INTRODUCTION

The fungus *Roesleria hypogaea*, associated with a rootrot of grapes, has long been known, especially in the grape-growing regions of Europe. It has not been definitely proved that this fungus is the primary cause of the rot, but there is no question that, once established, it contributes largely toward the killing of the roots. Disagreement among mycologists as to the identity of some well-known fungus is often due to a lack of knowledge of its life history. Confusion has more than once arisen because of an accidental similarity of fruit bodies or spores. A case in point is that of *Roesleria* and *Pilacre*.

We are indebted to Brefeld (52) for the beautiful illustrations of *Pilacre friesii* B. & C., a fungus which he believed to represent a very primitive Basidiomycete. His figures of the fruiting bodies on bark show a "gleba" composed in part of short septate branches, each bearing a definite number of spores. Such a branch he considers a simple basidium. *Roesleria hypogaea* Thüm. and Pass., an ascogenous fungus whose fruiting bodies have a strong superficial resemblance to those of *P. friesii*, is, on this account, most interesting. *Calicium pallidum* Pers., well known to lichenologists, also has fruiting bodies similar in general appearance to those of *Roesleria* and *P. friesii*. Since Rehm (27, p. 396) considers *Coniocybe pallida* (Pers.) Fr. a synonym of *R. hypogaea*, and Bayliss-Elliott and Grove (5) suggest that *R. hypogaea* is merely the ascogenous stage of *P. friesii*, and since Viala and Pacottet (55) report conidial and chlamydosporic forms of *Roesleria* in culture, it is not surprising that we find the greatest confusion of these names in the literature. The writer has had the opportunity recently to study *R. hypogaea* and *P. friesii* in culture and has also compared them with specimens of the lichen, *Coniocybe pallida*, with the result that we find that there is no basis for considering these fungi identical or genetically related.

From a review of the literature it will be seen that the grape rootrot fungus has been described under a number of different names and combinations. The following list includes such as appear from original descriptions and illustrations to refer to a Discomycete identical with *Roesleria hypogaea* of von Thümen and Passerini.

*Pilacre subterranea* Wein. 1832. (36, p. 458.)

*Pilacre friesii* Wein. (in Flora) 1832. (36, p. 458.) Not Wein. 1834.

*Onygena friesii* Wein. 1834. (37, p. 413-414.)

*Vibrissa flavipes* Rab. 1852 (26, p. 286.)

*Sphinctrina coremioides* B. & Br. 1872. (2.)

*Roesleria hypogaea* Thüm. & Pass. 1877. (32.)

1 Received for publication Nov. 25, 1923.

* Reference is made by number (italic) to "Literature cited, pp. 615-616."

Journal of Agricultural Research,
Washington, D. C.

Vol. XXVII, No. 8
Feb. 23, 1924
Key No. G-355

(609)
Vibrissea hypogaea (Thûm. & Pass.) Rich. 1881. (28.)
Coniocybe pilacrimoris Rehm 1892. (20, p. 56.)
Roesleria pilacrimoris (Rehm) Henn. 1895. (16.)
Pilacre pilacrimoris (Rehm) Boud. 1907. (4.)
Pilacre pallida Boud. 1907. (4.) Not Calicium pallidum Pers. 1794 (23, p. 20), nor P. pallida E. & E. 1900. (9, p. 59.)

A lichen Calicium pallidum Pers. 1794 (23, p. 20), Coniocybe pallida (Pers.) Fr. 1824 (31, p. 65) has also been confused with Roesleria hypogaea. The specific name "pallida" has been used frequently in combination with the generic name Roesleria (R. pallida (Pers.) Sacc. (30, p. 299) to refer to a fungus thought at the time to be one of the Stilbaceae but which is in reality Roesleria hypogaea.

Another fungus, nonascogenous, commonly called Pilacre jaginea, or P. petersii in this country, has been regarded erroneously by various authors as a conidial stage of Roesleria hypogaea. It appears to have been first described as Onygena jaginea Fr. The synonymy of this fungus so far as can be ascertained by a study of original descriptions and the literature is as follows:

Onygena jaginea Fr. 1818. (10, p. 25.)
Onygena decoricata Schwein. 1822 (31, p. 65.) Not Onygena decoricata Pers. 1799. (24.)
Phleogena jaginea (Fr.) Link 1833. (21, p. 369.)
Pilacre friesii Wein. in Linnaea. 1834. (37, p. 413–414.) (Not P. friesii Wein. in Flora 1832. (36, p. 458.)
Botryochaete jaginea (Fr.) Corda 1854. (7, p. 9, pl. 95.)
Pilacre jaginea (Fr.) B. & Br. 1850. (3, v. 5, p. 365, pl. 11, fig. 5.)
Ecchyna jaginea Fr. 1857. (13, p. 151.)
Pilacre petersii B. & C. 1855. (3, v. 3, p. 362.)
Stilbum pilacrimorm Rich. 1889. (29), not Coniocybe pilacrimoris Rehm (20, p. 56.)

