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Field observations and inoculation experiments to determine the nature of the carpophoroids associated with *Entoloma abortivum* and *Armillaria*

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Abstract: Carpophoroids traditionally attributed to *Entoloma abortivum* ("Aborted Entolomas") repre*sent* malformed *Armillaria* fruiting bodies permeated by *E. abortivum* hyphae, as shown by our field observations and preliminary laboratory work. This contradicts the generally accepted hypothesis that carpophoroids are *E. abortivum* fruiting bodies colonized by *Armillaria.* Carpophoroids possess many of the structural characteristics of *Armillaria* fruiting bodies, including growth and development from rhizomorphs and the production of *Armillaria*-like spores on basidia. Our inoculation experiments demonstrate the ability of *E. abortivum* to abort the development of *A. tabescens* fruiting structures in vitro. In rare instances the introduction of *E. abortivum* disrupts *A. tabescens* fruiting structures to the point where they macroscopically resemble immature carpophoroids as observed in nature. If *E. abortivum is* a parasite of *Armillaria* species under natural conditions, *E. abortivum* may contribute to the regulation of *Armillaria* populations and could be investigated as a candidate for the biological control of destruc*tive Armillaria* species. We recommend "Abortive Entoloma" be used as the common name for *E. abortivum.*

Key Words: Aborted Entoloma, Abortive Entoloma, agaric, basidiomycete, biological control, fungi, fungicolous, mycoparasitism

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INTRODUCTION

Entoloma abortivum (Berk. & Curt.) Donk is a forest-inhabiting agaric that has been described as occurring in two forms. The first form is a typical agaricoid fruiting body with a gray stipe, a gray pileus, and pink, short-decurrent to adnate gills (FIG. 1A), while the second form is a white carpophoroid ("aborted") form lacking well formed gills (FIG. 1B). This second form often occurs in close association with *Armillaria* basidiomes (FIG. 1C). For a complete description of these fungi, see Hesler (1967) and Wading (1974).

Prior to 1974, carpophoroids were believed to be *E. abortivum* fruiting bodies that never developed properly. They were described as "the gastroid or atavistic aberration of an otherwise gymnocarpic fungus" (Wading 1974). Wading (1974) changed this interpretation by reporting that the carpophoroid form of *E.* abortivum is not comprised solely of *E. abortivum* hyphae. Through his studies of cultures prepared from agaricoid and carpophoroid forms of *E. abortivum,* Wading (1974) concluded that there was a second fungus present in the carpophoroids that was absent in the agaricoid fruiting bodies. This second fungus usually sectored out in culture and produced a darker mycelium with black rhizomorphs. Through an analysis of these rhizomorphs, the luminosity of his cultures, and the results of hyphal fusion experiments, Wading (1974) identified the second fungus as *Armillaria mellea* (Vahl : Fr.) Kummer s.1. Considerable work in the past 15 years has revealed that this is a species complex, and *A. mellea* has been split into 5 species in Europe, 10 species in North America, and approximately 35 species worldwide (Anderson and Ullrich 1979, Burdsall and Volk 1993, Hintikka 1978, Korhonen 1978, Volk and Burdsall 1995); this has made the exact identity of Wading's isolates unclear. This species complex will be referred to here as *A. mellea* s.1. or simply *Armillaria.*

Wading (1974) postulated that *A. mellea* s.1. was attacking and parasitizing *E. abortivum* fruiting bodies, thereby causing carpophoroid formation through the disruption of normal developmental patterns. Although never proven, this hypothesis has become widely accepted (e.g., Cha and Igarashi 1996, Jeffries and Young 1994, Redhead et al 1994), particularly in popular literature and field guides (e.g.. Huffman et

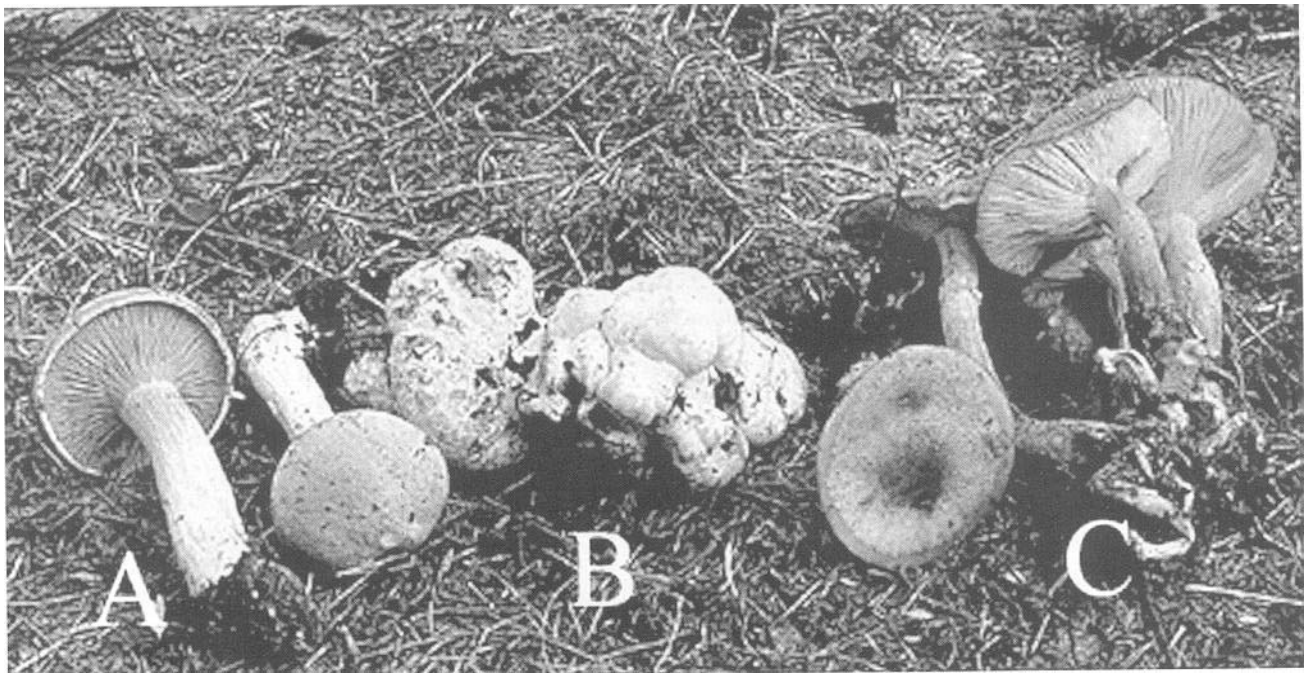

FIG. 1. The two agaric species involved in carpophoroid formation. A. Agaricoid *E. abortivum.* B. Carpophoroids ("Aborted Entolomas") often found associated with agadcoid *E. abortivum* and agaricoid *Armillaria.* C. Agaricoid *Armillaria.*

