cork cells of the bark. With the scanning electron microscope (SEM) the thallus gives the appearance of loosely woven hyphae; at the margin of the thallus individual hyphae can be seen appressed to the bark (Figure 64).

The cortex of the thallus consists of a 15 to 20 micron thick layer composed of short-celled, rounded hyphae. The algal layer below the cortex is 60 to 75 microns thick with short-celled hyphae appressed to the algae; haustoria were never observed. The hyphae below the algal layer, although fewer in number, do not differ appreciably in size and shape from upper cortical hyphae (Figure 3).

The first evidence of fructification is the appearance of an ascocarp initial, a mass of closely woven hyphae, in or below the algal layer (Figure 4). The initial usually has a diameter of 30 to 35 microns and stains uniformly and darkly with iron hematoxylin. The hyphae of the initial are half as wide as other hyphae, and their cells are not as rounded. The initial enlarges by peripheral
growth until it reaches 50 to 55 microns in diameter. At this point the hyphae on the surface of the initial are oriented tangentially to the initial and are relatively prominent (Figures 5, 6).

When the initial has reached 75 microns in diameter, it is no longer densely packed, and intercellular spaces appear. The hyphae toward the center of the initial are not oriented in any particular direction at this time (Figures 7, 8, 9, 10). When the young ascocarp has reached 100 microns in diameter, the central hyphae are oriented predominantly vertically, and their apical ends are unattached (Figures 12, 13). The tangentially oriented enveloping hyphae that surrounded the young ascocarp appear to have increased in number, especially toward the top of the young ascocarp (Figures 13, 14, 15). At this point the young ascocarps are 150 micron wide dome-like protrusions of the thallus; the texture of the dome's surface is similar to that of the thallus (Figure 59).

The ascocarp now begins to break through the cortex, and the cortex appears fragmented in the region of the fracture (Figure 16). This fracture appears as a small depression in the center of the dome-like protrusion (Figure 59). The tangentially oriented hyphae are intact and still enclose the primary paraphyses (Figure 16). The tangentially oriented enveloping hyphae at the top of the ascocarp below the fracture in the cortex stain intensely
and appear to grow more rapidly than the surrounding hyphae (Figures 15, 16). Subsequently, the enclosure of tangentially oriented hyphae ruptures just below the fracture in the cortex (Figures 18, 19) and exposes the primary paraphyses. The ends of the actively growing hyphae in the enclosure that ruptured are now oriented nearly radially with respect to the geometric center of the ascocarp (Figures 18, 19, 20). This region is the marginal sheaf, and the component hyphae grow by sympodial branching (Figure 31). Hyphae produced on the internal face of the marginal sheaf become secondary paraphyses, and hyphae produced on the external face become ectal exciple. The marginal sheaf of a young ascocarp curves outward laterally to expand the young ascocarp and then curves gradually upward as the apothecium grows in diameter (Figures 19, 20, 21, 22, 23, 26).

When the young ascocarp is 70 to 180 microns in diameter, dark staining structures composed of three or four cells may appear in the ascocarp. The cells of these structures are approximately three times wider than the normal hyphal cells (Figures 9, 10, 11) and contain large nuclei (1.5 microns in diameter) with large nucleoli (1.0 micron in diameter) (Figure 25). Both ends of these structures are continuous with hyphal cells of normal size. If the ascocarp has ruptured the cortex, the hyphae
attached to the apex of the structures may extend upward among the exposed vertical hyphae (Figure 24).

Regardless of the stage at which these structures appear, slightly smaller dark staining ascogenous hyphae begin to appear in the immediate vicinity. At this point the general appearance is that of a spherical mass of hyphae, centrally or peripherally located, surrounded by vertical hyphal elements (Figure 18). Hyphae proliferate from this mass centrifugally and acropetally (Figures 20, 21, 22). Ascii form from these dark staining hyphae by crozier formation. The walls separating the apical and subpenultimate cells of the crozier partially dissolve, allowing nuclear transfer, and a second ascus may develop (Figure 57).

Spermogonia develop as small areas approximately 50 microns in diameter in the algal layer of the thallus (Figure 36). There is no well developed spermogonial wall; the spermatiophores themselves form cavities enclosing the free spermatia (Figures 38, 39). Sites of spermatial production are not clearly visible. Spermatiophores are slightly narrower than vegetative hyphae, stain intensely, and exhibit a variety of forms (Figure 37). Spermatia are two microns long and one micron wide; they are spindle shaped to oval (Figures 37, 39). There is no definite opening from which the mass of spermatia is released. The spermogonia are irregular in outline,
and as they enlarge, they eventually break through the cortex, and the spermatia are released (Figure 40).

The exciple is prominent when the young ascocarp has reached 300 microns in diameter (Figure 59). The ectal exciple of the apothecium consists of elongated hyphae with thick and agglutinated walls (Figures 28, 29). The longitudinal axes of these hyphae are oriented perpendicularly to the excipular surface. Loosely woven hyphae and algae may be present also. Occasionally the ectal exciple contains large, thin-walled, globose hyphae with no visible interhyphal spaces (Figure 30). Externally, the exciple is marked by transverse ridges and appears so dense that individual hyphae cannot be detected (Figure 61).

Mature asci are clavate, are inoperculate with no apical pore, and are ten microns wide and 40 microns long (Figures 34, 35). Ascus walls are generally thick (1 micron) and may be relatively thicker near the apex (Figure 35). The two layered ascus wall (Figures 48, 49, 51) has an outer electron dense layer nearly half a micron thick. Microfibrils radiate from this layer (Figures 48, 49). When two asci are close together, the microfibrils interdigitate, and it is difficult to tell where one ascus wall ends and the other one begins (Figure 53). The inner layer of the ascus wall is relatively electron transparent and is
nearly 3/4 micron thick, may be lamellate, and may possess some microfibrils. The inner boundary of the inner layer with the cytoplasm is smooth (Figures 49, 50, 51).