The grape rootrot fungus has been reported growing on the roots of various hosts such as Vitis, Malus, Pyrus, Cydonia, Prunus (almond and cherry), Salix, Tilia, Rosa, and Paliurus.

From a résumé of the literature, it would seem that the advocates of the parasitic nature of Roesleria as opposed to its saprophytic nature of growth are almost evenly divided. The following consider it parasitic: Lemonnier (19), d' Arbois de Jubainville (1), Hennings (16), Jolicoeur (22, p. 289), and Bayliss-Elliott and Grove (8). Gillot (14), von Thümen (33, p. 210–212), and Prillieux (25) believe it to be somewhat parasitic, while it is thought to be nonparasitic by Berkeley (2), Cooke (6), Laurent (18), Viala and Pacottet (35), Hartig (15, p. 83), and Verge (34). The results of the writer's experiments show that when ascospores are sown in wounds the fungus can establish itself in living roots.

CULTURES

Cultures were made on various media from ascospores from the fruiting bodies of Roesleria on apple roots (Pl. 1, A) collected in New York City in October, 1920, by Dr. Dodge. Plates of cleared corn-meal agar were

---

* 3 Dodge, B. O. A Root-Rot Disease of Apple Seedlings. (Title) in Amer. Assoc. Adv. Sci. Program. 71st meeting, p. 32. 1918. A number of French crab-apple seedlings, obtained through a nursery, had been grown in pots for two years, then set out in the garden. During this time the plants had been inoculated with the pear-blight organism, and had been attacked somewhat by wooly aphids in the garden. Early in November in 1917 it was noticed that several of the little trees were falling over, due to the fact that their root systems had been destroyed by some rot. By digging down in the soil a few inches, partially decayed pieces of roots were found bearing numbers of ascocarps of Roesleria. The greenish mycelium of the fungus was discovered at least a foot beneath the surface of the ground. Fruiting bodies of the fungus were found on roots of plants the remainder of the root systems of which appeared to be perfectly healthy.
inoculated on October 18, 1920, with ascospores from the fruiting heads. In two days numerous spores germinated. Growth was slow, the germ tubes having reached a length of not more than 20μ. Viala and Pacottet (35) described fairly accurately the method of germination, showing that a few spores became septate during germination but more of them were undivided. Single spores were marked in the plates, and when they had germinated each spore was transferred to a tube of slanted corn-meal agar. All of the writer's cultures discussed in this paper were derived by transfer from these single ascospore cultures. Eight single spore cultures were made and kept at room temperature until the mycelium had made good growth, and they were then placed in a refrigerator where the temperature averaged 10 to 12° C. This provided uniformly cool, moist and dark conditions for growth.

CORN-MEAL AGAR CULTURES

On the corn-meal agar the fungus grows slowly forming a thin layer of felty mycelium, white to buff at first, becoming green at the center, the green color gradually spreading over the surface. It becomes blackish green with age, sometimes taking on a grayish tinge. None of these first transfers fruited, but later transfers were made to the same medium, making in all 28, and at the end of five months stalked fruiting bodies were found on three cultures, one culture having three at the base of the agar. These were small and white or grayish white ascocarps with heads scarcely wider than the stalks. The heads contained asci and paraphyses which were slender and extended out beyond the asci.

OATMEAL PASTE AGAR CULTURES

Agar in which the nutrient medium is an oatmeal paste was found very satisfactory for the development of Roesleria. Forty-five transfers were made at different times from single ascospore cultures to tubes of this medium; 37 of these were kept at least part of the time in the refrigerator at 10° to 12° C. The mycelium spreads very slowly, forming a compact growth, cream or buff colored at first which becomes a bright malachite green, darkening with age. The growth is more felty, more luxuriant, and of a brighter green than that on the corn-meal agar. In from five to seven months ascocarps appeared in a large number of the tubes. They occurred singly or in groups of from 2 to 15 (Pl. 1, B, F, G). The stalks were white to grayish with mouse-gray heads. These fruiting bodies, 4 to 4½ mm. × 1 mm. were somewhat larger than those occurring in nature on the roots, the stalk being thicker and the heads larger, 3 mm. wide in the largest ones.