al 1989, Lincoff 1981, Phillips 1991). The hypothesis that *Armillaria* could be a mycoparasite may have seemed plausible to many mycologists because *Armillaria* is a notorious pathogen of a wide variety of gymnosperms and angiosperms. Unfortunately, at the time of Wading's study the difficulty of fruiting either *Armillaria* or *E. abortivum* in pure culture made it impossible to gather direct evidence for the parasitism hypothesis. Because of these limitations, Koch's postulate was never demonstrated and it was never conclusively shown that one fungus was causing a "disease" of the other. Refinement of fruiting techniques in the intervening years has made it possible to fruit *Armillaria* spp. on a limited basis (Darmono et al 1992, Reaves and McWilliams 1991), but to date we know of no reports of *E. abortivum* fruiting in culture.

Due to a lack of direct evidence, knowledge of the ecology and the interactions of these two fungi has been based almost exclusively on anecdotal field observation and collection. This relationship has been primarily explained in terms of macroscopic appearance of carpophoroids and in terms of their spatial associations: carpophoroids are vaguely reminiscent of distorted *E. abortivum* fruiting bodies, and they are often found adjacent to normal *E. abortivum* fruiting bodies. Singer's (1962, 1975, 1986) and Wading's (1974) reports of entolomatoid spores in carpophoroids further suggested the *Entoloma* origin of carpophoroids. All of this evidence seemed to indicate that carpophoroids were malformed *E. abortivum* fruiting bodies, and such evidence was used to sup port the idea that *E. abortivum* was a host that could be parasitized by *Armillaria* spp.

In other cases of mycoparasitism between agaric species, the determination of which species is the host and which the parasite does not need to be pieced together in this way. The fungal parasite is often easily identified due to its ability to fruit directly from or on the fruiting structure of the host fungus. This situation is seen in the interaction between fungi such as *Psathyrella epimyces* (Peck) Smith on *Coprinus comatus* (Müll. : Fr.) S.F. Gray (Buller 1924) or *Volvariella surrecta* (Knapp) Singer on *Clitocybe nebularis* (Batt. : Fr.) Kummer (Shaffer 1957). In the extreme example of *Squamanita* spp., the parasitized basidiomes become so altered that they were long thought to only be the bulbous base of the parasite (Redhead et al 1994). In the cases listed above, one agaric is fruiting directly from the disrupted basidiome of another (a basidiome that often lacks spores or has a severely reduced reproductive capacity), thus making it seem clear that one species is benefiting at the expense of the other. In the case of *A. mellea* s.1. and *E. abortivum,* however, an analysis of the spatial positioning of carpophoroids and normal fruiting bodies does not reveal any obvious trends that could help elucidate which fungus is most likely the parasite and which the host. While agaricoid *E. abortivum* and carpophoroids are often found growing togeth-

er, we have also commonly found agaricoid *E. abortivum,* carpophoroids, and agaricoid *Armillaria* spp. growing closely together or even clustered in all possible combinations, as well as in separate fruitings.

Because the spatial positioning and basic morphology of basidiomes and carpophoroids tell us so little about the nature of the relationship between these two fungi, Watling's (1974). report that both *A. mellea* s.1. and *E. abortivum* hyphae are present in carpophoroids is one of the few well proven aspects of the interaction between these two fungi. In contrast to Walting (1974), we have collected evidence suggesting that the scenario of parasitism should be reversed, with *E. abortivum* hyphae aborting the development of young *Armillaria* basidiomes. We propose this revised scenario based on two lines of evidence: (i) our field observations together with our structural and cultural analysis of carpophoroids, and (ii) our experimental work done by inoculating fruiting cultures of *Armillaria tabescens* (Scop.). Emel with *E. abortivum* mycelium.

MATERIALS AND METHODS

Field observations and culture.–Collections and cultures of *E. abortivum,* carpophoroids, and accompanying *Armillaria* spp. were made during the autumns of 1994-1995. Specimens were initially cultured and grown on 2% malt agar amended with 1 mg benomyl and 100 mg streptomycin per liter of medium, and were maintained on 2% malt agar at 24 C in the dark. Cultures and specimens were deposited in the culture collection and herbarium of the Center for Forest Mycology Research (CFMR) located in the USDA-Forest Service Forest Products Laboratory (Madison, Wis consin). All isolate numbers refer to cultures maintained by CFMR.

Specimens examined. USA. WISCONSIN: Dane Co., 2 Oct 1994, *TJV94-120.* 6 Oct 1994, *TJV94-122, TJV94-123.* 10 Oct 1994, *TJV94-126, TJV94-127.* 19 Oct 1994, *TJV94-140, TJV94-141, TJV94-142, TJV94-148.* Grant Co., 2 Oct 1994, *TJV94-119.* INDIANA: Brown Co., 30 Sept 1995, *TJV95-72, TJV95-74, TJV95-75, TJV95-76, TJV95-77, TJV95-78, TJV95-79, TJV95-80.* OHIO: Mahoning Co., 16 Oct 1994, *TJV94-135, TJV94-136. TJV94-137, TJV94-138, TJV94-139.*

