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Tilletia sabaudiae, a new smut fungus (Tilletiales) and some observations on the gelatinoid sheath of the *Tilletia* spores.

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Abstract

Zogg, H. 1983. *Tilletia sabaudiae,* a new smut fungus (Tilletiales) and some observations on the gelatinoid sheath of *Tilletia* spores. Bot. Helv. *93:* 91-98. A new species, *Tilletia sabaudiae,* parasitizing the ovaries of *Poa nemoralis* L. is described. The striking differences in size and shape of its spores studied both by light microscopy (LM), and by scanning electron microscopy (SEM) led to some reflections on the gelatinoid sheath, on the outermost membrane of ripe *Tilletia* spores as well as on studies on the violent opening of the spore corona (i. e. spore wall ornamentations) in water or other mounting media. This violent opening of the spore corona was also found in a series of other *Tilletia* species, but its mechanism is not yet known.

1. Description

Tilletia sabaudiae Zogg spec. nov. – Sori in ovariis totae plantae, semina infecta plus minusve atra, disrumpenti parieti, paene inflata, in glumis intactis contenta, leviter prominentia. Massa sporarum levis, pulverulenta, rubiginosobrunnea. Sporae permagnae, plus minusve globosae, rubiginosae, indistincte reticulatae, superficies sporarum corona alarum erectarum vel lateraliter curvatarum ornata, membrana tenuis coronam obducenti (an membrana ipsa paries cellulae formantis sporam ?). Sporae (ornamentis parietis inclusis) (38)43-54(58) × (35)41-52(55) μm, sine ornamentis (26)30-38(41) × (26)29-35(38) μm, ornamenta 6-9 μm alta. Cellulae steriles hyalinae vel flavidae, ad 20 μm magna, parieti 2-3 μm crassa, levigata. – Hab. in ovariis *Poae nemoralis* L. – Typus: In Alpibus Sabaudiae, Gallia, leg. J. Mueller-Argoviensis, 1851 (NEU). – Etym.: Sabaudia (= Savoie, = Savoy), provincia Gallica.

Sori destroying all ovaries of a plant, covered by the delicate pericarp, somewhat swollen up, partially hidden by the glumes; spore mass dark reddish-brown, powdery. – Spores very large, subglobose to globose, reddish-brown, indistinctly reticulate, with a thick, slightly yellowish tinted corona provided with erect or, in one direction, curved

ridges covered with a thin membrane (the wall of the former cells of the sporogenous hyphae ?); spores, wall ornamentations (corona) incl., (38)43-54(58) × (35)41-52(55) μm in diam., spores, wall ornamentations (corona) excl., (26)30-38(41) × (26)29-35(38) μm in diam., wall ornamentation (corona) alone 6-9 μm high (plate 1). Sterile cells hyaline to slightly yellowish, about 20 μm in diam., wall smooth, 2-3 μm thick. – Hab. in ovaries of *Poa nemoralis* L. – Type: In the Alps of Savoy, province of France (NEU). – Etym.: Sabaudia = Savoy.

Among the described *Tilletia* species on *Poa* , *T. sabaudiae* may resemble somewhat *T. transiliensis* Kuznetzova et Schwarzman in Schwarzman, Crypt. Flora of Kasakhstan II, Alma-Ata, 1960:240 (cit.): «... sporis globosis vel subglobosis, 37,5-56(57) × 37,5-50(52) μ, saepe 48 × 44,4 μ, atro-brunneis, nigrescentibus, reticulatis, sexangularis flagelliferis, 3-4 μ lat., et atris projecturis dauciformis, 3,7-5,6 μ, rarissime 7 μ elat; episporio stratum hyalinum formato ...», on *Poa nemoralis* L., Kazakhstan, USSR.

