

## STUDIES ON PSEUDONECTRIA ROUSSELIANA

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### ABSTRACT

*Pseudonectria rousseliana* (Mont.) Seaver is fully described and the presence of a hypostroma connecting this fungus to the host is reported.

*Volutella buxi* (Cda.) Berk. is proved to be the imperfect stage of *Pseudonectria*. *Verticillium buxi* (Link) Auersw. is not a conidial stage of *Pseudonectria*. It is renamed *Paecilomyces buxi* (Link ex Fr.) comb. nov.

### INTRODUCTION

During my stay at the Phytopathological Laboratory in Baarn, I received in August 1962 a collection of *Buxus sempervirens* L. composed of living and dead plants. The material was collected by Dr. J. A. von Arx in Oberbuchsitzen (Switzerland), where *Buxus sempervirens* is a common forest shrub. It had been attacked by a number of fungi, which had to be identified.

On the living leaves were found *Puccinia buxi* DC. and *Mycosphaerella limbalis* (Pers.) v. Arx. The latter fungus is the cause of white, distinctly margined leafspots. On dead leaves and stems were observed stromata or fructifications of *Dothidea puccinioides* Fr., *Ceuthospora buxi* (Fr.) Petrak, *Macrophoma mirbelii* (Fr.) Berl. & Vogl. and *Dothiorella candollei* (Berk. & Br.) Petrak.

Two hypocreaceous fungi could be found only on dead leaves, viz. *Hyponectria buxi* (DC.) Sacc. with perithecia immersed in the leaf tissues and *Pseudonectria rousseliana* (Mont.) Seaver with fructifications growing superficially. The latter fungus was chosen for a thorough study and for pure culture experiments. On some leaves could also be found two imperfect fungi, which were determined as *Volutella buxi* (Corda) Berk. and *Verticillium buxi* (Link) Auersw. These two fungi were regarded by TULASNE (1865), FÜCKEL (1869), SACCARDO (1877) and others as the different conidial stages of *Pseudonectria rousseliana*. However, this supposition has never been proved by culture experiments.

The fungus first described by MONTAGNE (1856) as *Nectria rousseliana* Mont. was placed by FÜCKEL (1869) in the genus *Stigmatea* because of the superficial and isolated development of the perithecia on the leaves. SACCARDO (1877) created for it the new genus *Nectriella*. As a later homonym of *Nectriella* Nits., this name could not be maintained and was changed by SEAVER (1909) to *Pseudonectria*. CLEMENTS & SHEAR (1931), on the other hand, did not accept this name. They used as a genus name *Notarisiella* (Sacc.), a subgenus of *Nectriella* Sacc. VON ARX & MÜLLER (1954) rejected the view of Clements & Shear and

maintained the genus name *Pseudonectria* with *P. rousseliana* as the single species.

#### DESCRIPTION OF THE FUNGUS

The mycelium does not grow superficially except in a small area around the fructifications. Generally the germ tubes of the spores penetrate the leaf through the stomata. The intercellular immersed mycelium is composed of flexuous, septate, hyaline hyphae, the cells of which are 15–30  $\mu$  long and 2,5–4,5  $\mu$  broad. In the substomatal cavities the hyphae branch to form a parenchymatous mass of hyaline, elongated, vertically arranged cells. These "hypostromata" reach a diameter of 26–44  $\mu$ . The upper part forms small plugs of narrow and elongated cells, forcing apart the guard cells of the stomata and producing superficially both sporodochia and perithecia. This is the reason that these fructifications are spread uniformly over the lower surface of the leaf.

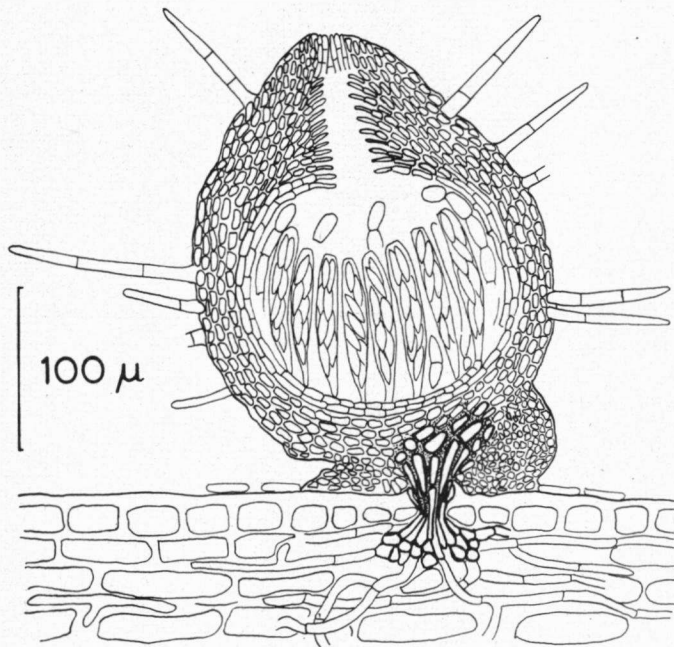


Fig. 1. *Pseudonectria rousseliana*, cross section of a perithecium on a hypostroma.

