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Studies in Heterobasidiomycetes. Part 24[1]). On *Ustilago violacea* (Pers.) Rouss. from *Saponaria officinalis* L.

By

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With 16 figures

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Within the genus *Ustilago* the anther smuts of Caryophyllaceae are a small natural group of parasites. *U. violacea* s.l. is characterized by the infection of species of a single host family, the formation of sori in the anthers of their hosts, and the reticulate surface ornamentation of the teliospores. Liro (1924) splits *Ustilago violacea*, based on field observations under natural conditions and extensive infection experiments into 11 different species. While Ciferri (1938) and Savulescu (1957) largely agree with the opinion of Liro (1924), Zundel (1953) recognized only *U. violacea* and *U. major*. Savile (1953) included all of the anther smuts of Caryophyllaceae in *U. violacea*; he maintained, however, the varieties *violacea, major,* and *stellariae*. Brandenburger and Schwinn (1971) described *Ustilago violaceo-irregularis* and Durrieu (1972) proposed *U. gaussenii,* which mainly differ from *U. violacea* by an echinulate surface ornamentation of the teliospores. Durrieu and Zambettakis (1973), taking into consideration the ultrastructure of the spore ornamentations, divide the anther smuts of Caryophyllaceae into 5 species.

Prior to the examination of *Ustilago violacea* s.l., we want to characterize the anther smut of Caryophyllaceae from its type host *Saponaria officinalis* in the present investigation.

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[1]) Part 23: Metzler, B.: Basidiosporenkeimung und Infektionsvorgang beim Birnengitterrost. Phytopath. Z. 103, 126—138 (1982).

Material and Methods

Ustilago violacea on *Saponaria officinalis* was collected several times in the area of Tübingen.

For light microscopy, living and infected hosts as well as herbarium specimen were used. Spore formation was studied in young anthers before the teliospores became mature. Anthers were incubated in 1 drop of 1 N KOH for about 15 minutes. Then potassium hydroxide was removed by rinsing several times with water. Preparations were squashed carefully in a 1 : 10 glycerine-water solution (V/V), to separate hyphae from the host cells without destroying sporogenous hyphae. The slides were then examined by phase contrast microscopy.

Germination of the teliospores was studied on water agar or a malt extract-yeast extract-peptone (MYP) medium (BANDONI 1972) using a slide culture technique (VAN UDEN 1951). These preparations were also used for isolating pure cultures of *Ustilago violacea*. The cultures were then characterized by standard yeast identification tests (VAN DER WALT 1970) and enzymatic activities on solid media (HANKIN and ANAGNOSTAKIS 1975). For comparison a strain of *Ustilago hordei* was tested too.

Details of the nuclear behavior in spore formation and germination were investigated by HCl/Giemsa staining. For the study of spore formation whole anthers were air dried for at least one week and then fixed in a 3 : 1 mixture of 92 % ethanol and acetic acid (V/V) for three days. The investigations on germinating teliospores were done with slide preparations as described for the germination experiments. Only here slides were dried at 40 °C for 4 hours, followed by fixation for 12 to 15 hours in the same solvent as described above. After rinsing several times with water for a total of 30 minutes, both preparations were: a) hydrolysed in 60 °C 1 N HCl for 6 minutes, b) rinsed in one change of water and 5 changes of phosphate buffer, pH 7, for a total of 30 minutes, c) stained 2 hours in 1 part Giemsa stock solution and 9 parts phosphate buffer, d) rinsed with phosphate buffer, dipped in water, and e) dried again. For light microscopy the germinated teliospores were embedded in synthetic resin and mounted with a cover-glass. The stained and air dried anthers were transferred into a drop of synthetic resin, broken up with needles, mounted with a cover-glass, and squashed carefully to separate hyphae from the host cells.

For scanning electron microscopy (SEM), separated anthers were fixed with 2 % glutaraldehyde and 1 % osmium tetroxide, washed with distilled water, dehydrated in an alcohol series, followed by critical point drying according to SAUTER's procedure (1977). Prior to examination in a Cambridge Stereoscan S 4-10, anthers were fixed on a specimen holder, broken, and coated with gold-palladium.

Evaluation of the teliospores were made with a Leitz A.S.M. System Socos using the Uni-3/M respectively ** SSP **/P statistic program.