Laboratory experiments.–A monosporous isolate from an *A. tabescens* basidiome was found to fruit readily under cultural conditions (Isolate 928881). This isolate was fruited in Petri plates by placing a small amount of mycelium on 2% malt agar and allowing it to grow in the dark for 2 wk at 24 C. After this time small cubes of agar ca 1 mm^3 were excised from the growth margin and placed in 100 × 15 mm plastic Petri plates that had been filled to a depth of 5 mm with squeezed orange juice (SOJ) agar. SOJ agar was prepared according to Darmono et al (1992), who found this a reliable substrate for fruiting *A. tabescens.* Isolates placed on SOJ agar were then moved to a 28 C incubator and left to grow in the dark. After 2 wk, when the growing edge of the mycelium was about 1 cm from the edge of the Petri dish, the isolates were transferred to an incubator at 19 C with a day/night cycle of 12 h. Fruiting bodies appeared sporadically within the next 6-8 wk. *Entoloma abortivum* cultures (Isolate 11665 FP-102438-T) were grown in the dark on 2% malt agar at 24 C. These were used to inoculate *A. tabescens* fruiting bodies by excising three cubes of agar ca 8 mm^3 from the growth margin of an *E. abortivum* culture and placing the cubes against developing clusters of primordial *A. tabescens* fruiting bodies.

Armillaria isolates were also fruited in 500-mL Erlenmeyer flasks. This was accomplished by placing one small, coarsely chopped orange in each flask. Flasks were plugged with cotton, covered with aluminum foil, and autoclaved for 26 min at 121 C. After cooling, either *A. tabescens* (Isolate 11853 SG-3) or *A. gallica* (Isolate TJV94-140) was introduced into the flasks by chopping the growing edge of a ten day old colony into fine pieces with a scalpel, adding the inoculum to the flask, and shaking. Flasks were placed in a dark incubator for 2 wk at 28 C and then transferred to room temperature with indirect sunlight. Fruiting bodies of *A. tabescens* appeared sporadically within 3-4 mo, whereas *A. gallica* did not fruit Inoculation of *A. tabescens* fruiting bodies was accomplished by excising cubes of agar from the growth margin of an *E. abortivum* culture and placing the cubes against developing clusters of primordial *A. tabescens* fruiting bodies.

We studied the temporal and spatial distribution of culturable *E. abortivum* and *Armillaria* hyphae in field-collected carpophoroids by culturing from specific areas within many individual carpophoroids in different growth stages. Cultures from a particular area could yield either *Armillaria, E. abortivum,* both, or neither, thus giving a rough picture of where each species was active in a carpophoroid. Carpophoroids were broken open and small pieces of tissue were removed from three locations: from as close to the outer edge as possible, from within the tissue that represents deformed gill tissue (the "rind"), and from within the inner matrix of the carpophoroid (see FIG. 2). Each piece was placed on 2% malt agar amended with 1 mg benomyl and 166 mg streptomycin per liter of medium and grown at room temperature. After each sample had grown for 2-3 wk, subcultures were taken from at least three places within each culture. Cultures were made from a total of 13 separate collections, which formed 57 primary cultures and approximately 175 subcultures (TABLE I). After another 2 wk, the original cultures and the matching subcultures were examined macroscopically and microscopically to determine whether hyphae of *Armillaria, Entoloma,* both, or neither were present. *Entoloma* hyphae could be distinguished from *Armillaria* hyphae by the presence of clamp connections, the common presence of a distinctive three-way branching system, and by the general form and shape of the hyphae.

Armillaria gallica and *E. abortivum* were also grown together on glass microscope slides to observe possible hyphal interactions. A small amount of SOJ agar was poured onto microscope slides to create a strip of agar ca 1 mm deep. *A. gallica* and *E. abortivum* were inoculated 1 cm apart, and a cover slip was placed over the two inoculations. The slides

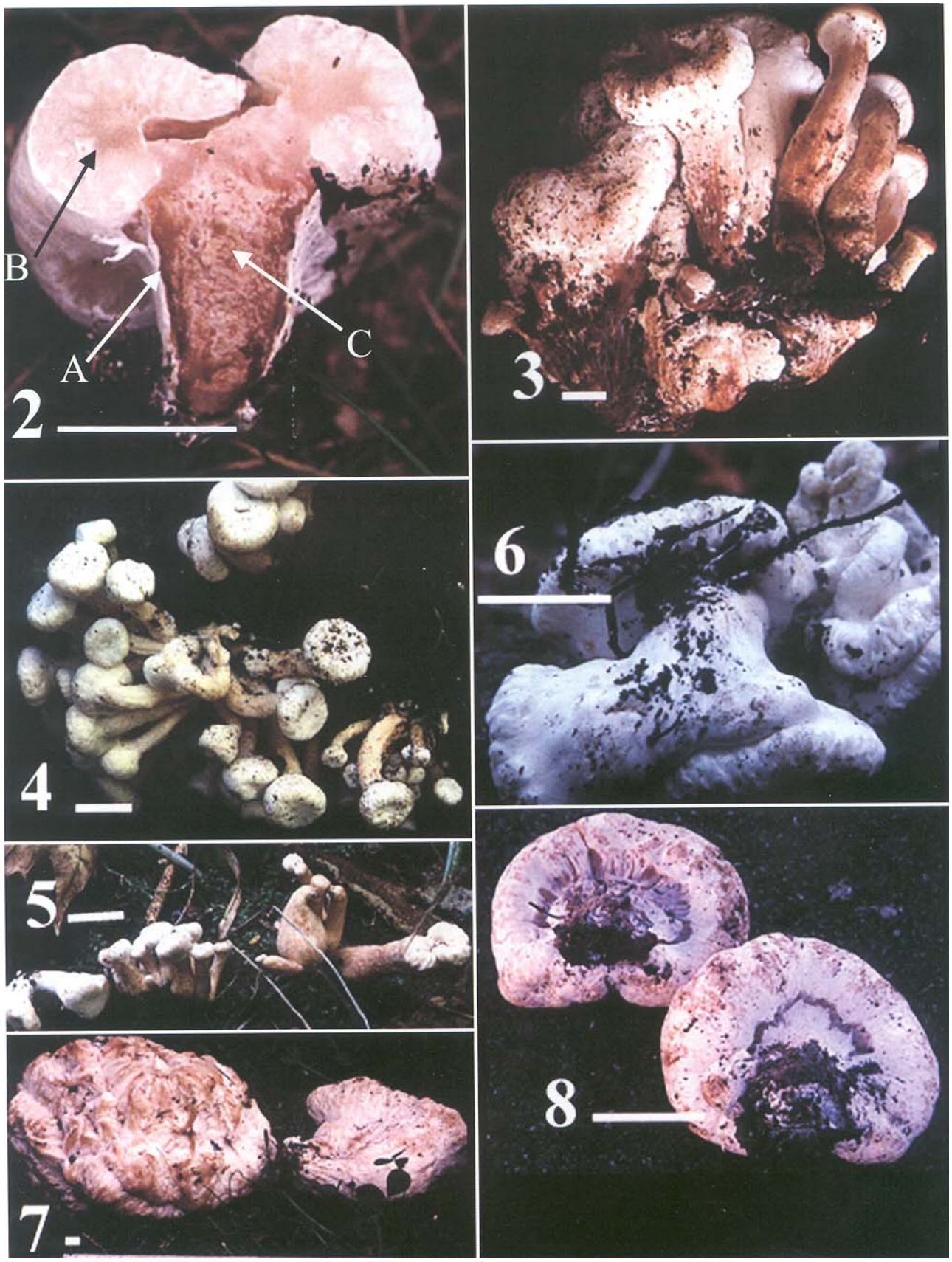
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TABLE 1. Cultural identification of *Entoloma abortivum* and *Armillaria gallica* from carpophoroids