The perithecia are pear shaped, light orange to greenish, setose, 135–190  $\mu$  in diameter and 180–250  $\mu$  high. The conical beak of each perithecium is filled with hyaline, delicate periphyses; the wall is 18–25  $\mu$  thick and is composed of several layers of flattened, thick-walled cells, more or less hyaline and 7–19  $\mu$  long. The irregularly distributed setae are tapered towards their tips, septate, thick-walled,

hyaline, 50–110  $\mu$  long and 5–7  $\mu$  wide. The cylindrical clavate asci measure 50–70  $\times$  8–10  $\mu$ . Each has a thin membrane and contains 8 ascospores. There are no typical paraphyses, but the asci are surrounded by rows of rounded, very thin-walled, delicate cells, 9–14  $\mu$  long. The ascospores are fusoid, rounded at both ends, hyaline, thin-walled, one-celled, 11–17  $\mu$  long and 3–5  $\mu$  broad.

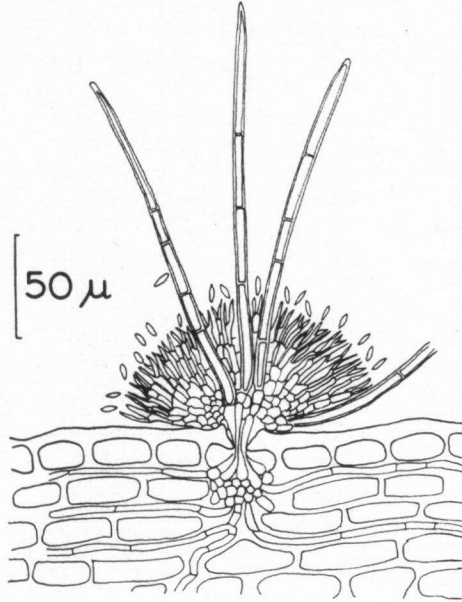


Fig. 2. *Pseudonectria rousseliana*, cross section of the *Volutella* conidial stage.

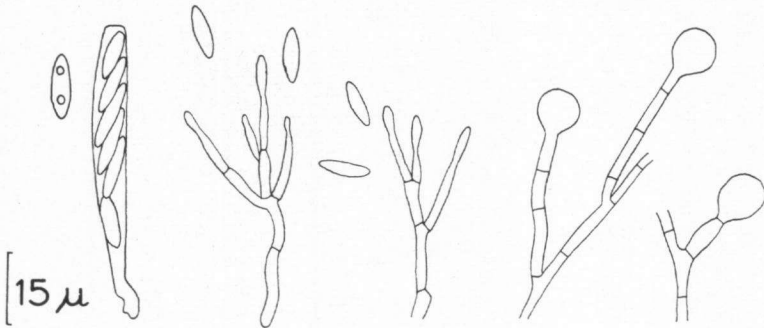


Fig. 3. *Pseudonectria rousseliana*, ascospore, ascus containing ascospores, conidiophores, conidia and chlamydospores, the last from a pure culture.

The sporodochia are small, more or less hemisphaerical, light reddish to pink and 60–110  $\mu$  in diameter. The setae look like the perithecial ones but can reach a length of 190  $\mu$ . The basal parts are composed of elongated or rounded cells. The conidiophores are flexuous, branched,

septate, 2,5–3,5  $\mu$  broad and 45–75  $\mu$  long. The conidia are formed singly at the phialide-like apices of the conidiophores; they are fusoid, hyaline, smooth, one-celled, 8–12  $\times$  2,5–3  $\mu$  and are united in a pinkish-red, slimy mass of spores.

A fungus fitting the description of *Verticillium buxi* could also be found on the material. It produces a superficial mycelium composed of hyaline, white or yellowish, septate, branched, 2–3  $\mu$  broad hyphae. The erect conidiophores are 65–110  $\mu$  long and 3–5  $\mu$  broad, branched, and verticillate above. The branches bear truncate, tapered phialides, 6–9  $\mu$  long and 2,5–4  $\mu$  broad. The phialospores contain two oil drops, are catenulate, elliptical to fusoid, hyaline, 5–8  $\mu$  long and 2–2,5  $\mu$  broad.