Observations and Interpretations

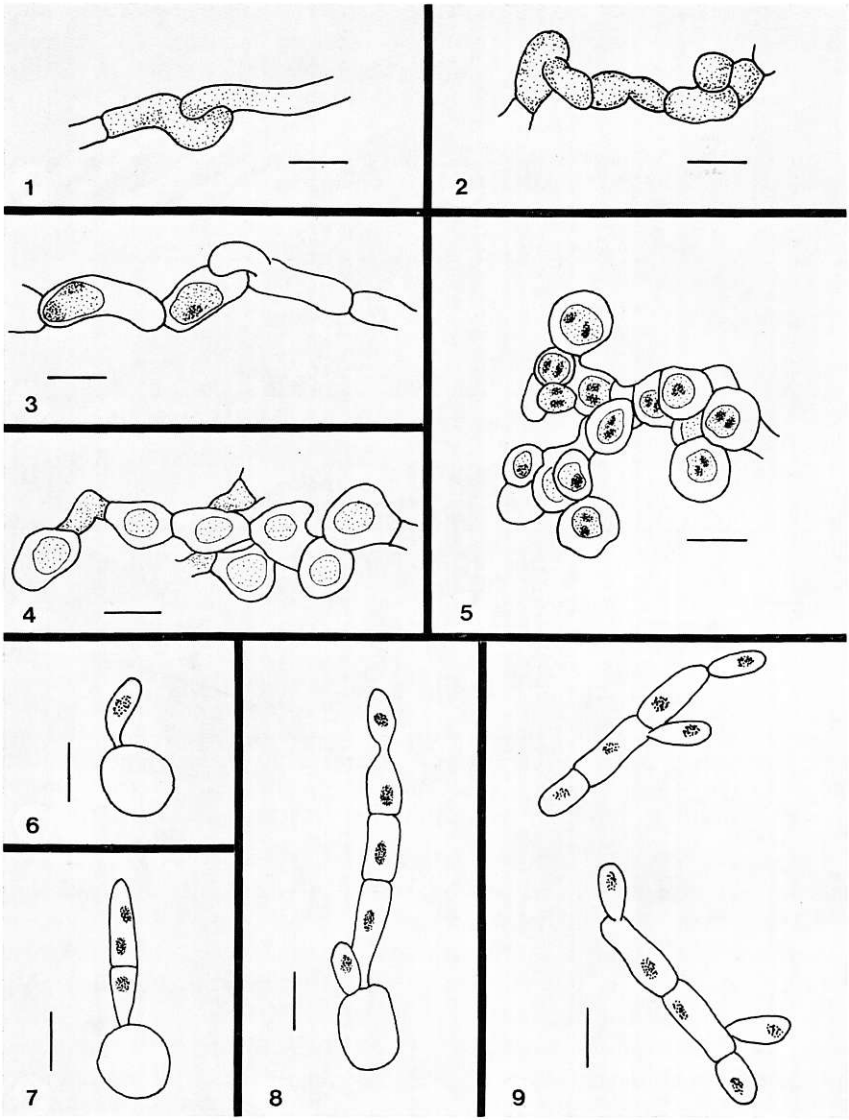
Sori

Ustilago violacea produces the sori in the anthers of its host. Usually all of the flower buds are infected by the parasite. Mature teliospores are already visible in early ontogentic stages of host, when the flowers are still closed. In the blossoms anthers are opened, and the teliospores spread out over the flowers. Often germinated teliospores can be found in the sori.

Spore formation

Within young sori there are still few non-sporogenous hyphae. They are regularly septate and clamps are rarely present (Figs. 1 and 2). The cells are more or less straight (Fig. 13). In the beginning of spore formation the cells become more rounded (Figs. 3 and 14). At that stage the cell wall of sporo-

genous hyphae starts to gelatinize, and simultaneously the cytoplasm within the cells seems to be contracted and becomes more spherical (Fig. 4). As a result, many spore initials are formed in large clusters. Then the volume of the spore initials increases and the surface structure of the teliospores becomes



