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STUDIES IN THE DISCOMYCETE GENERA CRUMENULA DE NOT. AND CENANGIUM FR.

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CONTENTS

SUMMARY

Conifers, especially species of *Pinus*, may suffer from attack of the Discomycete genus *Crumenula*. In literature there exist many reports on the "Cenangium disease" of coniferous hosts, but it appears that the apothecia of *Cenangium ferruginosum* were often confused with the apothecia and even the pycnidia of *Crumenula*. *Cenangium ferruginosum*, however, is a common saprophyte. Representatives of both genera were thoroughly studied and an attempt was made to distinguish them even when immature. The asci and ascospores were studied, but special attention was given to the apothecial structure.

The study of the latter provided characteristic differences, on the

* The pathological part of the work was carried out by the senior author, the taxonomical part by the junior author.

basis of which it became possible to distinguish the species one from another. The necessity arose to transfer the species *Crumenula abietina* Lgbg. and *Crumenula laricina* Ettl. to the genus *Scleroderris* (Fr.) de Not.

Cultures of the fungi treated in the present paper were also studied, especially with the aim of investigating the connection between the ascigerous and the imperfect stages. Cultures were made of both ascospores and pycnospores. A conidial form belonging to *Crumenula sororia* Karst. was newly described as *Digitosporium piniphilum* Gremmen gen. nov. spec. nov. This form was found in nature and also cultivated in vitro from ascospores. Probably it is a new member of the family of the *Excipulaceae*. Inoculations were executed with a culture of the ascospores of *Crumenula sororia*, a mono-pycnosporic culture of *Scleroderris abietina* and a mono-ascosporic culture of *Cenangium ferruginosum*. Experiments with *Cr. sororia* were successful, whilst those with *Cenangium* always failed.

ACKNOWLEDGEMENTS

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PART I

a. Purpose and methods

Representatives of both genera *Crumenula* and *Cenangium* occur on coniferous hosts and some of these species cause serious losses. When there are mature fructifications it is possible to distinguish them by means of the sporological data, but when these are lacking, identification is hardly possible. More especially, confusion is likely to occur in case of the common fungus *Cenangium ferruginosum*, the apothecia of which strongly resemble the apothecia and even the imperfect stage of *Crumenula abietina*. Apothecia of the species involved were collected and hand-sectioned. The apothecia were particularly studied with the aim of finding structural differences, whereas measurements of asci and ascospores were compared with those in literature. Moreover, culture studies were executed in the laboratory in order to observe the growth of these Discomycetes and, more especially, to investigate whether the perfect form could be obtained. The following species were studied:

a. *Crumenula pinicola* (Fr.) Karst. sensu Karst.
b. *Crumenula sororia* Karst.

c. *Scleroderris abietina* (Lgbg.) Gremmen comb. nov.
d. *Scleroderris laricina* (Ettl.) Gremmen comb. nov.
e. *Cenangium ferruginosum* Fr. ex Fr.
f. *Cenangium acicolum* (Fuck.) Rehm

b. On the taxonomy of the genus Crumenula de Not.

Review of literature

Crumenula de Not. was established by DE NOTARIS in 1863, probably based on a rediscovery of material of *Cenangium urceolus* A. et S., which he had collected on *Calluna vulgaris* Hull. NANNFELDT (16), however writes "Ob die Art de Notaris" die echte *urceolus* ist, erscheint höchst unsicher". In 1869 KARSTEN used the name *Crumenula* for a subgenus of *Peziza* (Mon. Pez. Fenn.), but in 1871 he brought it to generic rank (Myc. Fenn.) in the subfamily of the *Cenangieae*. Once more, in 1885, KARSTEN changed the name *Crumenula* to *Godronia* (Rev. Mon. p. 144), with the exclusion of *Cr. pinicola* and *Cr. sororia* which he placed in the subgenus *Heteropeziza* Fr. In1896, REHM (18) again used *Crumenula* de Not. for the above mentioned species, *Cr. pinicola* and *Cr. sororia*, placing this genus in the *Dermateaceae* sub *Cenangieae*, together with the genera *Cenangium*, *Cenangella*, *Tryblidiella* and *Godronia*. NANNFELDT (16), in 1932, studying the genus *Crumenula*, described as a new subfamily the *Scleroderridoideae*, including *Crumenula*, *Godronia* and *Durandia* in the genus *Scleroderris* (Fr.) de Not. According to this author, there is no marked difference in the apothecial structure and he also asserts the congeniality between the imperfect stages. The type species of *Scleroderris* (Fr.) de Not. is *Scleroderris ribis* (Fr.) Lind. ETTLINGER (8) pointed out the contradictions in the arguments of NANNFELDT. The former did not observe relations with *Scleroderris*, but considered his four species of *Crumenula* a distinct group. In 1951, SEAVER (20) again transferred *Cr. pinicola* and *Cr. sororia* to the genus *Godronia* in the sense of KARSTEN (Rev. Mon. 1885).

New taxonomical conceptions on the genus Crumenula

In the studies by NANNFELDT (16) concerning the identity of *Crumenula* and *Scleroderris*, the taxonomy of the inoperculate Discomycetes is based on the apothecial structure. Up till now the taxonomy of the Discomycetes was mainly based on sporological data. Modern mycology should try to find a natural system based on apothecial structure, culture studies and sporology, perhaps in future combined with genetical studies.