Some microfibrils of the inner layer of the ascus wall show a linear orientation in an ascus that has shot its spores. In addition, the outer dense layer may not be as prominent after spore release (Figure 53). After the spores are expelled from the ascus, some epiplasm containing much endoplasmic reticulum remains in the ascus (Figure 53).

Around the margin of the apothecium, the interascal elements consist primarily of branched paraphyses (Figures 34, 35). However, in the older, central areas, the interascal elements consist of old asci that have shot their spores and collapsed and of a few paraphyses (Figure 33). In the lower part of the apothecium ascogenous hyphae and bases of paraphyses are tightly packed, and no interhyphal spaces are visible (Figure 32). A microfibrillar substance radiates from these cell walls and fills all available space (Figure 57). The medullary exciple below this area consists of loosely woven hyphae and algae (Figure 32).

Within a single hymenium, asci in all stages of development occur. Refractile granules up to one micron in diameter appear in young asci that are otherwise homo-
genous (Figure 34). One to several layers of unit membrane are appressed to the ascus wall; large quantities of circular and concentric membrane and membrane-bounded, electron dense bodies are also visible in the cytoplasm (Figure 47).

The surface of the epithecium, formed by branched paraphyses and old asci, may be ten to 20 microns above the tips of the mature asci. The epithecium consists of a number of closely fitting units with narrow crevices between them and appears very much closed (Figures 60, 65, 66, 67). Crystals are evident on the epithecium as well as on the exciple. After treatment with acetone, the crystals are not visible, and the relief of the epithecium is greater although the epithecium is still very closed (Figure 69).

Ascospores are eight to ten microns long and five to seven microns wide (Figures 35, 41, 42) with polymorphic nuclei (Figures 41, 42). Spores are generally polarilocular and single-celled, but a spore may occasionally show a definite cross wall. The spore walls consist of several different electron dense layers. Delimiting spores are elliptical in outline, and the spore wall thickens centripetally. Spore walls lack any surface markings (Figure 70).

When spores are collected on agar after being shot from an ascus, all eight spores usually clump together.
Spores germinate by one or two germination tubes located at the ends of the spore after five to seven days on water agar. The spore wall is intact after germination. Cells of the young germination tube are beaded. Vegetative growth is slow, requiring a year for the colony to reach a spherical mass two millimeters wide (Figure 46).

**Caloplaca cerina**

The thallus of *Caloplaca cerina*, 160 to 180 microns thick, consists of tightly fitting ellipsoidal masses of interwoven hyphae and algae closely appressed to the substrate (Figure 72). When growing on tree bark, the thallus may develop under the outermost cells of the bark (Figure 73). The thallus, whether on bark or rock substrate, appears to be broken into units or squamules separated by prominent crevices (Figure 110). The thallus appears fibrous (Figure 104). The cortex is a ten to 15 micron thick layer consisting of thin-walled, closely appressed hyphae. The algal layer is 60 to 70 microns thick and consists of loosely woven hyphae and algae; no haustoria were ever observed. Hyphae below the algal layer are not well developed (Figure 74).

The first indication of fructification is the appearance of the ascocarp initial, a dark staining mass of hyphae 40 microns in diameter (Figure 73). Generally,
Figures 1-12. Light microscopy of *Caloplaca ulmorum*

Figure 1. General aspect of thallus and apothecia (6x)

Figure 2. Cross section of thallus (126x)

Figure 3. Higher magnification view of thallus (315x)

Figure 4. Ascocarp initial (510x)

Figure 5. Tangentially oriented hyphae on external face of ascocarp initial (510x)

Figure 6. Slightly older ascocarp initial (510x)

Figure 7. Intercellular spaces beginning to appear (495x)

Figure 8. Continued enlargement of ascocarp (495x)

Figure 9. Possible ascogonium (arrow) visible with phase contrast optics (495x)

Figure 10. Same as Figure 9 but with bright field optics (510x)

Figure 11. Possible gametangia (arrow) visible with bright field optics (510x)

Figure 12. Continued ascocarp enlargement with central hyphae beginning to show some vertical orientation (495x)
Figures 13-20. Light microscopy of *Caloplaca ulmorum*

Figure 13. Tangentially oriented hyphae enclosing vertical hyphal elements and possible ascogonium (510x)

Figure 14. Dark staining, rapidly growing hyphae just below break in cortex (495x)

Figure 15. Continued proliferation of hyphae below break in cortex (495x)

Figure 16. Areas of intense hyphal growth increase in width and thickness (315x)

Figure 17. Bright field photo showing small mass of dark staining ascogenous hyphae among primary paraphyses (315x)

Figure 18. Bright field photo showing centrifugal and acropetal spread of ascogenous hyphae and rupture of rapidly growing hyphae to expose primary paraphyses (315x)

Figure 19. Same as Figure 18 but with phase contrast optics (315x)

Figure 20. Shows continuous spread of ascogenous hyphae centrifugally and acropetally (315x)
Figures 21-27. Light microscopy of *Caloplaca ulmorum*

- **Figure 21.** Young ascocarp showing outward lateral movement of marginal sheaf (315x)
- **Figure 22.** Continued outward lateral movement of marginal sheaf (315x)
- **Figure 23.** Further opening of the ascocarp and a knot of ascogenous hyphae (possible ascogonium at arrow) (315x)
- **Figure 24.** Older ascocarp showing possible ascogonia at arrows (495x)
- **Figure 25.** Enlarged segment of Figure 24 (1275x)
- **Figure 26.** Older ascocarp showing marginal sheaf, prominent exciple, and absence of asci from hymenium (126x)
- **Figure 27.** Cross-section of typical old ascocarp (126x)
Figures 28-35. Light microscopy of Caloplaca ulmorum

Figure 28. Apothecium collected in Iowa showing ectal exciple (495x)