Area from which cultures were made	Number of primary cultures[a]	Species identified in culture
Young carpophoroids		
outermost tissue[b]	3	*E. abortivum* (3 cultures)
inner matrix	25	*E. abortivum* (6 cultures)
		A. gallica (10 cultures)
		Mixed (9 cultures)
Old carpophoroids		
outermost tissue[b]	2	*E. abortivum* (2 cultures)
gill tissue (the rind)	11	*E. abortivum* (1 culture)
		A. gallica (2 cultures)
		Mixed (8 cultures)
inner matrix	16	*E. abortivum* (16 cultures)

[a] Most primary cultures were made from separate fruiting bodies.

[b] Most cultures made from the outermost tissue of carpophoroids were lost to contamination.

were incubated at 24 C in the dark at high humidity, As the hyphae of the two species approached one another and intermingled, observations were made using an Olympus BH-2 compound microscope at ×400 and under oil immersion at ×1000.

RESULTS

Field work.–Numerous observations of *Armillaria* and *E. abortivum* populations were made between 1993 and 1995 that do not agree well with the hypothesis that carpophoroids are *E. abortivum* mushrooms parasitized by *Armillaria.* For example, a cluster of *Armillaria gallica* basidiomes was found in which some of the fruiting bodies appeared normal, but most were aborted into carpophoroids (FIG. 3). All of the carpophoroids in this cluster were arising from one continuous rhizomorph, just as *Armillaria* fruiting bodies arise from rhizomorphs. This particular cluster had other carpophoroids scattered near it, as well as agaricoid forms of *E. abortivum.* Since that time, we have observed at least 10 other fruitings of what appear to be "half-aborted" *Armillaria* spp. fruiting bodies (FIG. 4), usually in close association with carpophoroids and unaborted fruiting bodies of both *Armillaria* spp. and *E. abortivum.* In some collections, a continuum can be seen from well formed *Armillaria* fruiting bodies, to well formed *Armillaria* stipes with aborted caps, to complete carpophoroids (FIG. 5). In the rare instances where a carpophoroid develops to the point where rudimentary gills are formed, we have observed that the few basidia that mature produce *Armillaria*-like spores, not the angular spores characteristic of *E. abortivum.*

In addition to exceptional fruitings that distinctly appear to represent malformed *Armillaria* fruiting bodies, we have observed other commonly reported phenomena, such as carpophoroids usually, if not always, growing with black rhizomorphs attached at their bases (FIG. 6). Walting (1974) explained the location of these rhizomorphs by hypothesizing that *Armillaria* was sending rhizomorphs up through *E. abortivum* fruiting bodies via their bases. However, this phenomenon distinctly resembles the way in which *Armillaria* itself produces fruiting bodies from black rhizomorphs. In a section, the similarity between the base of a carpophoroid and the base of an agaricoid *Armillaria* is readily apparent. Both bases possess a melanized layer of hyphae that usually leads to the attachment point for a rhizomorph, and both display the yellowish and pinkish coloration characteristic of *Armillaria* stipes. In contrast, the base of an agaricoid *E. abortivum* fruiting body is almost entirely white, lacking melanized hyphae as well as pink and yellow coloration. In our experience, carpophoroids appear to develop from rhizomorphs in the same fashion as *Armillaria* fruiting bodies, and the parental rhizomorphs are often incorporated into the bases of the carpophoroids to the point where carpophoroids can he detached from the substrate simply by pulling on the surrounding rhizomorphs.