Figs. 1—9. Formation of the teliospores: Figs. 1 and 2. Non-sporogenous hyphae in very young anthers. • Figs. 3 and 4. Early stages of sporogenous hyphae. • Fig. 5. HCl/Giemsa stained preparations of sporogenous hyphae; during gelatinazation of the cell walls, the developing teliospores are dikaryotic. • Germination of the teliospores: Fig. 6. The nucleus migrates into the developing promycelium. • Fig. 7. A second nuclear division only occurs in the apically cell. • Fig. 8. A three-celled promycelium produces the basidiospores. • Fig. 9. Promycelia separated from the producing teliospores. Standard for all figures is 5 μm

visible (Fig. 10). Until this stage HCl/Giemsa staining shows the dikaryotic stage of the cells (Fig. 5). When the young spores have almost increased to their final size, nuclear division takes place. Scanning electron microscopy shows only remnants of the gelatinized hyphal coat (Fig. 15). Most of the monokaryotic teliospores are globose and 6.7 × 6.1 µm in diameter, but some of them are occasionally angular. Table 1 shows the results of the teliospore measurements; they base on at least 100 separate measurements each. The surface of the teliospores has a reticulate ornamentation (Fig. 16, Table 2).

Table 1
Teliospore measurements*) of the anther smuts from *Saponaria officinalis*

	Collection		
	758	933	1052
Length	5.38—7.60	5.12—7.65	5.10—8.21
Breadth	5.15—7.12	4.90—7.45	4.76—7.95
Average	6.54×6.20	6.50×6.13	6.71×6.25

*) The measurements base on 100 teliospores each; unit of length = µm.

Table 2
Evaluation of teliospore ornamentation*)

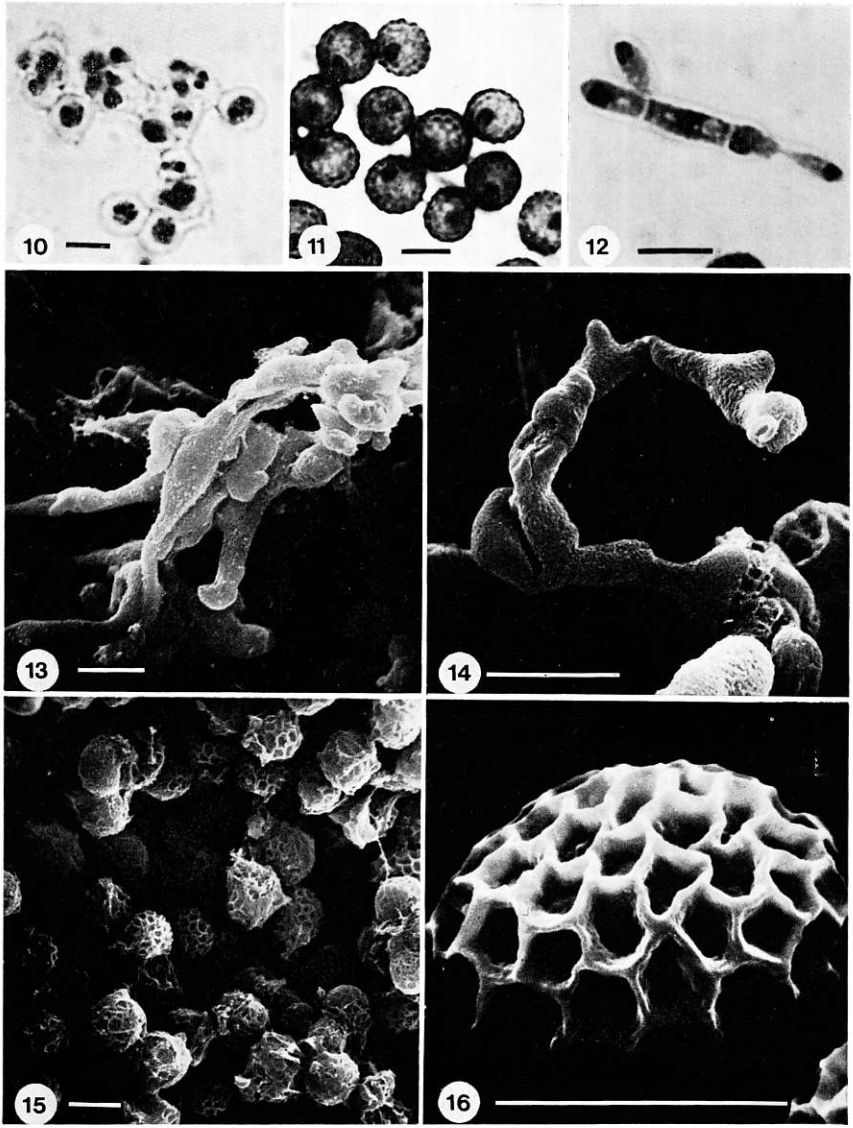
	Collection	
	933	1045
Width of meshes	0.88	0.79
Height of ridges	0.34	0.31
Diameter of ridges	0.17	0.15