The species *Cr. pinicola* (Fr.) Karst. sensu Karst. and *Cr. sororia* Karst. in the sense of REHM show great similarity in apothecial structure. Both possess a characteristic tissue, the textura intricata, as well as marginal hairs. However, inter se they differ to such an extent that it is perfectly justified to keep them separate specifically. Quite different from these two species are *Cr. abietina* Lgbg. and *Cr. laricina* Ettl. which are characterized by a textura prismatica whereas their apothecial structure shows a strong resemblance to that of

Scleroderris ribis (Fr.) Lind., the type species of *Scleroderris* (Fr.) de Not. Instead of marginal hairs we find some marginal scales. The ascospores are 2 — or 4 — celled, shorter than in *Scleroderris ribis* (Fr.) Lind. (Fig. 1), but not needle-shaped as in species of *Godronia* Moug. sensu REHM.

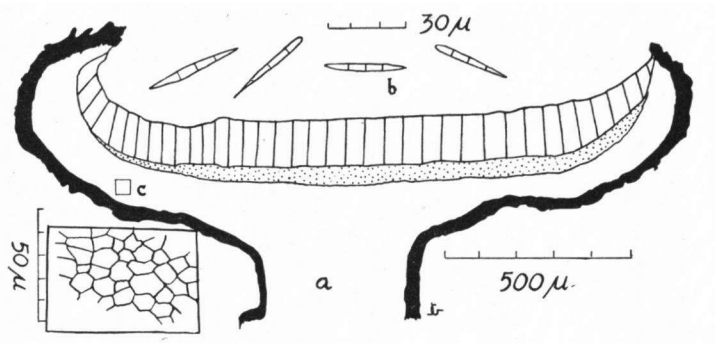

Fig. 1. *Scleroderris ribis* (Fr.) Lind. a. apothecium (partly schematic); b. ascospores; c. part of the tissue of the excipulum

Though ETTLINGER (8) proposed to conserve the name *Crumenula* sensu REHM (non *Crumenula* de Not.), in this paper, pending the conservation, alterations are not made in the name of the genus.

Up till now, the correct name of *Crumenula* sensu REHM is not known, since the junior author has not seen the type species of *Godronia* Moug., viz. *Godronia Mühlenbeckii* Moug. et Lév. and since there is no description of this species with regard to its apothecial structure. Therefore the species *Cr. abietina* and *Cr. laricina* are transferred to the genus *Scleroderris* (Fr.) de Not. on account of their apothecial structure. The species *Cr. pinicola* and *Cr. sororia* have no *Scleroderris*-characters and should provisionally remain in *Crumenula* sensu REHM.

c. On the taxonomy of the genus Cenangium Fr.

Review of literature

The name *Cenangium* was first used by FRIES (Syst. Myc. p. 177) in 1822 and again by KARSTEN in his Mycologia Fennica (p. 18 and p. 220).

REHM (18), who considered the *Cenangieae* to comprise several genera, divided *Cenangium* in the subgenera *Eucenangium* and *Encoelia*. Afterwards, most authors have accepted the same arrangement with some slight alterations, though the differences between both genera are extremely vague and difficult to define on a morphological basis.

VON HÖHNEL (12) asserted that *C. ferruginosum* Fr. ex Fr. is quite different from species of *Encoelia*. He classified this species in the *Tryblidiaceae* on account of its coriaceous structure.

Cenangium acicolum (Fuck.) REHM was placed by VON HÖHNEL in the genus *Encoelia*. This was radically altered by NANNFELDT (16), who founded the *Encoelioideae* in the *Helotiaceae* with the type genus *Encoelia* (Fr.) Karst. This subfamily is closely related to the *Ciborioideae* as

conceived by NANNFELDT on the basis of the apothecial structure. The same author was not able to classify the genus *Cenangium* Fr., so that in this case the taxonomy of REHM is still in use. The type species of this genus is *C. ferruginosum* Fr. ex Fr., as selected by NANNFELDT.

New taxonomical conceptions on the genus Cenangium Fr.

Because of the insufficient differences between *Cenangium* Fr. and *Encoelia* (Fr.)Karst. as to their apothecial characters, some authors use the former name, others the latter. The correct name, however, is *Cenangium* Fr., since it is the earlier name.

The junior author did not observe any characteristic difference which could serve to clearly separate both genera and therefore the fungi were studied in pure culture to see whether imperfect stages might give any clue. Always black spermogonia with bacilliform, hyaline spermatia were obtained in cultures of *Cenangium ferruginosum* Fr. ex Fr., whereas slimy sporodochia were found in the mycelia of *Encoelia fascicularis* (Fr.) Karst. These sporodochia formed globular microconidia of the same type as were also detected by DRAYTON in the genus *Sclerotinia* c.s. (DRAYTON, 1934).

The conclusions of NANNFELDT (16) on the relationship between *Encoelia* and *Ciboria* are thus confirmed by culture studies. The growth of both mycelia is so different as to safely assume that they do not belong to the same genus. In vitro, species of *Encoelia* grow rather quickly, those of *Cenangium*, however, develop very slowly. Also the colour of the mycelia is quite different. For the time being, therefore, it seems best to apply the name *Encoelia* (Fr.) Karst. to quickly growing species with slimy groups of microconidia and *Cenangium* Fr. to slowly growing fungi.

PART II

Description of the fungi in relation to taxonomy and phytopathology

a. **Crumenula pinicola** (Fr.) Karst. sensu Karst., Myc. Fenn. I. p. 210: 1871.