Later on this species was treated by Ulyanishchev (1968) as well as by Kochman and Majewsky (1973) as a synonym of *T. poae* Nagorny, Sci. Papers Appl. Sec. Tiflis Bot. Gard. *5:* 170, 1926 (in the ovaries of *Poa nemoralis* L. var. *svanetica* Hack., Caucasia). – Unfortunately it was impossible to obtain for comparison the type materials of either *T. poae* nor *T. transiliensis.* – The drawings of the spores of *T. transiliensis* published together with the original description show that the ornamentation of the spore wall can scarcely be the same as in *T. sabaudiae.* The latter, new species, possesses a much denser arrangement of the wall projections which are, moreover, distinctly higher than those of *T. transiliensis.* These facts may justify the erection of a new species.

2. Observations on the gelatinoid sheath and the outermost membrane as well as the violent opening of the corona of *Tilletia* spores

The discrepancy between the micrographs of the spores of *T. sabaudiae* taken by means of light microscopy (LM) and scanning electron microscopy (SEM) are striking. Under LM the spores mounted in Shear's mounting fluid modified by Graham (1959) show the beautiful ornamentations of the spore surface as described above. Under SEM the spores look like blackberry fruits with coarse, rounded warts, about 1.5-1.8 μm in diam., intermixed with super-fine verruculations and with a delicately wrinkled surface. The diameter of the spores under SEM conditions varies between 27-33(40) μm, thus corresponding to the size of the spore proper (without corona) in a mounting fluid under LM conditions.

Air-dry spores of the 130 year old herbarium material brought between the slide and the cover slip without mounting fluid showed under LM exactly the same size and shape as those prepared for and studied under SEM. If a droplet of water (or another mounting medium such as concentrated lactic acid or Shear/Graham's mounting medium) was

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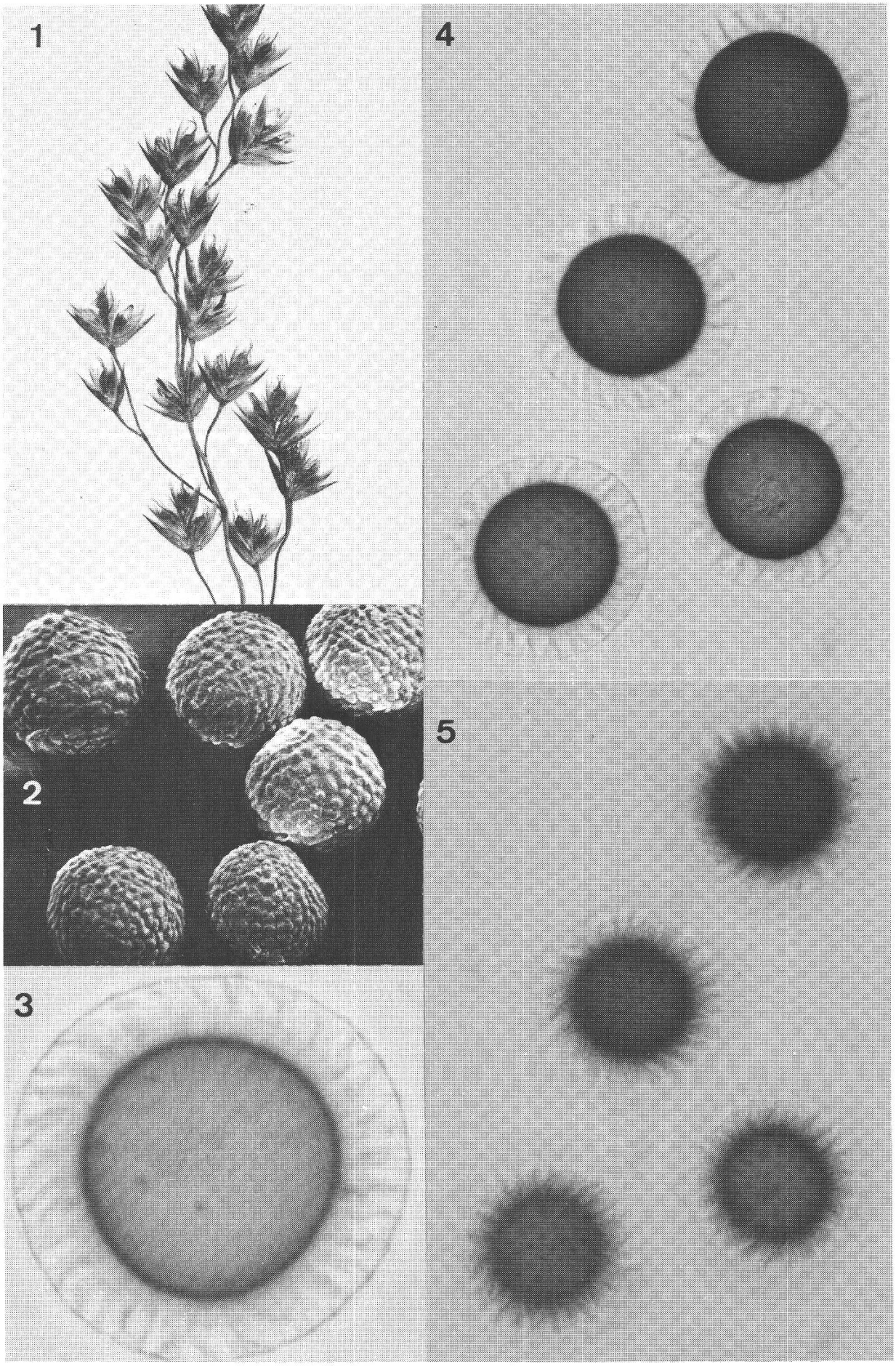
Plate 1. Tilletia sabaudiae Zogg spec. nov. on *Poa nemoralis* L. – Fig. 1: Symptoms (x 2.5); fig. 2: spores, SEM (x 600); fig. 3: spore, LM (x 1100); fig. 4: spores LM (x 550, median view); fig. 5: spores LM (x 550, surface view).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added to the spores under the cover slip the corona unfolded violently within less than one second as soon as a spore was completely surrounded with water. A high content of humidity alone remained ineffective. After evaporation of the water the corona joined closely the surface of the spore proper assuming the same feature as in the previous dry state. By adding water once again to these spores the corona opened likewise. This process could be repeated several times with the same spores by adding and evaporating the water alternately. The mechanism of this process is not yet known, but it cannot be explained (solely) by the existence and action of a gelatinoid material which would melt away gradually in the surrounding water as can be demonstrated for example with gelatine chips in water. Furthermore the melted gelatine sticks the slide and the cover slip together after evaporation of the water. In contrast to this such an adherence could never be observed using pure *Tilletia* spore powder.