CULTURES ON APPLE ROOTS

Transfers were made from each of the single-spore cultures to autoclaved apple roots in large test tubes, with a few cubic centimeters of water in the bottom. These cultures were kept in the refrigerator at 10 to 12° C. The fungus grew well, covering the roots with a felty or fluffy mycelium, white to buff at first, later becoming bright malachite or fluorite green (Ridgway). In from 6 to 12 months, when the cultures had dried somewhat, fruit bodies containing mature asci appeared

singly or more often in groups (Pl. 1, C, E). As many as 14 ascocarps developed in one group. The larger ascocarps developed to maturity, but some of the smaller ones had not matured by the time the cultures dried.

**CULTURES ON RUBUS STEMS**

Four similar tubes containing blackberry stems with a few leaves were sterilized, inoculated, and treated in the same manner as previously described for apple roots. The mycelium which developed was light to dull green and felty but less luxuriant than on the apple roots. The cultures fruited sparingly, one having several small ascocarps on the midrib of a leaf, another with several fruiting in different stages of development at the base of the stem. Even the smallest ascocarps matured.

**CULTURES MADE FOR COMPARISON WITH THOSE OF VIALA AND PACOTTET**

In view of the fact that in the writer's cultures described above ascocarps were developed without the fungus producing any intermediate spore forms such as the conidia reported by Viala and Pacottet (35), she has endeavored to grow the fungus in such a way as to duplicate as nearly as possible the cultures described by them. The writer's methods were as follows: Two 2-liter flasks were filled to a depth of 6 or 7 cm. with kidney-bean juice with 1/10 per cent tartaric acid and 5 per cent sugar added. (The fungus did not grow on media made with 1 per cent tartaric acid as specified by Viala and Pacottet.) These were steam heated for three successive days. They were inoculated with bits of mycelium from single ascospore cultures. No. 1 was kept in the light at room temperature averaging 20 to 25°C, No. 2 was treated in the same way for five months, then put into a refrigerator with temperature at about 10 to 12°C. About a week after inoculation, one colony grew on the surface of each and within a day or two showed a greenish color. These colonies soon appeared warty, raised at the edges, and depressed in the center, which was yellowish green. Around the colonies the liquid was iridescent, forming a film. A month or so later the colonies appeared heavy and fell to the bottom. In flask No. 1 the colony grew considerably and formed numerous rounded protuberances covered with whitish mycelium, giving them a fluffy appearance. Some of the nodules were as large as 1 to 1 1/2 cm. in diameter. The main part of the colony was dark, with some brownish accretions or precipitate from the liquid. Several months later flask No. 2, which had been in the refrigerator for some time, showed 12 or 15 immersed colonies, while the other flask had only 3 or 4. Some had greenish to deep-green zones, while one or two appeared deep green and warty only at the center. None of the colonies adhered to the glass, as was the case in Viala and Pacottet's cultures, but all remained separate and free in the liquid. They were subspherical or hemispherical, but had no stalks such as they describe. Microscopically, all showed mycelium with numerous rounded subhyaline swellings, often occurring singly or in rows of 3 or 4. The mycelium was of a slightly yellowish or yellowish-brown tinge. Viala and Pacottet called these bodies chlamydosporic fruits, and the rounded swellings chlamydospores. It is a well-known fact that the mycelium of many species of fungi which grow for a long time in culture becomes abnormal, and the cells become misshapen so as to resemble chlamydospores. Viala and Pacottet did
not show that they function as spores in any way. While the writer did not find these bodies quite so regularly developed in long chains as described by these authors, there can be no doubt that they are identical.

One 2-liter flask of kidney-bean juice with 2 per cent agar and pieces of steamed grapevine added was autoclaved and inoculated with bits of mycelium from a single-spore culture. In a few days compact round colonies buff in color appeared. These increased in size and when about one-half inch in diameter were raised in the center, felty, and pale green with rim of white or buff. The green color deepened and brightened, and when the colonies were 5 cm. across they were deep grayish green with wide white or cream colored margins. The colonies coalesced with distinct lines of demarkation. Soon concentric rings appeared with the centers glaucous, green, and felty. The other rings were in order, buff color and white, the newest growth being white. The whole growth was superficial, and no fruiting bodies of any kind were ever formed.