Description (after 118)

Apothecia about 1.5–2 mm across. Stipe short, 150–190 μ. The excipulum consists of two layers with a reddish brown colour (textura intricata). The first layer has a compact tissue of 30–40 μ; the second layer a tissue with large cavities, 80–120 μ thick. The excipulum-wall, 70–100 μ thick, is also made up of a compact structure which further externally becomes quite opaque, bearing cell-protuberances on the outside. At the margo red-brown hair-like excrescences are found. Hypothecium 10–15 μ thick, colourless. Hymenium about 70 μ thick, beige-coloured. Epithecium wanting. Asci 75 $\times$ 9–11 μ. Ascospores 17.1–30.4 $\times$ 3.8–4.8 μ, colourless, 1- and 2-celled, markedly acuminate. Paraphyses filiform. (Fig. 2).

Culture results

Many isolates were obtained from ascospores which had naturally been ejaculated. The colour of the mycelium strongly resembled that of *Cr. sororia* Karst. varying from grey to greyish brown on cherry decoction agar. Many cultures were studied on different media, but

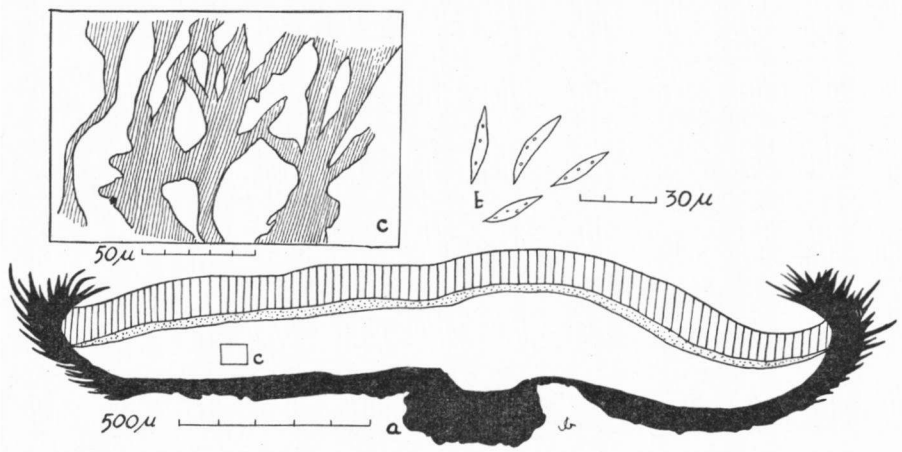

Fig. 2. *Crumenula pinicola* (Fr.) Karst. sensu Karst. a. apothecium (partly schematic); b. ascospores; c. part of the tissue of the excipulum

the junior author failed to find an imperfect stage of this Discomycete. On sterilized wheat-grains mixed with small bits of branches of *Pinus*, dark brown bodies were seen to develop after about 4 months culturing, probably being the primordia of the apothecia.

Remarks

It seems impossible nowadays to get a clear idea of what *Peziza pinicola* really is. According to NYLANDER (cit. ETTLINGER), *Peziza pinicola* Rebent. (1804) is *Pseudographis pinicola* (Nyl.) Rehm.

FRIES, in his Syst. Myc. II. p. 113: 1823, described *Peziza pinicola* as follows "obovatus, extus fibrosa-striata, rugosa, fusconigricans, margine inflexo-fimbriata, disco expallente" and cited his exsiccatum no. 162. His description does not exclude the identity with *Crumenula pinicola* (Fr.) Karst., but only a renewed study of the Fries's exsiccatum may solve this problem. KARSTEN, afterwards, has given a clear diagnose of *Crumenula pinicola* (exs. no 726 in Fungi Fennici). The citation of the latter author only, as was done by ETTLINGER is incorrect; FRIES must be cited between brackets.

BOUDRU (2) recorded damage due to this species to young corsican pine in Belgium. He succeeded in finding the apothecia on the infested branches. An imperfect form was never detected, which is confirmed by JØRGENSEN (13) and KUJALA (14).

In the Netherlands, VOORBEYTEL CANNENBURGH (21) recorded a serious injury of the stems of corsican pine, caused by a *Crumenula* species which he regarded as hitherto undescribed. No herbarium

specimen was left, but from his description of the fungus we have no doubt that he had found *Crumenula pinicola*.

It was supposed that this species has a saprophytical life. Sometimes, however, canker-like wounds were noticed on the stems and branches of *Pinus sylvestris* L. which were covered with numerous apothecia of this fungus. The same phenomenon was observed in corsican and austrian pine. In many cases wounds were seen to form a considerable amount of resin. Investigations on the pathology of this species were started, but up till now no proof of its parasitism could be given.

This Discomycete was collected from the following localities on *P. nigra* Arn. var. *austriaca* Asch. et Gr., *P. nigra* Arn. var. *corsicana* Schn. and on *P. sylvestris* L. The apothecia had not only developed on the bark of the stems, but in great numbers on the dead branches as well.

Specimens examined

Gelderland:

Ede, Ginkel, on P. sylvestris, 16-VI-1951, Gremmen 116
Loenermark, on P. nigra austr., 26-VI-1951, Gremmen 118
Loenermark, on P. sylvestris, 26-VI-1951, Gremmen 117
Nieuw-Soerel, on P. nigra cors., 16-IV-1942, v. Vloten 522
Vorden, Kieftenkamp, on P. sylvestris, 16-VII-1951, Gremmen 112
Wageningen, Oostereng, on P. sylvestris, 25-VI-1951, Gremmen 109

Utrecht:

Rhenen, Koerheuvel, on P. sylvestris, 16-VI-1951, Gremmen 96.

b. **Crumenula sororia** Karst., Myc. Fenn. I. p. 211: 1871.