Carpophoroids also often arise in places that are

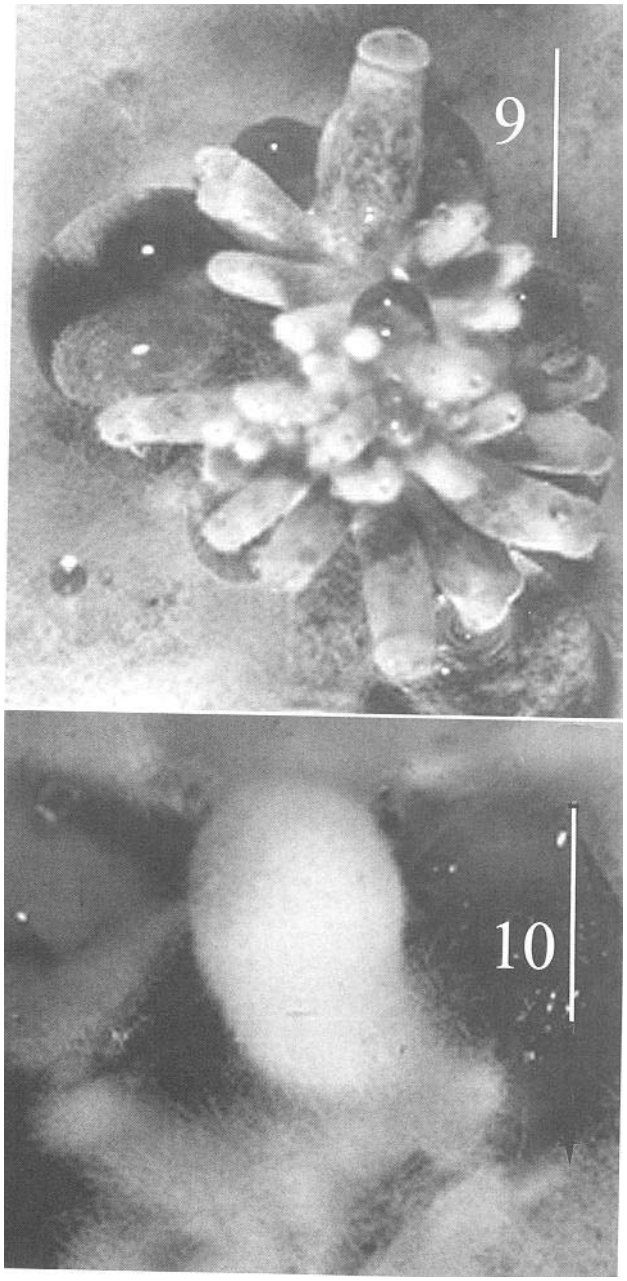
FIGS. 2-8. Detail and variation of carpophoroids. 2. Section of a carpophoroid showing outermost layer of tissue (A), area ("the rind") that represents malformed gill tissue (B), and the inner matrix (C). 3. Cluster of carpophoroids arising from a rhizomorph. Although most mushrooms in the cluster developed into carpophoroids, the 4 mushrooms on the right are agaricoid *Armillaria gallica.* 4. *Armillaria* fruiting bodies with carpophoroid caps, but stipes that are morphologically unaffected. 5. A continuum can sometimes be seen from fully developed carpophoroids (on the left), to fruiting bodies with carpophoroid caps or no cap and *Armillaria* stipes (on the right), to agaricoid *Armillaria* with fully developed caps (not visible in this photo). 6. Rhizomorphs attached to base of carpophoroid. 7. A massive cluster of carpophoroids comprised of many malformed mushrooms (the cluster on the left is approximately 25cm long). The stipes of all the mushrooms originate from a common point, usually a group of rhizomorphs. This type of fruiting is typical of *Armillaria* spp. 8. Carpophoroids sometimes produce a rudimentary partial veil. Scale bars: 2-8 = 1 cm.

more associated with the fruiting of *Armillaria* spp. than *Entoloma* spp. It is common to find carpophoroids growing from wood that is hardly decayed (sometimes even from the pseudosclerotial plates of Armillaria), or even from wild grape vines (*Vitis riparia*). Substrates such as woody grape vines are commonly inhabited by *Armillaria* spp., while it would be unusual to find an *Entoloma* sp. fruiting from such a substrate.

We have also observed the rare occurrence of massive clusters of carpophoroids, sometimes greater than 15 cm diam (FIG. 7). All of the carpophoroids in such a cluster seem to have malformed stipes that originate from a common point, a fruiting pattern commonly seen in *Armillaria* spp., but not *Entoloma* spp. This last observation may explain why *Clavaria gigantea* Schweinitz 1822 (= *Acurtis giganteus* Schw.: Fr.), which is believed by some to represent *E. abortivum* carpophoroids, was originally described as "a large receptacle, as large as a man's head" (Wading 1974). Another *Armillaria*-like aspect of carpophoroids is their tendency to produce tissue that is reminiscent of a rudimentary partial veil (FIG. 8). Partial veils. are structures formed by most *Armillaria* spp., but not by *E. abortivum.*

Laboratory experiments.–In the most successful inoculation trial, 20 SOJ plates were inoculated with the monosporous isolate of *Armillaria tabescens,* and 19 produced primordial fruiting structures. The one plate that did not produce primordia was discarded, 9 plates were left uninoculated as controls, and 10 were inoculated with *E. abortivum.* The 9 uninoculated plates all produced typical, albeit small, *A. tabescens* fruiting bodies (FIG. 9). The fruiting bodies were 1 to 3.5 cm long, grew in clusters of 2 to 7, and tended to produce more gilt tissue and less pileus tissue than fruiting bodies found in nature. Of the 10 plates where *A. tabescens* fruiting clusters were inoculated with *E. abortivum,* 5 were inoculated after the fruiting bodies had developed to the point where gills could be seen. In all of these cases, *E. abortivum* grew over the fruiting clusters, covering them with white mycelium. In the 5 cases where *E. abortivum* was inoculated at the point where fruiting clusters still resembled small white "lumps," 4 grew into fruiting structures covered with white mycelial fluff, while one formed a white peg-like structure 11 mm high and 6 mm diam. This structure morphologically resembled a young carpophoroid (FIG. 1.0). In nature we have observed the formation of similar structures, some of which matured into fully formed carpophoroids over the course of 4-7 d, and some of which remained in this primordial state until they decayed.

In other inoculation experiments, young *Armillaria* basidiomes covered by *E. abortivum* mycelium were often produced, but carpophoroid-like structures were only produced three times, and only in the one instance described above did the structure develop to a point where it clearly resembled a young car-



FIGS. 9-10. Cultural production of carpophoroids. 9. *Armillaria tabescens* fruiting bodies produced on SOJ agar. These are control mushrooms that were not inoculated with *E. abortivum.* 10. Carpophoroid produced by inoculating *A. tabescens* primordia with *E. abortivum.* This structure is 11 mm long and 6 mm wide and morphologically resembles a young carpophoroid as observed in nature. This carpophoroid is positioned similarly to the uppermost fruiting body in FIG. 9. Scale bars: 9-10 = 1 cm.

pophoroid. That particular structure was observed for 2 wk to determine if it would develop further, which it did not. This could have been due to the timing of the inoculation, or it may have been due to the limitations of the cultural environment (e.g., a lack of nutrients, incorrect humidity or temperature, etc.).