Another 2-liter flask of kidney-bean juice containing 4 per cent agar with 0.1 per cent tartaric acid and 5 per cent sugar with pieces of steamed grape vine added was inoculated in the same way as the other flasks. This medium had a jelly-like consistency. Seven or eight compact felty buff-colored colonies appeared in less than two weeks, one showing a faint greenish-brown tinge. Two or three weeks later the colonies were quite irregular, felty, and greenish yellow, with wide white or buff colored margins, which soon coalesced. No fruiting bodies or chlamydospores were formed on this medium. Viala and Pacottet report that their cultures on similar media produced conidia.

INOCULATION OF GRAPE ROOTS

A number of roots of grape were inoculated in April, 1921, by placing mycelium in wounds made on the roots or by spraying them with ascospores produced in culture. A number of fruiting bodies were found eight months later on certain roots which had been sprayed with ascospores. These fruiting bodies were characteristic ascocarps, except that some of them had greenish stalks and heads, while some were buff colored with cinder-gray heads. They produced asci and ascospores of the usual sort.

DISCUSSION

The results of the culture experiments show that ascocarps were formed on all the media in tube cultures after four or five months, 37 cultures fruiting. None has been found in the large flasks of kidney-bean media, but these cultures comprised a much larger bulk and remained in a moist condition, and up to the time reported on were not dried down, as were the test-tube cultures. Ascocarps were developed abundantly in cultures from single ascospores and without any intermediate spore form such as Pilacre petersii or other imperfect stage. The best medium for the production of fruiting bodies was obtained by autoclaving apple roots in test tubes. These were large tubes giving a good supply of air, and when the culture medium had dried somewhat fruiting bodies formed in abundance. Rubus stems used in the same way proved much less satisfactory, while oatmeal-paste agar was second only to apple roots, and these cultures could be depended on to produce numerous ascocarps. A cool, humid atmosphere, such as obtains in the ordinary refrigerator, was apparently essential to the production of fruiting bodies. At room temperature in diffuse light only one culture was found with ascocarps.
Viala and Pacottet (35) report that they had no success whatever in germinating ascospores in culture. They used ascospores which germinated on heads developed from the old mycelium in roots kept several months in damp chambers. This method would open the way for contamination. The writer's cultures were all obtained from single ascospores germinated in Petri dishes and transferred each to a tube of nutrient agar; later cultures were obtained by transfer from these. Viala and Pacottet found no difference in the color of the mycelium on the different media used, all having shown a malachite green. The writer found this green color fairly constant except on the kidney-bean media to which tartaric acid and sugar were added. In this medium a slight green color was observed in the early stages of growth, but for the most part a greenish-yellow color obtained, noticeable particularly on the solid medium and to some extent in the liquid medium. The only yellow color mentioned by Viala and Pacottet was in connection with what they thought were conidiophores, which they noted were white to yellowish in the early stages, and their "chlamydomorphic fruits" which were yellow when young. The conidiophores described by them in some of their older cultures have not been observed in the writer's cultures at any time, although some of them are more than two years old. As for the chlamydomorphic fruits of Viala and Pacottet, structures with such appearances are often found in old cultures of fungi and have no significance.

SUMMARY AND CONCLUSIONS

Whether the name Pilacre should be applied to an ascomycete or not is impossible to say until the nature of Fries' specimen of \textit{P. weinmanni} is known (12). Without such an investigation, just as pointed out by Bayliss-Elliott and Grove (8), any attempt to settle definitely the question of priority with regard to the names which have been applied to the grape and apple rootrot fungus would be premature.

The Roesleria of von Thümen (32) is an ascomycete which can be grown easily in culture from ascospore to ascospore. The spores are sometimes septate on germination, producing one or two germ tubes. This fact has led some to suggest that it belongs in the family Geoglos-saceae. A fine septate felty mycelium is formed, which both in culture and frequently on the roots shows a characteristic malachite green. Ascocarps are formed in culture in the refrigerator, where the dark, cool conditions simulate the natural soil conditions where ascocarps mature in the fall of the year. Notwithstanding the fact that ascocarps were frequently formed only after the agar had dried out considerably, this dryness is clearly not a necessary condition, because fruit bodies were formed sometimes on the weft of hyphae floating on the water in the bottom of the test tubes containing sterilized roots which were used instead of an agar medium (Pl. 1, D). The strain from apple rootrot is evidently the same as that found on grape, since inoculations of grape roots with the strain from apple have resulted in the formation of similar ascocarps.