Description (after 119)

Apothecia about 1–2.5 mm across. Stipe 300–600 μ. The excipulum consists of a compact tissue, yellow-green coloured and about 40–60 μ thick (textura intricata). The excipulum-wall has a dense tissue of

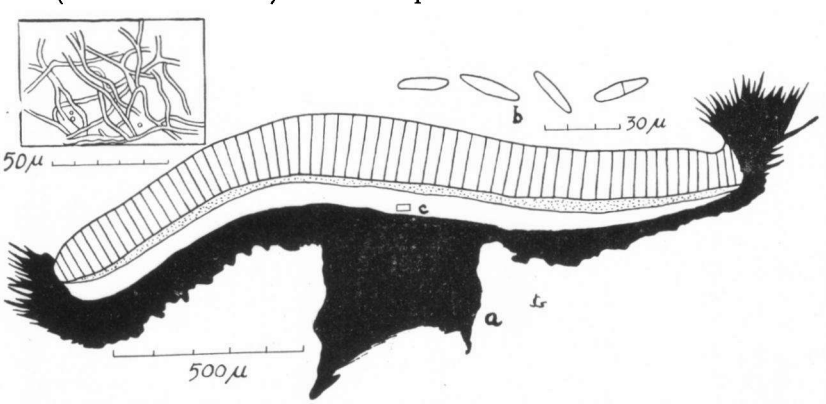

Fig. 3 *Crumenula sororia* Karst. a. apothecium (partly schematic); b. ascospores; c. part of the tissue of the excipulum

60–90 μ, becoming opaque externally and on the outside showing irregular cell-protuberances which towards the margo pass into hair-like projections up to 100 μ long. Hypothecium about 25–35 μ, yellow-green coloured. Hymenium about 130 μ thick, yellow-green. Epithecium wanting. Asci 86–114 × 11 μ. Ascospores 12.9–30.1 × 5.7 μ, colourless, 1-, 2-, 3- and 4-celled, ellipsoidal. Paraphyses filiform (Fig. 3).

Pycnidial Stage

This Discomycete species has a most characteristic imperfect stage which was often found and seems related to the genus *Sirothecium* Karst. (Symb. Myc. 20. p. 105).

The pycnidia are more or less globular, growing in groups or isolated on the substratum. They are black and measure 0.4 to 0.7 mm. The wall of the pycnidium is dark brown and consists of roundish, thick-walled cells with a diameter of 3.5 to 8.0 μ. A characteristic porus is lacking, the pycnidia open by rupturing of the cellwalls. On account of these features, this conidial stage may belong to the *Excipulaceae.*

The conidia (Fig. 4) are subhyaline or slightly green, mostly consisting of a system of many-celled branches. The main branch has 7 to 9 cells, mostly carrying 1 to 4 secondary branches, each of which consists of 1 to 5 cells. Sometimes a tertiary branch may be observed.

Each cell has rather thick walls and measures 5.5–5.7 × 3.5–3.8 μ. The main branch varies from 30 to 50 μ in length and is terminated by an acuminate cell. The conidium which strongly resembles the fingers of a hand is as a whole shed from a subhyaline tissue which forms the inner wall of the pycnidium.

Since this stage of the Discomycete *Crumenula sororia* Karst. seems to be undescribed and, in fact, as we never found such characteristic pycnospores in any of the existing genera of the *Excipulaceae*, the stage is considered to represent a new genus which the junior author proposes to name *Digitosporium.* The type species is *D. piniphilum* which was found on branches of *Pinus sylvestris* L. The type is deposited in the junior author's herbarium, the iso-type in the Leiden Herbarium.

Digitosporium piniphilum Gremmen gen. nov. spec. nov.

Ad familiam Excipulacearum pertinens. Corticola.

Pycnidia superficialia, atra, solitaria vel caespitosa, orbicularia vel oblongata, astomata, 0.4–0.7 mm in diam.

Corticis pycnidiorum cellulae, subglobosae, brunneo-atrae, 3.5–8.0 μ in diam.

Conidia magna, hyalina vel subhyalina, cellulis pluribus, digitiformibus interdum ramosa, 30–50 × 3.5–3.8 μ.

Stadium perfectum: Crumenula sororia Karst. Myc. Fenn. I. p. 211: 1871.

Habitat: In cortice ramorum *Pini sylvestris.*

Typus: In Herbario J. Gremmen, 6-XII-1952, ex Herbario Fungi Fennici, leg. V. Kujala, sub *Crumenula sororia* Karst., pycnidia cum conidiis digitatis, N. Helsinki, Kulosaari, in *Pino sylvestre*, in ramis vivis, 14-IV-1944.

Other specimen examined (from the Netherlands)

Gelderland: Nunspeet, Willemsbosch, on P. nigra austr., 10-III-1949, Gremmen 631.

Culture results

Cultures were obtained from ascospores. The colour of the mycelium was greyish, while the growth resulted in the formation of globular colonies on cherry decoction agar. Isolates obtained from pycnospores were similar to those from ascospores. After 2½ months black pycnidia with typical pycnospores were developed. After aging these cultures also showed abundant greyish aerial mycelium, whilst a black pigmentation was diffused into the agar.

Remarks

REHM (18), primarily kept *Cr. sororia* Karst. and *Cr. pinicola* (Fr.) Karst. separated, but united them again in 1912.

GUYOT (11), in 1934, also assumed the existence of only one species, on the grounds that he had collected an intermediate form. There are, however, sufficient reasons to consider *Cr. pinicola* and *Cr. sororia* as good species, viz. the structure of the excipulum, the form of the ascospores and moreover the imperfect stage in *Crumenula sororia* Karst.

With regard to the pathology of *Cr. sororia* Karst., the senior author already in 1933 proved beyond doubt its parasitism, and found the characteristic imperfect stage at the same time.