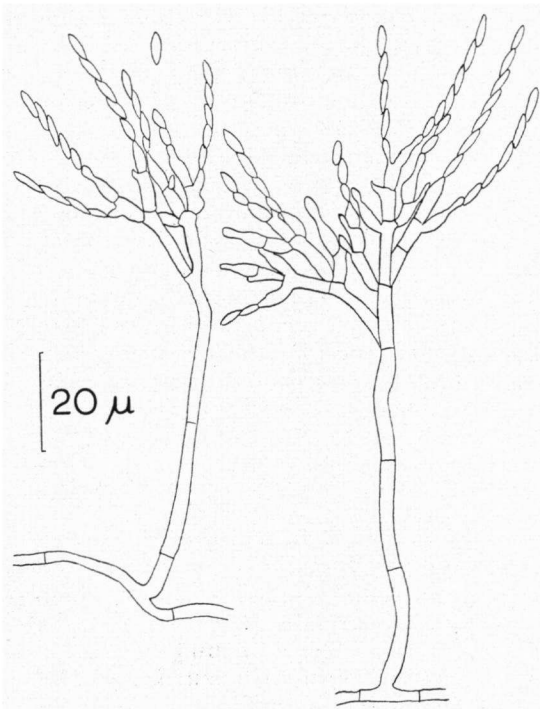


Fig. 4. *Paecilomyces buxi*, conidiophores and conidia as found on the host plant.

#### PURE CULTURE EXPERIMENTS

Cultures were obtained in different ways. To get pure cultures from ascospores, fresh, wet pieces of leaves bearing perithecia were fixed to the inner surfaces of covers of Petri dishes. The ejaculated ascospores fell onto the surface of the medium (potato-dextrose agar) and germinated. For the isolation of the conidial fungi, spore suspensions were made at different concentrations. These were seeded on potato-dextrose agar in Petri dishes.

The developing cultures were studied on oat-meal agar. The cultures growing from ascospores of *Pseudonectria rousseliana* agreed in all details with those obtained from conidia of *Volutella buxi*.

The mycelium grows immersed in the agar and produces scattered, superficial, small, white, rounded or irregular sporodochia. These soon change to a light orange color, when the slimy mass of the conidia is produced. The immersed mycelium is composed of abundantly-branched, septate, often anastomosed hyphae, 1,5–4,5  $\mu$  broad. Sporodochia, conidiophores and conidia look like those developed on leaves.

Principally at the margins of older cultures an aerial mycelium also developed. In it, the hyphae were more septate and there was a great number of chlamydospores. These were elliptic to globose, one-celled, hyaline, terminal, sometimes intercalary and 13–25  $\times$  13–22  $\mu$ .

On potato-dextrose agar the fungus grew very abundantly with an immersed and an aerial mycelium but no sporulation was observed.

*Verticillium buxi* also was obtained in pure culture. The colonies obtained from spore-suspensions developed very well on potato-dextrose agar.

The mycelium grows mostly immersed in the agar and produces conidiophores which sporulate very soon. On older cultures some aerial mycelium is also present. Both types of mycelium are composed of abundantly branched, septate, anastomosed hyphae, 1,5–4,5  $\mu$  broad. Chlamydospores were not observed. The conidia, which form in dry masses, give a pink coloration to the colonies.

Conidiophores, phialides and conidia have the same characteristics as those produced on leaves.

## CONCLUSIONS

It has been established by pure culture experiments that *Volutella buxi* is the conidial stage of *Pseudonectria rousseliana*. The fungus grows in *Buxus* leaves with an intercellular mycelium, which penetrates the stomata and produces superficially both perithecia and sporodochia. *Pseudonectria rousseliana* is a typical member of the Hypocreaceae, a family of the Sphaeriales, sensu MÜLLER & VON ARX (1962). The fungus has the following synonymy:

Perithecial stage: *Pseudonectria rousseliana* (Mont.) Seaver

Syn.: *Nectria rousseliana* Mont. — Syll. Crypt. 224 (1856)  
*Stigmatea rousseliana* Fuckel — Symb. Myc. 97 (1869)  
*Nectriella rousseliana* Sacc. — Michelia 1: 51 (1877)  
*Pseudonectria rousseliana* Seaver — Mycologia 1: 48 (1909)

Conidial stage: *Volutella buxi* (Cda.) Berk.-Outl. brit. Fungi 340 (1860)

Syn.: *Chaetostroma buxi* Cda. — Icon. fung. 2: 30 (1838)  
*Tubercularia buxi* DC. — Fl. gall. 6: 110 (1815)  
*Psilonia rosea* Fr. — Scler. Sueciae, 6, no. 220 (1821)

*Verticillium buxi*, on the other hand, does not participate in the life-cycle of *Pseudonectria rousseliana*. This fungus is not a typical member of the genus *Verticillium*, but it belongs to *Paecilomyces* Bainier, as this genus was characterized by BROWN & SMITH (1957). This fungus could not be identified as one of the species enumerated by these authors, especially because its phialides can produce phialospores also laterally. This character makes this species very distinct from the other species of *Paecilomyces*. However, this genus is the most suitable one to include this fungus. Therefore the following new combination is proposed:

PAECILOMYCES BUXI (Link ex Fr.) comb. nov.

Syn.: *Fusidium buxi* Link — Spec. Plant. 2: 97 (1825)

*Fusisporium buxi* Fr. — Syst. Mycol. 3: 447 (1832)

*Verticillium buxi* (Link) Auersw. — Hedwigia 6: 9 (1867)

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