In discussing the relationship between Pilacre and Roesleria, Bayliss-Elliott and Grove (8) state:

Moreover it became evident that it would do no violence to the facts if it were concluded that \textit{Pilacre faginea} and \textit{P. petersii} were also identical with each other, and that both resembled the Roesleria so much in character as to make it seem not unlikely that Pilacre is only a stage of Roesleria.
And later:

The conclusion at which we have arrived is that Pilacre is a conidiophorous fungus, not in any sense a Basidiomycete, and that it is not in the remotest degree allied to the Auriculariae and Tremellineae, but is a stage of the Discomycetous genus Roesleria. This suggestion can no longer be entertained. No conidial stage has ever appeared in the life cycle of Roesleria which the writer has grown to maturity many times in single ascospore cultures. Conidia are produced freely in cultures of the Basidiomycete Pilacre petersii. Roesleria is hypogaeous, developing large quantities of ascospores which can not be discharged into the air in any way comparable to that prevailing in most other Ascomycetes, the ascii deliquesce allowing the spores to mass together in the head, which is at first covered with a peridium-like weft of hyphae. This is soon broken away by the crowding of the spores. Spore distribution is probably brought about by disturbances of the soil by insects, earthworms, or by cultivation.

It has been pointed out that there are three distinct fungi having fruit bodies which are more or less similar in appearance: One, a lichen growing on bark, Calicium (Coniocybe) pallidum; another, a Discomycete growing on roots, Roesleria hypogaea; and third, Pilacre petersii, the primitive Basidiomycete of Brefeld. If Pilacre is an ascogenous genus, P. petersii does not belong with it. Certainly R. hypogaea is not a lichen. There is no basis for considering either Coniocybe pallida or Pilacre petersii synonymous with Roesleria hypogaea.

LITERATURE CITED

(15) Hartig, Robert
xvi, 331 p., 159 fig. London, New York.

(16) Hennings, Paul

(17) Jolicoeur, Henri
Rheims.

(18) Laurent, Émile
xvi, 331 p., 159 fig. London, New York.

(19) Hennings, Paul

(20) Joucoeur, Henri
Rheims.

(21) Joucoeur, Henri
Rheims.

(22) Joucoeur, Henri
Rheims.

(23) Joucoeur, Henri
Rheims.

(24) Joucoeur, Henri
Rheims.

(25) Joucoeur, Henri
Rheims.

(26) Joucoeur, Henri
Rheims.

(27) Joucoeur, Henri
Rheims.

(28) Joucoeur, Henri
Rheims.

(29) Joucoeur, Henri
Rheims.

(30) Joucoeur, Henri
Rheims.

(31) Joucoeur, Henri
Rheims.

(32) Joucoeur, Henri
Rheims.

(33) Joucoeur, Henri
Rheims.

(34) Joucoeur, Henri
Rheims.

(35) Joucoeur, Henri
Rheims.

(36) Joucoeur, Henri
Rheims.

(37) Joucoeur, Henri
Rheims.
A.—Ascocarps of *Roesleria hypogaea*, collected October, 1920, on root of French crab seedling grown in the garden of Columbia University, New York City. The writer's cultures were obtained from ascospores from this specimen.

B.—Culture of *Roesleria* on oatmeal paste in tube showing ascocarps, 10 months after inoculation. Natural size.

C.—Apple root from tube culture showing numerous small ascocarps, 9 months old. Slightly magnified.

D.—Apple root in tube showing ascocarps produced on the hyphal weft formed on the water at the bottom. Culture scarcely 5 months old. Natural size.

E.—Apple root from tube culture showing a group of ascocarps somewhat older (9 months). The "peridium" in each has broken away. × 1.4.

F.—Oatmeal paste agar culture removed from the tube showing groups of large ascocarps. Culture a year old. × 1.4.

G.—Oatmeal paste agar culture removed from the tube showing ascocarps on upper part of slant. Culture 5 months old. Natural size.
Life History of Grape Rootrot Fungus

PLATE 1

Journal of Agricultural Research
Washington, D. C.