Inoculation experiments were also done on *Armillaria tabescens* fruiting in flasks. *Entoloma abortivum* was inoculated on the *A. tabescens* fruiting structures when they were between 0.5 and 8 cm tall and had cap diameters between 0.2 and 1.5 cm. Gills were visible on the largest caps, but the majority were still in the "pin" stage. Unfortunately, this inoculation system did not work well because the *Armillaria* fruiting structures matured normally and gained full height, ca 14 cm, before the *E. abortivum* inoculation had a chance to grow. Microscopic analysis revealed that *the E. abortivum* mycelium had grow over the spent *A. tabescens* fruiting bodies within 2 wk, but no carpophoroids were produced.

For our cultural study of carpophoroids collected in the field, we divided our sample of carpophoroids into two categories based on presence or absence of rudimentary gill tissue: "young" (before formation of gill tissue), and "old" (after a significant amount of gill tissue had formed). This tissue, referred to as a "rind," appears to be a layer of malformed gill tissue that covers the entire upper surface of the aborted fruiting body, This condition resembles small *A. tabescens* basidiomes grown in culture that produced excess amounts of gill tissue and little pileus tissue, possibly due to an excess of carbon dioxide. If this condition were taken to an extreme so that only gill tissue were formed with no pileus tissue, it would be reminiscent of what appears to happen in the rind formation of a carpophoroid.

The cultural analysis, supplemented with microscopic evaluation of hyphal characters, suggests that young carpophoroids are made up of a thin outer layer of *Entoloma* hyphae, with the center being comprised of a mixture of *Armillaria* and *Entoloma* hyphae. *Armillaria* was isolated slightly more often than *Entoloma* from the center of young carpophoroids (TABLE I). The smallest carpophoroids from which cultures were taken were already almost 1 cm long, so it is not clear if one species is present before the other in carpophoroid primordia, or if both species are present in a primordium from the start. In older carpophoroids, the outside layer of hyphae again appeared to be comprised almost entirely of *Entoloma* hyphae, while the gill tissue was a mixture of both species. The inner matrix of older carpophoroids yielded only *Entoloma* cultures (TABLE I).

Species identification of our *Armillaria* tissue isolates from carpophoroids was also completed. Of 10 isolates (see TABLE I) from southern Wisconsin tested using the dip-hap pairing protocol of Rizzo and Harrington (1992), all were identified as *A. gallica.* Two isolates from carpophoroids collected in New Jersey (Isolate DLC99-3 and DLC99-4) were also identified as *A. gallica,* while one isolate from lower Michigan (Isolate DLC99-2) was identified as *A. ostoyae.* In addition, we have observed carpophoroid formation in clusters of *A. tabescens* basidiomes in nature, and carpophoroid formation near clusters of *A. mellea* s. s. fruiting bodies, although it is still unclear whether *A. mellea* s. s. can enter into this relationship. In work by Cha and Igarashi (1996) in Hokkaido, Japan, three isolates of *Armillaria* found associated with *E. abortivum* were identified to species. One isolate was identified as *A. gallica,* while the other two were identified as *A. jezoensis* Cha and Igarashi.

In our experiments to observe hyphal interactions between *A. gallica* and *E.* abortivum on microscope slides, hyphae of *A. gallica* and *E. abortivum* grew up to and past one another without coiling, penetration, or any other obvious interaction. As a hypha of one species approached a hypha of the other species, there was no discernible change in direction toward or away from the foreign hypha. The hyphae of the two species grew together thickly until it was no longer possible to positively identify a particular hypha to species. This same interaction was observed when *A. gallica* and *A. tabescens* isolates were grown with *E. abortivum* on SOJ agar in Petri plates. In contrast, Cha and Igarashi (1996) found that the growth of *Armillaria* isolates was severely inhibited by the presence of *E.* abortivum colonies. This inhibition was found to be dependent on the type of growth medium used, and primarily occurred on PDA. On other media, the inhibitory effect of *E. abortivum* was found to be weaker, and in some cases mutual inhibition was seen (Cha and Igarashi 1996). Our use of SOJ agar may account for our inability to observe hyphal interactions and mycelial inhibition. It is likely that successful observations of microscopic hyphal interactions will not he accomplished until these fungi are grown and observed on a variety of media with varying nutrient levels.

DISCUSSION

Our laboratory work demonstrates the ability of *A. tabescens* to form carpophoroids in vitro, and our field observations support the ability of *Armillaria* spp. to form carpophoroids in nature. Our inoculation experiments with *A. tabescens* illustrate that under laboratory conditions *E. abortivum* is capable of disrupting the development of *Armillaria* fruiting

bodies, although it has yet to be established definitively that such a phenomenon occurs in nature. Specifically, the null hypothesis that was disproved by our lab work was that the fruiting mechanisms of *E. abortivum* are always involved in carpophoroid formation; in our case, we formed carpophoroids using *A. tabescens* fruiting bodies and vegetative *E. abortivum* mycelium. We were able to reisolate *E. abortivum* from the carpophoroid formed in culture, making our work a demonstration of Koch's postulate in terms of *E. abortivum* being capable of causing "disease" in *A. tabescens* fruiting structures.

It is important to note that historically other mycologists have also reported the ability of *Armillaria* to form carpophoroids. Peck (1889, 1890, 1893) and Singer (1970a, b) both reported the ability of *A. mellea* s.1. to form carpophoroids similar to those of *E. abortivum,* and McIlvaine (1900), Harper (1916), and Wading (1974) all mention this phenomenon briefly. Peck (1889, 1890, 1893) and Singer (1970a, b) describe the abortive form of *Armillaria* as being quite similar in appearance to that of *E. abortivum, al*though neither author suggested that the carpophoroids associated with *Armillaria* may in fact be the same as the carpophoroids associated with *E. abortivum.* Singer (1970a) observed carpophoroids of *A. mellea* s.1 in Illinois and Florida, and interpreted the carpophoroids as "gasteromycetation" of *A. mellea.* He noted that the abortive form was almost entirely white and that spores were produced late, in reduced quantities, and inside the fruiting bodies (Singer 1970a). Peck (1893) felt that *Armillaria* carpophoroids were "in no way distinguishable" from the carpophoroids formed by *E. abortivum,* and McIlvaine (1900) echoed this by reporting that the abortive form of *E. abortivum* was "in every way similar" to that of *A. mellea* s.1. (McIlvaine 1900). For Peck and McIlvaine, identification of a carpophoroid to species appears to have been contingent on the presence of agaricoid fruiting bodies. In contrast, Singer reported that *A. mellea* s.1. carpophoroids could be differentiated from *E. abortivum* carpophoroids "through few and small macroscopic qualities and through the form of the spores" (Singer 1970a).