*) The data are the average of 3 measurements on 30 teliospores each; unit of length = µm.

Germination

The teliospores of *Ustilago violacea* germinated well without resting period on both water agar and MYP-medium. 8—12 hours after inoculation upto 90% of the spores had germinated. Figures 6—9 show some stages of the germination using HCl/Giemsa stained preparations to show the nuclear behavior. Firstly all of the teliospores are monokaryotic (Fig. 11). About 4 hours after inoculation the promycelium breaks through the spore wall, the one nucleus is then visible in the promycelium (Fig. 6). After nuclear division the promycelium becomes septate. A second nuclear division occurs only in the apically cell (Fig. 7). After septation of this cell development of the three-celled promycelium of *Ustilago violacea* is finished (Fig. 8). Now basidiospores are produced from the basidium. After a more or less simultaneous mitotic nuclear division, one of the nuclei in each cell migrates into the spore, and the second remains in the promycelium. In many cases the basidium is now separated from the teliospores (Figs. 9 and 12). While the primary basidio-

spores are released from the basidium, by repetition several more basidiospores can be produced by each basidial cell. The basidiospores propagate by budding. Pure cultures were isolated from the budding cells. All of the strains were monokaryotic.



Figs. 10—16. Micrographs of HCl/Giemsa stained preparations: Fig. 10. Developing teliospores are dikaryotic. • Fig. 11. Mature monokaryotic teliospores. • Fig. 12. The cells of the promycelium as well as the basidiospores are monokaryotic. • SEM micrographs of teliospores development: Figs. 13 and 14. Young sporogenous hyphae; the cells are somewhat rounded. • Fig. 15. Almost mature teliospores; remnants of the hyphal coat are on their surface. • Fig. 16. Mature teliospore shows reticulate ornamentation of the teliospore. Standard for all figures is 5 μm

Characterisation of the cultures

The description of *Ustilago violacea* is based on 7 strains of three collections.

Growth in malt extract at 22 °C:

After three days the cells are elipsoidal (2.5 × 4.5)—(3.5 × 6) µm, single, in pairs, or few-celled clusters; a ring or a film are not present; there is a light sediment.

After one month no pellicle is formed; there is a light ring and a sediment.

Growth on malt extract at 22 °C:

After 7 days the culture is cream coloured to yellowish, smooth, and shiny. Cell morphology is similar to that in malt extract.

Dalmau plate culture at 22 °C:

Neither pseudohyphae nor true mycelium are formed.

No kind of spores are produced.

Reactions:

Fermentation: negative.

Assimilation of carbon compounds, as follows:

Glucose	+	D-Xylose	—	Mannitol	+
Galactose	+	L-Arabinose	+	Melizitose	—
L-Sorbose	—	D-Arabinose	+	Inulin	—
Sucrose	+	D-Ribose	+	Soluble starch	—
Maltose	—	L-Rhamnose	+	α-Methyl-D-glucoside	+
Cellobiose	+	Ethanol	—	Salicin	+
Trehalose	+	Glycerol	+	DL-Lactic acid	+
Lactose	+	Erythritol	+	Succinic acid	+
Melibiose	—	Ribitol	+	Citric acid	—
Raffinose	—	Galacitol	—	Inositol	—

Splitting of arbutin: positive.

Assimilation of nitrogen compounds:

Nitrate	+	Nitrite	—	Ethyl amine hydrochloride	+

Growth in vitamin-free medium: positive.

Growth on 50 % (W/W) glucose yeast extract agar: negative.

Growth at 27 °C: positive; at 30 °C: negative.

Starch formation: negative.

Hydrolysis of urea: positive.