Figure 29. Apothecium collected in Minnesota showing ectal exciple (495x)

Figure 30. Apothecium showing unusual ectal exciple (495x)

Figure 31. Apothecium showing sympodially branching marginal sheaf (495x)

Figure 32. One micron thick section showing hymenium and marginal sheaf (315x)

Figure 33. One micron thick section of hymenium showing old collapsed asci (top arrow) and paraphyses (bottom arrow) (495x)

Figure 34. One micron thick section showing paraphyses, young asci, collapsed asci, and agglutinated hyphae in medullary exciple (495x)

Figure 35. One micron thick section showing central part of apothecium with young and old, collapsed asci (495x)
Figures 36-46. Light microscopy of *Caloplaca ulmorum*

Figure 36. Very young spermogonium (495x)

Figure 37. Young spermogonium with accumulating spermatia (495x)

Figure 38. Spermogonium showing accumulation of spermatia between spermatiophores (495x)

Figure 39. Spermogonium beginning to rupture through the cortex (495x)

Figure 40. A typical older spermogonium (126x)

Figure 41. Propio-carmine stain showing nuclei (arrow) in spore (495x)

Figure 42. Propio-carmine stain of spore showing cruciform nuclear configuration (495x)

Figure 43. Whole mount of ascus with apical and subpenultimate cells attached (315x)

Figure 44. Whole mount of ascus showing homogeneous cytoplasm and second ascus arising after fusion of apical and subpenultimate cells (315x)

Figure 45. Whole mount showing spores germinating on water agar (375x)

Figure 46. Fungal colonies after one year of growth on agar (3x)
Figures 47-52. Transmission electron microscopy of *Caloplaca ulmorum* (line scales represent one micron)

Figure 47. Longitudinal section of a young ascus showing ascus wall (A) and cytoplasm (C)

Figure 48. Longitudinal section of a mature ascus showing ascus wall (A), epiplasm (E), and microfibrils (M)

Figure 49. Longitudinal section of a mature ascus

Figure 50. Cross section of an ascus with spores (S)

Figure 51. Longitudinal section of an ascus

Figure 52. Cross section of an old ascus
Figures 53-58. Transmission electron microscopy of *Caloplaca ulmorum* (line scales represent one micron)

Figure 53. Longitudinal section of two ascus walls (A)

Figure 54. Longitudinal section of paraphysis (P)

Figure 55. Longitudinal section of bacteria (B) on epithecium

Figure 56. Longitudinal section of paraphysis near marginal sheaf

Figure 57. Cross section of ascogenous hyphae (AH)

Figure 58. Cross section of medullary exciple showing microfibrillar material (M) and free air spaces
Figures 59-62. Scanning electron microscopy of *Caloplaca ulmorum*

Figure 59. General aspect of thallus collected in Iowa with young ascocarps (arrows) (57x)

Figure 60. Older ascocarp collected in Minnesota (114x)

Figure 61. Exciple of older ascocarp (342x)

Figure 62. Crystals (arrow) on epitheciun (3420x)
Figures 63-70. Scanning electron microscopy of *Caloplaça ulmorum*

Figure 63. Thallus (142x)

Figure 64. Individual hyphae of thallus on bark substrate (1140x)

Figure 65. Epithecium of lichen collected in Iowa (1140x)

Figure 66. Crystals on epithecium collected in Iowa (1140x)

Figure 67. Crystals on epithecium collected in Minnesota (3400x)

Figure 68. Normal lichen epithecium (300x)

Figure 69. Epithecium treated with acetone to remove lichen acid crystals (342x)

Figure 70. Spore (3420x)
very few algae are associated with the initial. The hyphae towards the center of the initial become generally vertically oriented by the time the initial is 75 microns in diameter (Figures 76, 77). On the external face of the initial there is little evidence of tangentially oriented enveloping hyphae. At this stage young asco-carps appear as dome-like protrusions of the thallus when viewed with the SEM (Figure 110).

Within an initial some darkly staining structures appear at irregular time intervals, each consisting of several linearly arranged large cells connected to a long thin hypha that extends upward (Figure 76). Dark staining masses of hyphae appear in the immediate vicinity of these structures (Figures 78, 79), and proliferate centrifugally and acropetally (Figures 81, 82, 83, 84). Asci arise from these dark staining ascogenous hyphae by crozier formation (Figure 96).

The young fructification initially enlarges with no apparent damage to the cortex. When the ascocarp is about 100 microns in diameter, a fracture develops in the cortex above the ascocarp. Externally this fracture appears as a depression in the dome-like protrusion of the thallus. The top of the ascocarp just below the fracture in the cortex stains more intensely as hyphae in this region grow more rapidly (Figures 84, 85). These hyphae become generally tangentially oriented to the top
of the young ascocarp. When the ascocarp is about 120 microns in diameter, the tangentially oriented hyphae rupture below the break in the cortex and expose the primary paraphyses to the outside (Figures 86, 87). The ends of the hyphae that separated are now oriented nearly radially with respect to the geometric center of the ascocarp. This marginal mass of rapidly growing hyphae is the marginal sheaf and is composed of sympodially branching hyphae (Figure 93). Secondary paraphyses develop on the inside, and ectal exciple cells develop on the outside. The marginal sheaf of the young ascocarp curves outward laterally to expand the young ascocarp and then gradually upward as the apothecium grows in diameter (Figures 87, 89, 90). As the ascocarp increases in diameter, the level of the hymenium also rises upward away from the substrate (Figure 90).