FERDINANDSEN & JØRGENSEN (9) again reported on its parasitism, and also mentioned a pycnidial stage with dactyloid spores.

KUJALA (14) found this Discomycete to be very common in Finland, where it causes wounds and a formation of resin on the infested wounds. According to this author the conidial stage is much more common than the perfect form.

In the Netherlands, this Discomycete is always found on resinous cankers, occurring on stems and branches of *P. nigra* Arn. var. *austriaca* Asch. et Gr., on *P. nigra* Arn. var. *corsicana* Schn. and on *P. sylvestris* L.

Specimens examined

Gelderland:

Loenermark, on P. sylvestris, 6-III-1951, Gremmen 119
Nieuw-Soerel, on P. nigra cors., 16-IV-1942, v. Vloten 523
Nunspeet, on P. nigra austr., 10-III-1949, v. Vloten 111.

c. **Scleroderris abietina** (Lgbg.) Gremmen comb. nov.

syn. *Crumenula abietina* Lgbg., in Svenska Skogsvårdsfören. Tidskr. p. 9–44. 1913.

Description: (after 521)

Apothecia about 1 mm across. Stipe short, about 200 μ. The excipulum consists of polygonal, dark brown, thick-walled cells, 6–15 μ in diameter (textura prismatica). At the base this tissue is

70 μ, tapering towards the margo into the opaque excipulum-wall. On the outside the excipulum bears irregular cell-protuberances. At the margo there are scale-like projections, instead of hairs. Hypothecium 20 μ thick, colourless. Hymenium 60–70 μ thick, subhyaline.

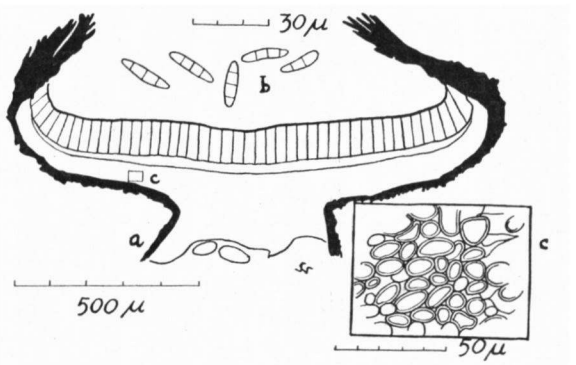

Fig. 5. *Scleroderris abietina* (Lgbg.) Gremmen. a. apothecium (partly schematic); b. ascospores; c. part of the tissue of the excipulum

Epithecium well-developed, 20–30 μ thick, brown. Asci 117.8–121.6 × 11.4 μ. Ascospores 15.2–16.1 × 3.8–4.7 μ, colourless, typically 4-celled, ellipsoidal. Paraphyses filiform (Fig. 5).

Pycnidial Stage

A pycnidial stage was always found in great numbers. This imperfect stage, *Brunchorstia pinea* (Karst.) v. Höhn. (confr. Fragmente zur Mykologie, p. 142: 1915) has dark brown pycnidia with falcate spores. These pycnospores ooze out in delicate whitish to creamy tendrils and measure 26.0–47.7 μ. Out of 100 spores, 15 were 1-celled, 17 were 2-celled, 66 were 3-celled and 2 were 4-celled.

Culture results

In many cases *Cr. abietina* was isolated from the diseased twigs. Cultures obtained from ascospores produced characteristic pycnidia with pycnospores. The mycelium was distinctly coloured and varied from green via yellow-green to greyish green.

Remarks

In 1926 a damage on corsican pine was reported in the Netherlands (Frederiksoord). Some years afterwards, in 1930, once again serious injury was observed in austrian and corsican pine ("de Delle" and Nieuw-Soerel). In 1944 a number of papers published by BOUDRU (3, 4, 5) appeared in Belgium dealing with this fungus. In one of these papers (5) the author mentioned 55 coniferous hosts of this Discomycete, most of which proved to be new. Curiously enough, he also described about 16 species of hardwoods (4) on which he found the fungus. In his opinion the species would be very common and certainly of saprophytical nature. These informations strongly suggest an error

on the part of the author, since hitherto *Crumenula abietina* is only known to occur in species of the genera *Picea* and *Pinus*. Fortunately, part of the material identified by BOUDRU was still available in the Forest Institute at Groenendael in Belgium for renewed study. Without exception the apothecia of the material on conifers proved to belong to *Pezicula livida* (B. et Br.) REHM, whereas those on hardwood were *Pezicula cinnamomea* (Fr.) Sacc. In none of these cases apothecia of *Crumenula abietina* Lgbg. could be detected, which was confirmed on re-examination of the material. We were almost certain that BOUDRU did not know *Cr. abietina*, which may account for the great number of new hosts recorded for this fungus. As far as we know, both *Pezicula*-species mentioned are saprophytes indeed.

ETTLINGER (8) studied a die-back of *Pinus* -species in Switzerland which was caused by *Cr. abietina* and KUJALA (14) described a serious attack by the same Discomycete on *Pinus Murrayana* Balf. in Finland.

In 1949 this fungus was again found in the Netherlands on *Pinus nigra* Arn. var. *austriaca* Asch. et Gr., where it occurred on the dying twigs. The trees showed the characteristic symptoms of the disease.

Specimen examined

Gelderland: Nunspeet, Willemsbosch, on P. nigra aust., 27-IV-1952, v. Vloten 521.

d. **Scleroderris laricina** (Ettl.) Gremmen comb. nov.
syn. *Crumenula laricina* Ettl., Diss. E. T. H., Zürich, 1945.