Enzyme production on solid media:

Table 3 shows the results of the tests for enzymatic activity on solid media.

Interpretation

The spore initials of *Ustilago violacea* from *Saponaria officinalis* are formed singly inside the sporogenous hyphae. During the maturation of the

Table 3
Enzymatic activities on solid media

Enzyme	Collection			
	anther smuts			*U. hordei*
	933	1045	1052	935
Amylase	—	—	—	—
DNAase	—	—	—	+
Lipase	+	+	+	+
Polygalacturonase	—	—	—	—
Pectatlyase	—	—	—	—
Phosphatase	+	+	+	+
Protease	+	+	+	+
RNAase	—	—	—	+
Urease	+	+	+	+

teliospores the hyphal coat gelatinizes and the spore surface ornamentations develop. This is in agreement with the results of HUTCHINS and LUTMAN (1938) for *U. maydis*, LANGDON and FULLERTON (1975) for some cereals smuts, AUDRAN and BATCHO (1980) for *Ustilago violacea* from *Silene dioica*, TRIONE (1980) for *U. scitaminea*, and DEML, NEBEL and OBERWINKLER (1981) for *U. pustulata* and *U. scabiosae*. It can be assumed, therefore, that the general features of teliospore formation of *Ustilago* species from different host plants are similar. We cannot decide the taxonomic value of this character for the smut fungi in general, because in *Entorrhiza casparayana* quite another type of teliospore formation was shown by DEML and OBERWINKLER (1981). In contrast to the morphological similarity the nuclear behavior during ontogeny seems to be different within the genus *Ustilago* (TRIONE 1980). In some *Ustilago* species, such as *U. striiformis*, and *U. scitaminea*, the ontogeny of the parasites takes place mononucleate and presumably diploid (TRIONE 1980). In *Ustilago violacea*, however, up to almost mature spores the cells are dikaryotic. This result agrees with the findings of HARPER (1900). If there are correlations of taxonomical range between host-parasite interaction, site of the sori, the surface ornamentation of the teliospores and nuclear behavior within the genus *Ustilago* must be proven by detailed studies on both, ontogeny and karyology of related species.

The germination of *Ustilago violacea* is characterized by formation of a three-celled basidium, which is usually separated from the producing teliospore. This is in agreement with the results of BREFELD (1883). The type of germination separates *U. violacea* from many other *Ustilago* species, for which four-celled basidia have been reported. In addition nuclear behavior during the germination of *U. violacea* teliospores is different from other *Ustilago* species.

The anther smuts of Caryophyllaceae, originally placed in *Uredo* by PERSOON (1801) and transfered to *Ustilago* by ROUSSEL (1806), do not belong

within this genus. They are distinguished from the typical *Ustilago* species of Poaceae, (1) by infection of hosts of a single family only, Caryophyllaceae, (2) formation of sori in the anthers, (3) reticulate surface ornamentation of the teliospores, which in a few cases can be reduced to an echinulate structure, (4) three-celled promycelium, which (5) commonly separates from the producing teliospore, and (6) the dikaryotic ontogeny within the host. These morphological differences were supported (7) by the results of a screening for siderophore formation (DEML and OBERWINKLER 1980, 1982). If the production of siderophores takes place in low-iron cultured smut fungi, all of the anther smuts of Caryophyllaceae form rhodotorulic acid, *Ustilago* species from other host plants, however, ferrichrome A and/or ferrichrome. There are also differences in the relative electrophoretic mobilities of (8) aminopetidase (BRADFORD, JONES and GARBER 1975) and (9) urease (BAIRD and GARBER 1978). BLANK and DEKKER (1975) have shown RNAse activity in cultures of some *Ustilago* species from Poaceae. In the single *Ustilago violacea* strain they used, RNAase activity was not detectable. This is in agreement with our results on enzymatic activities on solid media.

The anther smuts of Caryophyllaceae, on the mode of teliospore formation and the septate promycelium belong to Ustilaginaceae. Because of the criteria listed above, they can, however, no longer remain in the genus *Ustilago*.

Reputed synonyms of *Ustilago violacea*

Various authors have placed the anther smuts of Caryophyllaceae in *Uredo* Persoon, *Farinaria* Sowerby, *Caeoma* Link, *Erysibe* Theophrastus ex Wallroth, and *Microbotryum* Léveilllé.