Mature asci are clavate, are relatively thick-walled (1 micron), are inoperculate without an apical pore, and are approximately ten microns wide by 60 microns long. The ascus tip may be conspicuously thickened (Figures 94, 95). The ascus wall of Caloplaca cerina consists of an electron dense outer layer half a micron thick and a relatively electron transparent inner layer nearly 3/4 micron thick. Microfibrils radiate from the outer layer of the wall (Figures 100, 101); the microfibrils of adjacent asci interdigitate (Figure 101).
Some microfibrils also occur in the inner wall layer (Figure 100). After spore expulsion, some of the microfibrils in the inner wall layer are oriented parallel to the longitudinal axis of the ascus (Figure 101). The outer electron dense layer of an ascus that has shot its spores ends abruptly, and the inner transparent layer extends beyond it (Figure 101). Bacteria may grow next to old collapsed asci (Figure 101).

One to several unit membranes may be appressed to the smooth ascus wall (Figure 102). There are also large quantities of circular and concentric membranes in the cytoplasm. One or two layers of unit membrane are appressed to the ascospores (Figure 102). Three or four different electron dense layers are visible in the spore wall (Figure 102). An old ascus that has shot its spores, contains much residual epiplam with rough endoplasmic reticulum.

Within any hymenium there are asci in all stages of development; however, no meiotic divisions were ever seen. Secondary paraphyses branch, and in younger areas of the apothecium the asci are 20 to 25 microns below the surface (Figures 93, 94). However, in older parts of the apothecium, the interascal elements consist almost entirely of old asci that have shot their ascospores and have collapsed (Figure 95). The epithecium appears closed, and no crystals are evident. After treatment with acetone
the relief of the epithecium is greater, and small circular structures four microns wide with central depressions are evident (Figures 108, 109). The epithecium still appears closed. The ascogenous hyphal layer is 30 to 40 microns thick and is subtended by some long-celled, thick-walled, agglutinated hyphae (Figure 94). Intercellular spaces are filled by radiating microfibrils. The medullary exciple consists of loosely woven hyphae and algae; intercellular spaces are abundant.

The ectal exciple is not well developed; a few thin-walled, globose hyphal cells may be present. Some loosely woven hyphae and algae may be present also. The exciple of an apothecium from bark substrate is more prominent than the exciple of an apothecium from rock substrate (Figures 110, 113). The exciple extends slightly above the level of the epithecium (Figure 91, 92).

There are typically eight spores per ascus. The spores are seven to nine microns wide and ten to 12 microns long. Spores are typically polarilocular with a slightly polymorphic nucleus in each end (Figure 97). When spores are being delimited, they appear elliptical in outline, and the wall thickens centripetally. Mature spores are without any surface markings (Figure 114). Spores failed to germinate on water agar.

Spermogonia develop as small areas in the thallus. There is no well delimited wall; the spermatiophores are
variable in shape and sometimes extend transversely through the spermogonium (Figures 98, 99). Sites of spermatial production are not clearly evident, and relatively few spermatia are produced. Spermatia, usually spindle-shaped, are two microns long by one micron wide.

**Xanthoria elegans**

The thallus of *Xanthoria elegans* is loosely attached to its substrate toward the margin, often nearly crustose near the center, and is 300 to 400 microns thick. Thallus lobes may radiate from a common point, or the lobes may be scattered randomly (Figures 115, 116). Most of the thallus appears smooth except for a few pit-like areas which do not open into the interior of the thallus. Nothing on the thallus' surface is suggestive of hyphae, but some irregular scales and some small filaments 0.5 microns by several microns do occur. The cortex is approximately 20 microns thick and consists of hyphae oriented perpendicularly to the surface of the thallus (Figure 117). The algal layer is 80 to 100 microns thick and consists of loosely woven hyphae appressed to algal cells (Figure 136). Haustoria were never seen. The lower cortex is a 30 micron thick region consisting of closely appressed, globose hyphae (Figure 118). The terminal margin of a thallus lobe consists of much branched, narrow diameter hyphae oriented perpendicularly to the
Figures 71-80. Light microscopy of *Caloplaca cerina*

Figure 71. General aspect of apothecia and thallus on bark (3x)

Figure 72. Cross section of thallus on bark (126x)

Figure 73. Thallus developing under dead bark cells and very young ascocarp (right) (510x)

Figure 74. Cross section of young ascocarp showing dark-staining hyphae and vertical hyphal elements (510x)

Figure 75. Phase contrast photo showing young ascocarp (495x)

Figure 76. Phase contrast photo showing vertical hyphal elements and possible ascogonia (arrow) (495x)

Figure 77. Phase contrast photo showing some tangentially oriented enveloping hyphae (arrow) (495x)

Figure 78. Phase contrast photo showing knot of ascogenous hyphae (495x)

Figure 79. Same as Figure 78 but with bright field illumination (510x)

Figure 80. Phase contrast photo of an ascocarp the same size as the one in Figure 79 but with possible ascogonium (arrow) (495x)
Figures 81-91. Light microscopy of *Caloplaea cerina*

**Figure 81.** Bright field photo of cross section of young ascocarp showing knot of ascogenous hyphae and possible asco-gonia (arrow) (510x)

**Figure 82.** Bright field photo of a cross section of a larger ascocarp showing several knots of ascogenous hyphae and a possible trichogyne (arrow) (510x)

**Figure 83.** Same as Figure 82 but with phase contrast optics (495x)

**Figure 84.** Phase contrast photo of cross section showing ascogenous hyphae and tangentially oriented hyphae beginning to develop (495x)

**Figure 85.** Phase contrast photo showing further development of tangentially oriented hyphae (arrow) (495x)

**Figure 86.** Phase contrast photo showing cortex beginning to rupture (495x)

**Figure 87.** Phase contrast photo showing rupture of the enveloping hyphae (495x)

**Figure 88.** Bright field photo showing outward curvature of marginal sheaf (510x)

**Figure 89.** Same as Figure 88 but with phase contrast optics (495x)

**Figure 90.** Bright field photo of old ascocarp showing site where ascocarp initially began to develop (arrow) (126x)

**Figure 91.** Phase contrast photo of older apothecium showing marginal sheaf and ectal exciple (495x)
Figures 92-99. Light microscopy of *Caloplaca cerina*