Although mycologists had previously reported the ability of *Armillaria* to form carpophoroids, Wading's (1974) work was the first to associate *Armillaria* with the carpophoroids of *E. abortivum.* Wading (1974), however, interpreted carpophoroids as malformed *E. abortivum* structures and only commented briefly on Singer's (1970a, b) report of *Armillaria* forming carpophoroids. In a short article, Wading (1989) reiterated his proposal that *Armillaria* was capable of disrupting the development of *E. abortivum* fruiting bodies, despite the suggestion by an amateur mycologist, William H. Petty, that *Armillaria* was the host and *E. abortivum* the parasite (Petty 1989, 1992). In contrast to Wading (1974, 1989) and in support of Petty (1989, 1992), we hypothesize that all carpophoroids found associated with *E. abortivum* and *Armillaria* in nature represent parasitized *Armillaria* basidiomata permeated by *E. abortivum* hyphae, as supported by our field observations of natural carpophoroids and our cultural and structural analysis of carpophoroids. It is possible that "true" aborted *E. abortivum* fruiting bodies may exist, but we have found no evidence for such structures.

Although both Singer (1962, 1975, 1986) and Wading (1974) report finding basidia and entolomatoid spores in carpophoroids, which they felt indicated that true aborted *E. abortivum* does exist, Singer (1986) later stated: "It is correct to indicate that in *Acurtis*-forms [of *E. abortivum* carpophoroids] frequently endobasidia are formed which do not form *Entoloma* spores but *Armillaria* spores, or what looks very much like *Armillaria* spores. I have myself observed that in most collections at least some of the *Acurtis* carpophores contain such basidia and spores and that wherever *Acurtis* forms occur, *Armillaria polymyces* [= *Armillaria ostoyae*]–ordinarily later-fruiting–occurs nearby." Singer regarded this as an indication that Wading's parasitism hypothesis was probably correct and that such spores and basidia may be present due to the ability of the parasite, *Armillaria,* to fruit within the malformed fruiting body of its host, *E.* abortivum. Whatever his interpretation, Singer's comment is consistent with our observations of *Armillaria*-like spores being formed in carpophoroids.

Entoloma abortivum spores have a distinctive, angular appearance and are easily distinguished from the smooth, elliptical spores of *Armillaria.* Wading (1974) reports the carpophoroid form of *E. abortivum* as having basidia and basidiospores that are sparse, but "similar in all ways to agaricoid form [of *E. abortivum*]," and describes the spores as forming "in the cavities of the pitted pileus-surface." In our observations of carpophoroids collected in Wisconsin, Ohio, and Indiana (a total of at least 10 separate localities), the gills of large, well formed carpophoroids are usually very reduced and spores are absent. The type specimen of *Acurtis-giganteus* Schw. : Fr., based on *Clavaria gigantea* Schweinitz, which is assumed by some mycologists to be the carpophoroid state of *E. abortivum,* was also found to be sterile (Wading 1974). However, we have found that carpophoroids with the greatest gill development sometimes possess spores and basidia typical of *Armillaria* spp., not *Entoloma* spp.

The two most obvious explanations for our failure

to find entolomatoid spores being formed by carpophoroids are that: (i) carpophoroids with entoloma toid spores exist but we have yet to come across them, or (ii) reports of entolomatoid spores in carpophoroids are due to contamination. In the first case, carpophoroids producing entolomatoid spores could either be *E. abortivum* fruiting bodies attacked by *Armillaria,* or they could be the attempt of *E. abortivum* to fruit from an aborted *Armillaria* fruiting structure. It is possible that a long term search might turn up such carpophoroids. We suspect, though, that the second explanation, contamination, may, account for the reports of entolomatoid spores in carpophoroids. Contamination may be a likely explanation because carpophoroids and agaricoid *E. abortivum* have long been thought to be "the same species." Therefore, many people collect and carry carpophoroids and agaricoid fruiting bodies in the same container. Fertile, agaricoid *E. abortivum* fruiting bodies shed a tremendous number of spores, and we have had to discard many collections made by others because agaricoid fruiting bodies were placed alongside or on top of the carpophoroids. Even if a collector was careful to segregate specimens into different containers, it is possible that contamination may already have happened in the field. Agaricoid *E. abortivum* fruiting bodies are often found very close to carpophoroids, and collectors are more likely to collect samples when both forms are present. It is common to see the spores from one fruiting body lightly covering a neighboring pileus, and it would take only a light dusting to contaminate an entire sample. This could account for Wading's (1974) report of spores as forming "in the cavities of the pitted pileus-surface," because the pileus surface of a carpophoroid could easily be contaminated with entolomatoid spores even in the field.

In the course of our field work, we have found no evidence to support the claim of *Armillaria* as a mycoparasite. When dissected and microscopically examined, all of the mature carpophoroids that we have investigated appear to have the structure of malformed *Armillaria* fruiting bodies, possessing either pigmented bases with rhizomorphs or, in rare cases, forming *Armillaria*-like spores in their hymenium. Because evidence suggests that carpophoroids are a result of *E. abortivum* disrupting the development of *Armillaria* fruiting structures, we recommend the name "Abortive Entoloma" be adopted as a common name for *E. abortivum.* Although we can not directly address the question of *Armillaria* as a mycoparasite, it is the opposite question, whether *E. abortivum* is a mycoparasite, which may prove to be of greater interest. If *E. abortivum* is able to parasitize *Armillaria* mycelia, it may be possible to develop strains of *E. abortivum* suitable for biological control of plant pathogenic *Armillaria* species. *Armillaria* species are important plant pathogens in forest and orchard settings worldwide (Shaw and Kile 1991), and certain chemical control strategies for these fungi may soon be unavailable in the United States.