Description (After ETTLINGER, specimen collected on 18-VIII-1940)

Apothecia about 1 mm across. Stipe very short or lacking. The excipulum consists of a textura prismatica, 40–60 μ which is made up of more or less roundish, dark brown cells measuring 4–7 μ; it passes into the opaque excipulum-wall which bears slight cell protuberances.

Hypothecium 22–38 μ thick, hyaline. Hymenium 60–80 μ thick, hyaline.

Epithecium wanting. Asci 63–118 × 5–9 μ (cit. ETTLINGER). Ascospores 10–17 × 3–4 μ, colourless, 2-celled, ellipsoidal (cit. ETTLINGER). Paraphyses filiform (cit. ETTLINGER). (Fig. 6)

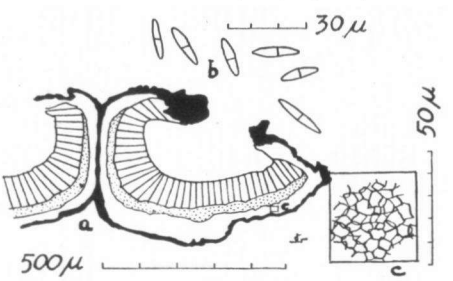

Fig. 6. *Scleroderris laricina* (Ettl.) Gremmen. a. apothecia (partly schematic); b. ascospores; c. part of the tissue of the excipulum

A characteristic imperfect form, *Brunchorstia laricina* Ettl. belongs to this fungus.

Culture results

Inoculation tests performed by ETTLINGER in Switzerland did not succeed. However, it was possible to isolate the fungus from fresh, diseased material.

Remarks

This species which was found in Switzerland (Kt. Graubünden and Kt. Bern) by ETTLINGER on branches of *Larix decidua* Mill. seems to be known from that country only.

Apothecia of this Discomycete were studied from the collections received from the "E.T.H." at Zürich.

e. **Cenangium ferruginosum** Fr. ex Fr., Syst. Myc. 2 (1). p. 187: 1822. syn. *Cenangium ferruginosum* Fr., Vet. Akad. Handl. p. 361: 1818.

Description (After 67)

Apothecia about 1–2 mm across, leathery. Stipe wanting. The excipulum consists of a loosely woven, hyaline tissue (textura intricata). At the base this tissue measures up to 400 μ, gradually tapering towards the margo (40 μ). Excipulum-wall dark brown, consisting of roundish to angular cells, 20–100 μ thick. Further externally this wall is opaque and occasionally shows some cell protuberances on the outside. Hypothecium 35–75 μ thick, yellow-brown. Hymenium 75–100 μ thick, colourless. Epithecium wanting. Asci 80 × 14–15 μ. Ascospores 12–13 × 5–6 μ, colourless, 1-celled, ovate. Paraphyses filiform, somewhat clavate at the apex, septate. (Fig. 7)

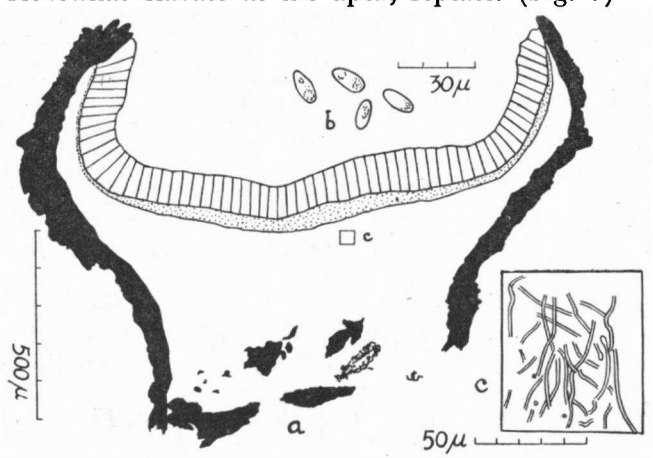

Fig. 7. *Cenangium ferruginosum* Fr. ex Fr. a. apothecium (partly schematic) b. ascospores; c. part of the tissue of the excipulum

Culture results

This fungus grew very slowly in vitro and had a green to yellowish green colour. After some time spermogonia with bacilliform spermatia

were formed, whereas under certain conditions it was possible to obtain mature apothecia in culture (GREMMEN, 1952).

Remarks

WEIR (22) successfully inoculated *P. ponderosa* Laws. with the ascospores of this fungus. It is possible that his spore suspension was contaminated with spores of *Brunchorstia pinea*, for this fungus was also observed on his trees in the field. It should be noted that at that time *B. pinea* was still considered the imperfect stage of *Cenangium ferruginosum*.

LIESE, in an oral communication, told the senior author that he always found the pycnidia of *B. pinea* on dying branches, whereas apothecia of *Cenangium* were never found. He, too thought *Brunchorstia* to be the imperfect form of *Cenangium* and therefore only used the name of the perfect stage, which is common practise when this stage is known. LIESE considered the occurrence of *Cenangium* due to previous damage by some *Cecidomyia*.

BAXTER (1) reported a damage caused by this fungus on exotics. FERDINANDSEN & JØRGENSEN (9) doubted the parasitic nature of this fungus, showing that in most cases *Brunchorstia* was found on the branches. BOYCE (6), however, considered the fungus a parasite, but rarely ascribed damage to this Discomycete. Sometimes this fungus caused the so-called "pruning disease". KUJALA (14) regarded it a saprophyte with a slightly parasitic nature.