Figure 92. Cross section of apothecium showing ectal exciple (495x)

Figure 93. One micron thick section of apothecium showing hymenium and marginal sheaf (550x)

Figure 94. One micron thick section showing agglutinated hyphae under ascogenous hyphae (550x)

Figure 95. One micron thick section showing interascal elements consisting primarily of collapsed asci (550x)

Figure 96. Whole mount showing branched paraphys and young ascus with homogenous cytoplasm (995x)

Figure 97. Propio-carmine stain of spores showing irregular nuclei at each end of spore (995x)

Figure 98. Cross section of young spermogonium (315x)

Figure 99. Cross section of older spermogonium showing irregular spermatothores (495x)
Figures 100-103. Transmission electron microscopy of *Caloplaca cerina* (line scales represent one micron)

Figure 100. Longitudinal section of a young and an old ascus showing ascus walls (A) and epiplasm (E)

Figure 101. Longitudinal section of an old ascus showing interdigitating microfibrils (M)

Figure 102. Longitudinal section of spore (S) and ascus walls

Figure 103. Microfibrillar material radiating from paraphysis wall
Figures 104-109. Scanning electron microscopy of *Caloplaca cerina*

Figure 104. Thallus on bark (604x)

Figure 105. Higher magnification view showing hyphae appressed to bark (604x)

Figure 106. Exciple of ascocarp (1140x)

Figure 107. Epithecium of ascocarp (1140x)

Figure 108. Epithecium treated with acetone to remove lichen acids (1140x)

Figure 109. Higher magnification view showing circular bodies with concave depressions (3400x)
Figures 110-114. Scanning electron microscopy of *Caloplaca cerina*

**Figure 110.** General aspect of thallus on bark collected in Minnesota with young ascocarps (arrows) (57x)

**Figure 111.** Young ascocarp (342x)

**Figure 112.** Older ascocarp (131x)

**Figure 113.** General aspect of thallus collected on rock in Iowa (114x)

**Figure 114.** Spores (1140x)
The first evidence of fructification is the appearance of the ascocarp initial, a ball of hyphae 25 microns in diameter. Hyphae of the initial stain darkly and are narrower than normal hyphae (Figures 120, 121). The outermost hyphae on the external face of the initial are oriented tangentially to its surface. By the time the initial has reached 60 microns in diameter, interhyphal spaces have appeared, and hyphae in the center of the initial are becoming vertically oriented (Figures 126, 127). The initial continues to increase in size until the young ascocarp reaches 100 to 125 microns in diameter, and then it begins to break through the cortex (Figures 129, 132). At this stage the cortex appears fractured, but the enveloping mass of tangentially oriented hyphae of the young ascocarp still enclose the central vertical elements (Figure 132). This stage appears as a dome-like protrusion of the thallus with a small depression at its center (Figure 166).

Tangentially oriented hyphae at the top of the ascocarp below the fracture in the cortex stain darkly, are tightly packed, and grow faster than the surrounding hyphae (Figures 129, 131, 132). This enveloping mass of hyphae ruptures below the fracture in the cortex, and the primary paraphyses of the ascocarp are exposed (Figure 134).

At any point between 40 to 60 microns in diameter,
dark staining, multicellular structures appear in the young ascocarp. Cells of these structures are twice the diameter of surrounding hyphae. Hyphal cells at either end of these structures taper down to the normal diameter of the surrounding cells (Figures 123, 124). Dark staining, small diameter hyphae develop in the immediate area and proliferate centrifugally and acropetally (Figures 131, 133, 134, 145). Asci arise from ascogenous hyphae by crozier formation (Figures 149, 159). The walls of the apical and subpenultimate cells may partially dissolve, nuclear transfer may occur, and a second ascus may develop (Figure 160).

The tangentially oriented enveloping hyphal mass that ruptured to expose the young ascocarp comprise the marginal sheaf. Hyphae produced on the internal face become secondary paraphyses, and those produced on the external face become ectal exciple. The marginal sheaf consists of sympodially branching hyphae (Figure 142). The marginal sheaf of a young, enlarging ascocarp curves outward laterally to expand the young ascocarp and then curves gradually upward as the apothecium enlarges (Figures 134, 135, 137, 138).

Mature asci are clavate, are thick-walled, are inoperculate without an apical pore, and are approximately 12 microns wide and 60 microns long. The ascus tip is conspicuously thickened (Figures 144, 145). The
ascus wall consists of an electron dense outer layer and a relatively electron transparent inner layer (Figure 155). Microfibrils radiate from the outer ascus wall layer. The inner layer may show a lamellate structure with some microfibrils (Figures 157, 158). The boundary of the epiplasm with the ascus wall is convoluted (Figures 157, 158), and several layers of unit membrane are appressed to the wall. Unit membranes are also appressed to the spore walls (Figure 156). A number of small vacuoles one to four microns in diameter are visible in the cytoplasm of the young ascus (Figures 144, 145, 155, 159, 160).

After spores are cut out, much epiplasm is left. The epiplasm contains large quantities of membrane and spherical membrane-bounded bodies (Figures, 157, 158). Ascospore walls typically show four different electron dense layers (Figure 157).

Within a hymenium there are asci in all stages of development; however, no meiotic divisions were ever seen. Secondary paraphyses are branched (Figure 144) and extend 20 microns beyond the ascus tips (Figure 145). The epithecium gives the appearance of tightly packed units, each consisting of an aggregation of granules. After treatment with acetone, some of the angularity of the epithecium seems to have been reduced, and the relief of the epithecium is greater (Figures 169, 170, 171).
The ascogenous hyphal layer is approximately 20 microns thick, and paraphyses arise from the layer below the ascogenous hyphae. All intercellular spaces in the ascogenous hyphal layer are filled with radiating microfibrils (Figures 155, 160, 162). The medullary exciple consists of loosely woven hyphae and algal cells (Figures 140, 145).