The genera *Uredo, Caeoma,* and *Erysibe* are typified by species of the Uredinales; they are therefore not available for smut fungi. SOWERBY (1803) created the genus *Farinaria* with 15 species. One of these, shown in Table 396, Figure 1, is *Farinaria stellariae,* on *Stellaria graminea.* The first species, however, which he named in Table 360 was *Farinaria seminaria,* from willow leaves. In 1832 FRIES placed *Farinaria seminaria* in synonymy with *Oidium erysiphoides.* Therefore AINSWORTH's statement (1963) "Farinaria = Ustilago sensu Fries 1832" cannot be accepted, and *Farinaria* cannot be used as generic name for smut fungi.

The genus *Microbotryum,* based on *Microbotyrum antherarum* Léveillé, was validly published. Although no host plant is indicated the original description of *M. antherarum* contains nothing that is inconsistent with being the same smut as that known as *Ustilago antherarum* Fries. Because FRIES (1832) indicated (1) no species of a host plant but only Caryophyllaceae, and (2) *Uredo violacea* Persoon as a synonym of his *Ustilago antherarum,* the first of PERSOON's host plants, *Saponaria officinalis* must be the type host of the genus *Microbotyrum.*

Our detailed studies on the anther smut of *Saponaria officinalis* show, that this smut has characteristics very different from those typical for *Ustilago* species from Poaceae.

Microbotryum Léveillé, Ann. Sci. Nat. Bot. III, 8: 372, 1847.

Sori in the anthers, soon rupturing and spreading out a powdery, dusty violet coloured mass of spores; spores pale lilac to almost hyaline, single, globose, spherical, occasionally somewhat irregular; surface ornamentation reticulate, rarely echinulate, then warts partly connected with ridges, thus sometimes reticulation is indicated; germination with a two to three-celled promycelium, with commonly separates during basidiospore formation.

Type species: *Microbotyrum antherarum* Lév.
Basionym: *Ustilago antherarum* Fries

Microbotryum violaceum (Pers.) G. Deml & Oberw.
Basionym: *Uredo violacea* Pers., Syn. Meth. Fungi 225, 1801
Synonyms: *Ustilago violacea* (Pers.) Rouss., Fl. Calavados ed. 2. 47, 1806
Uredo antherarum DC., Fl. Fr. 6: 79, 1815
Caeoma violaceum Nees von Essenbeck, Syst. Pilze 1: 14, 1817
Caeoma violacea Martius, Fl. Crypt. Erl. 315, 1817
Caeoma antherarum Schlechtendal, Fl. Ber. 2: 130, 1824
Ustilago antherarum Fries, Syst. Myc. 3: 518, 1832
Microbotryum antherarum Lév. Ann. Sci. Nat. Bot. III 8: 372, 1847
Erysibe antherarum Wallroth, Fl. Crypt. Germ. 2: 217, 1933
Ustilago violacea Fuckel, Jahrb. Nassau. Ver. Naturk. 15: 21, 1861
Ustilago dianthorum Liro, Ann. Acad. Sci. Fenn. A 17: 35, 1924
Ustilago superba Liro, Ann. Acad. Sci. Fenn. A 17: 37, 1924
Ustilago coronariae Liro, Ann. Acad. Sci. Fenn. A 17: 38, 1924
Ustilago silenes-nutantis Liro, Ann. Acad. Sci. Fenn. A 17: 43, 1924

Sori in the anthers, soon rupturing and spreading out a powdery and dusty violet coloured mass of spores; spores pale lilac to almost hyaline, singly, globose, spherical, occasionally somewhat irregular; surface ornamentation reticulate; 6.7—6.1 μm in diameter or 5.4—7.6 × 5.1—7.1 μm; ridges 0.42 μm high and 0.32 μm thick.

Type host: *Saponaria officinalis* L.