From our inoculation experiment, described below, it appeared that *C. ferruginosum* is a genuine saprophyte. Consequently, the die-back of *Pinus* in the above mentioned cases must be ascribed to *Scl. abietina*. (confr. Part III).

Usually this Discomycete is found on dead twigs of several species of the genus *Pinus*. The fructifications occur in small groups and when in dry condition resemble those of *Crumenula*.

Specimens examined

Gelderland:

Ede, Sliemer, on P. sylvestris, 28-IV-1947, Gremmen 23
Nunspeet, Willemsbosch, on P. nigra aust., 10-VIII-1948, v. Vloten 105
Otterloo, on P. sylvestris, 3-V-1951, Gremmen 4
Wageningen, Dorschkamp, on P. montana, 31-V-1948, Gremmen 67
Wageningen, Oostereng, on P. sylvestris, 25-VI-1951, Gremmen 5

f. **Cenangium acicolum** (Fuck.) Rehm, Krypt. Fl. p. 228: 1896.

Description (after 7)

Apothecia about 1–2 mm across. Stipe almost lacking. The excipulum consists of a hyaline textura intricata (a loosely interwoven tissue), at the base measuring up to 270 μ, at the margo about 40 μ thick. The excipulum-wall has dark brown roundish cells and is about 20–60 μ thick. Hypothecium about 40 μ thick, yellowish brown.

Fig. 8. Method of inoculation with pure culture; no. 2 has succeeded

PLATE VIII

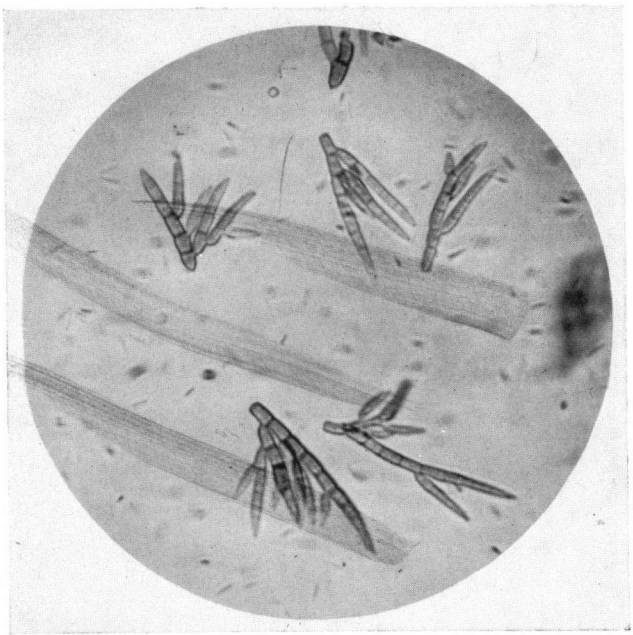

Fig. 4. *Digitosporium piniphilum* Gremmen gen. nov. spec. nov. Pycnospores and microconidia in a four months old culture

Hymenium about 80 μ thick, colourless. Epithecium wanting. Asci 70–96 × 7.5–11 μ. Ascospores 11.5–19 × 4–4.5 μ, colourless, 1-celled, sometimes 2-celled, acuminate, with 1 or 2 guttulae. Paraphyses with clavate apex.

Culture results

The growth of this fungus was very slow. On potato-dextrose agar the colour of the mycelium changed from white to cream, sometimes with greenish patches. After aging it turned via greyish blue to greyish white. Characteristic blackish brown ramifications were sometimes observed in the medium. On sterilized wheat-grains there was often a tendency of the mycelium to develop a yellowish colour. Neither spermogonia, nor imperfect stages were obtained in vitro, though this fungus was cultured for a long period.

Remarks

This species is a genuine saprophyte, occurring on dead needles of several species of *Pinus* and sometimes also on the twigs between the needles.

FUCKEL (Symb. Myc. p. 269: 1871–1875) considered this fungus a variety of *C. ferruginosum* Fr. ex Fr.

VON HÖHNEL (12) called this fungus *Encoelia acicolum*, but the whole structure of the apothecium shows a relationship with that of *C. ferruginosum*.

Specimens examined

Gelderland:

Nunspeet, Willemsbosch, on P. nigra austr., 28-VI-1948, Gremmen 7
Wageningen, Dorschkamp, on P. sylvestris, 19-X-1949, Gremmen 6

PART III

Investigations on the pathology of the fungi mentioned

The pathology of the species mentioned in Part II does not seem to have been studied in great detail. FERDINANDSEN & JØRGENSEN (p. 199: 1938) infected the tips of just opened shoots of *P. nigra* and *P. sylvestris* during June and July with conidia or mycelium of *Scleroderris abietina*. In the next year characteristic symptoms of the disease were observed.

THOMAS & TERRIER (cit. ETTLINGER) successfully executed bud and wound inoculations, from which they obtained the fungus *Scleroderris abietina* by means of re-isolation.

As regards *C. ferruginosum*, there is a short report by WEIR (22).

The senior author especially studied *Cr. sororia*, *Scl. abietina* and *C. ferruginosum* with regard to their pathology. For this investigation 21 trees from 6 to 7 years old were used, 7 of which were scots pine, 7 corsican pine and 7 austrian pine. Of each tree four equally developed

branches aged, 2, 3, 4 and 5 years were selected and wounded. The wounds were inoculated and labelled 1 to 4. In order to compare the results the four different inoculations were executed on one tree (fig.8). Before wounding, the part of the branch to be inoculated was cleaned with cotton-wool soaked in ethyl alcohol. Thereupon an incision was made with a flamed scalpel through the bark into the wood. The wounds were closed with sterile cotton-wool and paper of gutta-percha and the dressing tied with adhesive tape and raffia.