The ectal exciple is composed of hyphae that are oriented perpendicularly to the ascocarp surface; this region may be 20 to 25 microns thick (Figure 141, 142). Occasionally this orientation in the ectal exciple is not as evident (Figure 143). Some loosely woven hyphae and algae may also be present. The exciple is not marked by any lateral ridges and is composed of close fitting plates with no evident crystals (Figure 168).

Ascospores are polarilocular and are ten to 12 microns long by six microns wide. Spores are without surface markings (Figure 165). Delimiting spores are elliptical in outline, and the spore wall thickens centripetally. There is one nearly spherical nucleus in each end of the spore (Figure 151). When spores are collected on water agar after being shot out of the ascus, all eight spores usually clump together. Spores germinate in four to five days by a germination tube at one or both ends of the spore. The germination tube at first is beaded, but later the cells assume a more rectangu-
lar outline (Figures 152, 153). In the germination tubes, nuclei appear in pairs (Figure 153). Growth of the young colony is slow; after 30 days on potato dextrose agar, the colonies grow to only a few hundred microns in diameter (Figure 154).

Spermogonia arise within the algal layer of the thallus. A definite containing wall is not clearly visible, and the spermatiophores themselves form cavities containing the spermatia (Figures 147, 148). The spermatiophores often extend completely across the spermogonium, and the spermatia accumulate between them. Spermatia are approximately two microns long by one micron wide and are either spindle-shaped or oval-shaped (Figure 147). The spermogonia are spherical to roughly oval in cross section and open by rupturing the cortex.
Figures 115-123. Light microscopy of *Xanthoria elegans*

Figure 115. General aspect of thallus and apothecia collected in northwestern Iowa (4x)

Figure 116. General aspect of thallus and apothecia collected on dry ridge in northeastern Iowa (4x)

Figure 117. Phase contrast photo showing cortex of thallus (495x)

Figure 118. Phase contrast photo showing lower cortex of thallus (315x)

Figure 119. Phase contrast photo of terminal margin of thallus lobe (315x)

Figure 120. Bright field photo showing very young ascocarp in algal layer of thallus (510x)

Figure 121. Same as Figure 120 but with phase contrast illumination (495x)

Figure 122. Slightly larger ascocarp situated below algal layer (495x)

Figure 123. Median section of young ascocarp showing possible ascogonium (arrow) (495x)
Figures 124-132. Light microscopy of *Xanthoria elegans*

**Figure 124.** Same as Figure 123 but with bright field illumination (510x)

**Figure 125.** Cross section of young ascocarp showing intercellular spaces developing (495x)

**Figure 126.** Cross section showing central hyphae beginning to assume a vertical orientation (495x)

**Figure 127.** Cross section showing continued enlargement of young ascocarp (495x)

**Figure 128.** Cross section showing tangentially oriented hyphae (arrow) (495x)

**Figure 129.** Cross section showing relatively thick layer of tangentially oriented hyphae (495x)

**Figure 130.** Cross section of young ascocarp just beginning to rupture cortex (495x)

**Figure 131.** Median section of ascocarp of same size as the one in Figure 130 but with young asci and no sign of rupturing cortex (495x)

**Figure 132.** Median section showing the relative position of the young ascocarp in the lichen thallus (315x)
Figures 133-138. Light microscopy of Xanthoria elegans

Figure 133. Median section showing tangentially oriented enveloping hyphae beginning to rupture (315x)

Figure 134. Median section showing primary paraphyses exposed to the outside (315x)

Figure 135. Median section showing the beginning of lateral outward curvature of marginal sheaf (315x)

Figure 136. Median section showing the young ascocarp as a dome-like protrusion of the thallus (126x)

Figure 137. Median section showing outward curvature of marginal sheaf and paraphyses being formed on the internal face while ectal exciple forms on the external face (315x)

Figure 138. Median section of young ascocarp showing elevated position above the thallus (126x)
Figures 139-145. Light microscopy of *Xanthoria elegans*

**Figure 139.** Cross section of a medium aged asco-carpet (126x)

**Figure 140.** Cross section of a typical older asco-carpet (126x)

**Figure 141.** Cross section of apothecium showing marginal sheaf and exciple (315x)

**Figure 142.** Cross section of apothecium showing sympodial branching of marginal sheaf (arrow) (495x)

**Figure 143.** Cross section of apothecium collected on dry ridge top in northeastern Iowa (315x)

**Figure 144.** One micron thick section of marginal sheaf and hymenium (550x)

**Figure 145.** One micron thick section of central portion of apothecium (550x)
Figures 146-154. Light microscopy of *Xanthoria elegans*

Figure 146. Cross section of a young spermogonium (495x)

Figure 147. Cross section of older spermogonium showing irregular spermatiophores and spermatia (315x)

Figure 148. Cross section of spermogonium in lichen thallus collected on dry ridge in northeastern Iowa (495x)

Figure 149. Whole mount of asci showing apical, penultimate and subpenultimate cells (495x)

Figure 150. Whole mount showing branched paraphyses (495x)

Figure 151. Propio-carmine stain showing nuclei in both ends of spore (995x)

Figure 152. Whole mount of spores beginning to germinate on agar (495x)

Figure 153. Propio-carmine stain of older germinating spores with paired nuclei in hyphal cells (arrow) (495x)

Figure 154. Whole mount of lichen fungus colonies after one month of growth on potato dextrose agar (126x)
Figures 155-158. Transmission electron microscopy of *Xanthoria elegans* (line scales represent one micron)