Specimens examined: On *Saponaria officinalis,* Germany, Baden-Württemberg, Steinkirchen, leg. M. Nebel, 1978 (Herb. GD 758); Germany, Baden-Württemberg, Unterjesingen, leg. G. Deml, 1980 (Herb. GD 933); Germany, Baden-Württemberg, Unterjesingen, leg. G. Deml, 1981 (Herb. GD 1045); Germany, Baden-Württemberg, Tübingen, K. Brellochs & G. Deml, 1981 (Herb. GD 1052)

New combinations

Microbotryum lynchis-dioicae (DC.) G. Deml. & Oberw.
Basionym: *Ustilago lychnis-dioicae* (DC.) Liro, Ann. Acad. Fenn. Sci. A 17: 33, 1924

Microbotryum major (Schroeter) G. Deml & Oberw.
Basionym: *Ustilago major* Schroeter, Kr. Fl. Schles. I: 273, 1887
Synonym: *Ustilago clintoniana* Cif., Ann. Myc. 26: 64, 1928

Microbotryum silenes-inflatae (DC.) G. Deml. & Oberw.

Basionym: *Ustilago silenes-inflatae* (DC.) Liro, Ann. Acad. Fenn. Sci. A 17: 32, 1924

Synonym: *Uredo antherarum β silenes-inflatae* DC., Fl. franc. VI: 791, 1815

Microbotryum stellariae (Sowerby) G. Deml & Oberw.

Basionym: *Farinaria stellariae* Sowerby, Engl. Fungi, Pl. 396, fig. 1, 1803

Synonym: *Ustilago stellariae* (Sowerby) Liro, Ann. Acad. Fenn. Sci. A 17: 39, 1924

Microbotryum violaceo-irregulare (Brandenburger & Schwinn) G. Deml & Oberw.

Basionym: *Ustilago violaceo-irregularis* Brandenburger & Schwinn, Nova Hedw. 22: 279, 1971

Summary

Sporogenesis, germination, karyology, and cultural characteristics of *Ustilago violacea* (Pers.) Rouss. from *Saponaria officinalis* have been investigated. In sporogenous hyphae the spore initals are produced by condensation of the cytoplasm, followed by gelatinization of the cell wall. Developing teliospores are held together in clusters by the gelatinous matrix. HCl/Giemsa staining shows the dikaryotic stage of the cells during ontogeny up to almost mature teliospores; mature they are monokaryotic. The germination takes place without resting period and results in a three-celled promycelium, which produces the basidiospores. During the formation of the basidiospores the promycelium is commonly separated from the teliospore. The basidiospores propagate by budding. Monokaryotic strains were isolated and characterized by standard yeast identification tests and enzymatic activities on solid media. The anther smut on *Saponaria officinalis* differs in a number of characteristics from typical *Ustilago* species of Poaceae, therefore it should no longer be included in that genus. *Microbotryum*, a genus based on *Microbotryum antherarum* on *Saponaria officinalis*, is more appropriate for the smuts previously placed in *Ustilago*.

Zusammenfassung

Studien an Heterobasidiomyceten. Teil 24. Über *Ustilago violacea* (Pers.) Rouss. von *Saponaria officinalis* L.

Sporenbildung, Keimung und Karyologie, sowie Kulturmerkmale von *Ustilago violacea* wurden untersucht. Die Anlage der Teliosporen erfolgt in sporogenen Hyphen. Nach ihrer Reife werden sie durch Verschleimen der umgebenden Hyphenwand freigesetzt. HCl/Giemsa-Färbung zeigt, daß die sich entwickelnden Teliosporen zweikernig sind. In vollständig ausdifferenzierten

Teliosporen ist nur noch ein Kern nachzuweisen. Die Brandsporen sind sofort keimfähig und wachsen mit einem dreizelligen Promyzel aus. Während der Basidiosporenbildung trennt sich das Promyzel von der Teliospore. Die Basidiosporen vermehren sich durch Knospung; die davon isolierten Kulturen wurden durch die Standardtests der Hefebestimmung und einige Enzymaktivitäten charakterisiert.

Der Antherenbrand auf *Saponaria officinalis* unterscheidet sich in vielen Merkmalen von typischen *Ustilago*-Arten der Poaceen, er sollte daher nicht länger in dieser Gattung eingereiht werden.

We express our thanks to Prof. Dr. W. Sauer for making available a Leitz A.S.M. System Socos, to the Directors of the herbaria GZU and M for the loan of specimen. For technical assistance we are indebted to Mrs. Heindl and Miss Quellmalz. This work was supported by SFB 76 of the Deutsche Forschungsgemeinschaft.

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