In addition to *E. abortivum,* a number of other members of the Entolomataceae have been reported growing in association with the fruiting bodies of other fungi, suggesting that mycoparasitism may prove to be relatively common in this family. *Rhodocybe stangliana* (Bresinsky & Pfaff) Riousset & Joss., for example, is an agaric that has been variously described as arising from a "protocarpic tuber" (Laessøe and Rosendahl 1994), or as having "fleshy subterranean lumps at the base of the stipes" (Redhead et al 1994). Sandor (1957) originally suggested that *R. stangliana* was a parasitic agaric, but this idea was not widely accepted (Laessøe and Rosendahl 1994, Redhead et al 1994). As recently as 1992, Romagnesi interpreted the bulbs simply as volvas (Romagnesi 1992). Fresh material found by Laessøe (1990), however, has allowed modem methods of analysis to be used in the determination of these structures. Laessøe and Rosendahl (1994) compared the banding patterns of isozymes and denatured proteins from the stipe and pileus of *R. stangliana* and concluded that two taxa were involved in "basidiome" formation. This sup ports Sandor (1957). whose original work with a variety of chemical reagents suggested that the bulbs were comprised of tissue that was different from that of the fruiting bodies.

Another member of the Entolomataceae suspected of mycoparasitism is *Entoloma parasiticum* (Quél.) Kreisel. (= *Claudopus parasiticus* (Quél.) Ricken), a pleurotoid species sometimes placed in subgenus Claudopus (Noordeloos 1987). This species has been reported to fruit from the basidiomes of *Cantharellus cibarius,* as well as from living polypores such as *Trametes versicolor* and *Coltricia perennis* (Noordeloos 1987). However, some questions regarding the taxonomy and "host" preference of *Entoloma parasiticum* remain unanswered. Fitzpatrick (1915) described this fungus under the name *Claudopus subdepluens* Fitzp. and reported it fruiting from *Coltricia perennis* (= *Polyporus perennis*); Overholts (1929) found what he felt was the same species as Fitzpatrick's, but found it growing on *Cantharellus cibarius.* Noordeloos (1987) lists *Claudopus subdepluens* Fitzp. as a synonym of *Entoloma parasiticum* (Quél.) Kreisel. and lists the habitat of this species as including nonfungal substrates such as very rotten bark, earth, and living mosses (Noordeloos 1987). Noordeloos (1987) also reports *Entoloma pseudoparasiticum* nom. prov. as occurring on *Cantharellus cibarius* and *Craterellus lu-*

tescens. This raises the question of whether there are a number of "cryptic" taxa near or within the species described as Entoloma parasiticum, perhaps each specializing on particular substrates or whether *E. parasiticum* truly represents a single taxon with the ability to colonize a wide variety of fungal and non-fungal substrates.

If a particular *Entoloma* sp. were found to only fruit from the fruiting structures of other fungi, this would support but not prove that mycoparasitism was taking place. As Buller (1924) points out, ". . . it is as yet uncertain whether Claudopus subdepluens derives its nutriment form Polyporus perennis fruit-bodies or merely us the Polyporus fruit-bodies as an apparatus to which attach its own fruit-bodies. An experimental investigation alone can decide between these two alternatives... It is of course possible that *C. subdepluens* normally lives a saprophytic existence on wood, etc., and is only occasionally a parasite on *Polyporus perennis"* Another alternative is that these *Claudopus* spp. are entirely mycoparasitic, and are capable of directing parasitizing the mycelia of other fungi that grow in substrates such as soil and well decayed wood. Experimental studies including inoculation trials are needed to better understand the ecology of these fungi.

Clitopilus spp. have also been found fruiting on or in association with the fruiting bodies of other fungi. *Clitopilus daamisii* Noordel. has been found fruiting from *Hymenochaete tabacina* (Noordeloos 1984), while *C. passeckerianus* (Pilát) Sing. and *C. fasciculatus* Noordel. are associated with growing-beds of cultivated *Agaricus* (Noordeloos 1984, 1993). Despite the consistent association of *C. passecherianus* with *Agaricus* beds, ginger did nut seem to believe that *C. passecherianus* is found in this habitat because it is parasitizing *Agaricus.* Singer reported that *C. passeckerianus* is "...not detrimental to the crop of *Agaricus*..." (Singer and Harris 1987) and questioned whether *C.* passeckerianus really represents a species adapted to growing on *Agaricus* beds, or if it is a variant of a species that also occurs on natural substrates (ginger and Harris 1987). Further work needs to be done to determine if *C. passeckerianus* and *C. fasciculatus* are associated with *Agaricus* beds because they are parasitizing the abundant mycelia found in such habitats. If they are found to be mycoparasites, the small size of these fungi could explain why they do not appreciably affect yield.

Interestingly, McIlvaine (1909) reports that *Clitopilus prunulus* can also produce carpophoroids similar to those of *E. abortivum,* but it is difficult to interpret this report due to a lack of dried material or drawings. We have never observed this phenomenon in *Clitopilus prunulus* and it is possible that McIlvaine's report stems from a misidentification of *E.* abortivum as *C. prunulus.* There are also a few reports of fungi outside of the Entolomataceae forming carpophoroid-like structures. Watling (1974) observed *Inocybe geophylla* producing carpophoroids almost identical to those of E. abortivum, and the parasitism of *Helvella lacunosa* by *Clitocybe sclerotoidea* (Trappe 1972) produces a structure similar to that of a carpophoroid.

Carpophoroid formation may be more widespread in natural ecosystem than is presently recognized and could represent an important mechanism of interaction between fungal parasite and their hosts. Despite this, we know little about the ecological significance of carpophoroid formation, and critical experiments have yet to be carried out to determine if nutrient exchange is occurring at this interface of two organisms. Further experimental work, complimented by taxonomic and ecological studies of the species involved, is needed before we will fully understand these enigmatic structures.

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