**Figure 155.** Longitudinal section of a young and a slightly older ascus showing ascus walls (A)

**Figure 156.** Cross section of two asci showing spores (S), epiplasm (E), and interdigitating microfibrils (M)

**Figure 157.** Cross section of a mature ascus showing residual epiplasm with membrane bounded bodies

**Figure 158.** Cross section of mature ascus showing convoluted inner wall
Figures 159-162. Transmission electron microscopy of *Xanthoria elegans* (line scales represent one micron)

**Figure 159.** Section through ascogenous hyphae (AH) showing crozier and ascus (AS)

**Figure 160.** Section showing crozier and fusion of apical and subpenultimate cells

**Figure 161.** Cross section of ascus with spores (S) and residual epiplasm (E)

**Figure 162.** Section through ascogenous hyphae showing microfibrils and paraphyses (P)
Figures 163-166.  Scanning electron microscopy of *Xanthoria elegans*

Figure 163. General aspect of lobe of thallus (1140x)

Figure 164. Higher magnification view of pit-like areas of thallus (1140x)

Figure 165. Spores (1824x)

Figure 166. Apothecia in various stages of development (57x)
Figures 167-171. Scanning electron microscopy of *Xanthoria elegans*

Figure 167. Epithecium of apothecium (342x)

Figure 168. Exciple of apothecium (1140x)

Figure 169. Higher magnification view of normal epithecium (1140x)

Figure 170. Epithecium treated with acetone to extract lichen acids (300x)

Figure 171. Same as Figure 170 but higher magnification (1140x)
DISCUSSION

Lichens, even when dehydrated in TBA, are very brittle. Tissue embedded in 56°C Tissuemat and sectioned at 4°C shows substantial mechanical damage by the microtome knife. Best sectioning was done when embedded specimens were hydrated for a day before sectioning. However, hydration has limitations since water will penetrate a limited distance through a specimen embedded in paraffin. Sharpness of the microtome knife is critical, and the knife should be sharpened before each use.

Fixation of lichens for electron microscopy presents a problem. Some plasmolysis and membrane discontinuity was always present. Other workers have hydrated thalli up to 14 days before fixation believing that this reduced artifacts. Such hydration times are unnatural since the three species studied here do not grow in habitats where thalli remain wet for 14 days.

A multitude of terms are available for the description of the developmental morphology of Discomycetes. Corner (1929a) used few terms in his developmental descriptions. On the contrary, Letrouit-Gallinou (1968) and Bellémere (1967a, b) used a multiplicity of confusing terms. Furthermore, lichenologists have maintained a separate set of terms for apothecial parts when there have been acceptable mycological terms for the same parts. Since the International Code of Botanical Nomenclature
declares that lichen nomenclature is based on the fungus, it is logical to apply the long-accepted mycological terms.

Letrouit-Gallinou demonstrated for several species that all parts of a mature lichen apothecium, excluding ascogenous hyphae and asci, developed from one of two regions of a young ascocarp, the pericentrum and the carpocentrum. The three species studied here are similar. The central region of the ascocarp initial developed into primary paraphyses. The remainder of the mature ascocarp developed from tangentially oriented hyphae on the surface of the ascocarp initial.

Both Letrouit-Gallinou and Bellémere reported some species where sterile hyphae of the ascocarp were present before gametangia were present. Although Letrouit-Gallinou did not suggest it, Bellémere suggested that these forms might be ascostromatic. The three species studied here show probable development of ascogonia within a preformed mass of stromatic hyphae.

As reported here, ascocarp emergence is consistent with that reported by Hue (1906). Ascocarps were initiated in or slightly below the algal layer. Algae of the medullary exciple were derived from algae below the ascocarp initial, and algae of the ectal exciple were derived from algae to either side of the ascocarp initial. However, Hue left the impression that growing hyphae carried algae upward into new areas of the ascocarp. Nothing
was observed to indicate that this occurs.

The developmental pattern described here is also similar to that described by Corner for some non-lichenized Discomycetes. The lacuna, a space at the top of the young ascocarp into which palisade hyphae grow, is seldom evident in the lichen material studied here. The greatest deviation from Corner's description is the variable occurrence of gametangia.

In the three species studied here, dark staining structures consisting of several large cells appeared within the young ascocarp. In *Caloplaca ulmorum*, very large nuclei were visible in these cells. These structures are interpreted as ascogonia, although no nuclear fusions were ever seen.

Ascocarp development of these three species really consists of two separate series of events. The development of sterile supporting ascocarp tissue is one series. Development of gametangia, ascogenous hyphae, and asci is the other series of events. The most interesting aspect of this last series is the variable occurrence of gametangia with respect to ascocarp size.

If dikaryotization does not occur at an early stage, then either the ascogonium remains active or more ascogonia are produced. In the meantime, the sterile ascocarp tissue continues to develop, making it possible to find "apothecia" that contain gametangia and not asci.
The large quantity of microfibrillar material present in the ascocarp is best interpreted in terms of the environment. Microfibrils provide an enormous surface to which water molecules can adhere. If one assumes that the average ascus is ten microns wide and 60 microns long, that microfibrils are 150 Angstroms wide and half a micron long, and that they are placed on the ascus with a 200 Angstrom center-to-center separation, then one can calculate the surface area. One ascus would have a 203 \( \text{mm}^2 \) surface area. One can also calculate that there are approximately 1600 mature asci in an apothecium one millimeter in diameter. Thus, the total surface area of mature asci alone in such an apothecium is 3,200 cm\(^2\). If the microfibrils of collapsed asci, ascogenous hyphae, and paraphyses were considered, then the area would be considerably greater.

The cortical cells of other species are also reported to have microfibrils. One would interpret these interdigitating microfibrils, as well as the hymenial microfibrils, as forming a barrier to retard water evaporation.

The interdigitation of hymenial microfibrils is also responsible for the closed appearance of the hymenium in the SEM. The microfibrils are too small to be resolved individually. Thus, the hymenium appears closed. This is especially true of the ascocarps treated with acetone to remove the organic acids deposited on the epithecium.
The circular areas with central depressions which are visible on *Caloplaca cerina* are tips of old, collapsed asci; one of these shows in cross section in Figure 101.