The execution of the inoculations was as follows:

1. With sterile cherry decoction agar.
2. With a pure culture of *Cr. sororia* on cherry decoction agar of about 4 months old, which had been isolated from 6–8 ascospores from apothecia occurring on *P. sylvestris*.
3. With a pure culture of *Scl. abietina* on cherry decoction agar of about 5 months old, which was isolated from one pycnospore of a pycnidium occurring on *P. nigra* var. *austriaca*.
4. With a pure culture of *C. ferruginosum* on cherry decoction agar of about 3 months old. This culture was isolated from one ascospore of an apothecium occurring on *P. sylvestris*.

The branches which had been inoculated in October were checked for results in December the following year.

TABLE I

Hostplant	Sterile agar	Cr. sororia	Scl. abietina	C. ferruginosum
P. sylv.	6 inoc. 0 inf.	6 inoc. 6 inf.	6 inoc. 0 inf.	7 inoc. 0 inf.
P.n.aust.	4 inoc. 0 inf.	7 inoc. 5 (+ 1) inf.	6 inoc. 0 inf.	6 inoc. 0 inf.
P.n.cors.	3 inoc. 0 inf.	7 inoc. 4 (+ 2) inf.	7 inoc. 3 (+ 2) inf.	6 inoc. 0 inf.

All wounds inoculated with sterile agar were negative. In some cases, however, a small resin flow was observed.

Inoculations executed with a pure culture of *Cr. sororia* showed a serious injury. In 56 to 100 percent of the wounds, small cankers had been formed, most of which showed pycnidia.

Inoculations with the fungus *Scl. abietina* only succeeded in *P. nigra* var. *corsicana*, where 42 to 70 percent of the trees were infected. In a number of these infections pycnidia were found on the dead needles.

Inoculations on *P. nigra* var. *austriaca* and on *P. sylvestris* always failed.

Wounds treated with a pure culture of *C. ferruginosum* always showed a negative result.

References

1. Baxter, D. V., Development and succession of forest fungi and diseases in forest plantations. Mich. Univ. School Forestry and Conserv. Circ. 1 (1937).

2. BOUDRU, M., La crise du Pin Laricio de Corse en Belgique. Trav. Station de Recherch. de Groenendael. Sér. C., no 8 (1944).
3. BOUDRU, M., Contributions à la biologie de Crumenula abietina Lagerberg. Trav. Station de Recherch. de Groenendael. Sér. C., no 7 (1944).
4. BOUDRU, M., Crumenula abietina Lagerberg sur les essences feuillues. Trav. Station de Recherch. de Groenendael. Sér. C. no 9 et 10 (1944).
5. BOUDRU, M., Note sur la fréquence en Belgique de Crumenula abietina et note sur la découverte, en Belgique, de Crumenula abietina Lagerberg. Trav. Station de Recherch. de Groenendael. Sér. C., no 4 et 6 (1944).
6. BOYCE, J. S., Forest Pathology. New York, (1948).
7. DRAYTON, F. L., The sexual mechanism of Sclerotinia gladioli. Mycologia, vol. 26: 1. (1934).
8. ETTLINGER, L., Ueber die Gattung Crumenula sensu Rehm mit besonderer Berücksichtigung des Crumenula-Triebsterbens der Pinus-Arten. Diss., E.T.H., Zürich, (1945).
9. FERDINANDSEN, C. and C. A. JØRGENSEN, Skovtraeernes Sygdomme. København, (1938–1939).
10. GREMMEN, J., A preliminary study on the culture of Discomycetes, especially the perfect stage. Anthonie van Leeuwenhoek. Vol. 18: 2. (1952).
11. GUYOT, A. L., Note sur une maladie chancreuse du Pin sylvestre dans le Nord de la France. Rev. Path. Vég. et Ent. Agric. 21 (1934).
12. HÖHNEL, FR. von, Fragmente zur Mykologie. Mitt. 25. Sitzber. Akad. Wiss. Wien. Math.-Naturw. Kl. Abt. I. Bd. 132, Heft 4–6. (1924).
13. JØRGENSEN, C. A., Mykologiske Notitser. 3–10. (1930).
14. Kujala, V., Ueber die Kleinpilze der Koniferen in Finnland. Comm. Institut. Forest. Fenn. (1950).
15. LIESE, J., Zum Triebsterben der Kiefer. Deut. Forstwirt. 17 (1935).
16. NANNFELDT, J. A., Studien ueber die Morphologie und Systematik der nicht-lichenisierten inoperculaten Discomyceten. Nova Acta Reg. Soc. Scient. Ups. Ser. 4, vol. 8, no 2, (1932).
17. REBENTISCH, J. F., Prodromus Florae Neomarchicae. Berlin, 1804.
18. REHM, H., Hysteriaceen und Discomyceten in Rabenhorst' Kryptogamenflora. Abt. III: Ascomyceten. Leipzig, (1896).
19. REHM, H., Zur Kenntnis der Discomyceten Deutschlands, Deutsch-Oesterreichs und der Schweiz. Ber. Bayr. Bot. Ges. 13 (1912).
20. SEAVER, F. J., The North American Cup-Fungi (Inoperculates). New York, (1951).
21. VOORBEYTEL CANNENBURGH, W., Een ernstige ziekte in Corsicaansche den. Nederl. Boschbouwtijdschr. p. 242 (1942).
22. WEIR, J. A., Note on Cenangium abietis (Pers.) Rehm on Pinus ponderosa Laws. Phytopathology, vol. 11 (1921).