The exact nature of the spermatia reported for the three species is not clear. These structures were observed neither to germinate by germ tubes nor to transfer cytoplasmic contents to gametangia. Whether these structures function in asexual or sexual reproduction is not presently known. Since trichogynes are interpreted to be present in all three species, it is most probable that these structures function as spermatia or microconidia.

The lack of visible meiotic divisions in these three species is consistent with the report of Erbsch (1969a). It is not surprising that meiotic divisions are hard to find. Since asci mature year round, there is a small probability of finding a meiotic figure at any given time.

The cytoplasm of asci and ascogenous hyphae of *Xanthoria elegans* presents a contrast to that of the other two species. It appears vacuolate in both light and electron micrographs, which is unusual for asci and ascogenous hyphae in general. Since vacuolation was constant over a pH range and with two fixatives, it is probably not an artifact.

Judging from the literature and observations made during this study, bacteria commonly grow on or in
lichen thalli and ascocarps. Whether or not these bacteria influence either the fungus or the alga in some way is unknown. Lichenologists frequently use chemical tests in taxonomy, and until the possible influence of these bacteria on chemical substances present is understood, it might be well to use chemical taxonomy with caution.

Numerous workers have published detailed drawings of ascus apices of various lichens. In contrast to these reports, the paraffin and thick plastic sections revealed nothing that resembled the published drawings. Asci of all three species have no visible apical pore.

Spores of *Caloplaca ulmorum* and *Xanthoria elegans*, when collected on agar, germinate slowly. These organisms also grow very slowly in culture, where moisture presumably is not a limiting factor. The reproductive role of ascospores is not clear since moisture is limiting in a natural environment. If germinating spores and very small colonies are capable of drying out and rehydrating successively without fatal consequences, then they are very remarkable.

Germ tubes of *Xanthoria elegans* have two paired nuclei per hyphal cell. In the Buascomycetes it is often stressed that the ascocarp develops only under the association of two paired but unfused nuclei (Alexopoulos, 1966). Hyphae of *Xanthoria elegans* have septal pores, and nuclei can migrate from one cell to another; however, migration
should not result in a regular pairing of nuclei. The nuclear condition in the germ tubes of these three species should be studied further.

The taxonomy of some free-living Discomycetes is partially based on excipular tissue. However, all lichen excipular tissue does not readily lend itself to this kind of classification. Only the outermost areas of ectal exciple can be classified using Korf's terms because some areas of ectal exciple and the medullary exciple consist of loosely woven, irregular masses of hyphae and algae. Excipular character of the three species is consistent over a wide geographical range and does not seem to be subject to environmental modification (Weber, 1962).

In addition to excipular tissue, unitunicate and bitunicate asci are important in Ascomycete classification. Dennis (1968) indicated that asci of the Lecanorales, the largest lichen order, may be either clearly bitunicate or stain blue with iodine. Ziegenspeck (1926) indicated that some of the intensely blueing asci were bitunicate. Chadeau (1942) and his followers felt that all asci were two layered. The three species studied, members of the Lecanorales with intensely blueing asci, are unitunicate. Other unitunicate asci that have been studied have a uniformly electron transparent wall with some slight surface darkening; the lichen asci
studied here differ only in the intensity of the surface darkening.

On the basis of this study, *Caloplaca ulmorum* is interpreted to be distinct from *Caloplaca cerina*. The exciple of *Caloplaca ulmorum* is more prominent, and the ectal exciple most always consists of elongated, agglutinated hyphae. The ectal exciple of *Caloplaca cerina* is less well organized and most commonly contains globose hyphae. Furthermore, the tangentially oriented enveloping hyphae of the ascocarp initial develop later, are fewer in number, and are generally restricted to the top of the ascocarp in *Caloplaca cerina*. Crystals on the ascocarp, while absent in *Caloplaca cerina*, are abundant on the epithecium and exciple of *Caloplaca ulmorum*.

The thallus of *Xanthoria elegans* is more developed than the thalli of the other two organisms; the lower cortex is more prominent. Other than this, there is nothing exceptionally different between this *Xanthoria* and the two species of *Caloplaca*. While there is nothing to indicate that *Xanthoria elegans* should be a *Xanthoria* rather than a *Caloplaca*, perhaps further morphological studies should be undertaken. Studies of several more species of *Xanthoria* and some representatives of *Teloschistes*, a fruticose genus otherwise identical to *Caloplaca* and *Xanthoria*, might be very helpful.

*Caloplaca ulmorum, Caloplaca cerina, and Xanthoria*
elegans appear to have an interesting set of characteristics in common. They have unitunicate asci and a developmental pattern apparently suggestive of an ascostromatic form. Until more Discomycetes, free-living and lichenized, have been studied it is impossible to evaluate the apparent similarities in early ascocarp stages of the three species included in this study with ascocarp development of members of the Loculoascomycetes.
SUMMARY

Caloplaca ulmorum, Caloplaca cerina, and Xanthoria elegans were demonstrated to have a basically similar developmental pattern. In all cases the mature ascocarp was shown to develop from an ascocarp initial which was composed of a mass of central hyphae and a mass of tangentially oriented enveloping hyphae. In all cases the occurrence of gametangia with respect to ascocarp size was variable. The asci of the three species are unitunicate, inoperculate, and lack an apical pore. Hyphae of the ectal exciple were regarded as consistent over a wide geographical range.

The taxonomic position of the three species is not clear at this time since they appear to have an unusual combination of characters. It was concluded that the three species, which are interpreted to be distinct from one another, could be better evaluated after more developmental studies have been completed.


Bachmann, P. M. 1913. The origin and development of the apothecium in Collema pulposum (Bernh.) Ach. Archiv. Zellforsch. 10: 369-430.


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