The *Tilletia* spores are «... formed from intercalary cells of a sporogenous mycelium, or terminally on sporogenous hyphae, commonly encased in a hyaline to tinted, gelatinoid sheath...» (cit. after Duran and Fischer, 1961, p. 17). – In the genus *Neovossia* which is closely related to *Tilletia,* «... the spores are formed singly within the terminal cells of special sporogenous hyphae, and these continue to invest the teliospores. A fragment of the sporogenous hypha remains attached as an investing sheath and appears as a long, pedicel-like appendage of the spore...» (cit. after Duran and Fischer, 1961, p. 7). This permanent appendage of a spore can be observed also in ripe spores under SEM as well as under LM (Zogg and Schwinn, 1971, fig 24). They are formed by the cell wall of the sporogenous hyphae.

The outermost part of the corona of the spores of *T. sabaudiae* and other *Tilletia* species consists of a thin (elastic ?, hygroscopic ?) membrane. It is supposed that this membrane is identical with the cell wall of the sporogenous hypha. It does not gelatinize, in contradistinction to the successive gelatinizing of the wall of the sporogenous cells during maturation of the spores for example in some *Ustilago* species (Langdon and Fullerton, 1975; Deml, Nebel and Oberwinkler, 1981).

Concerning the spore wall ornamentations essential differences can exist between the micrographs taken by SEM compared with those taken by LM. In *Ustilago* and in many other genera the spore surface structures can be studied in detail under high magnification by SEM much better than by LM. In *Tilletia* and e.g. *Neovossia* the contrary must be stated because the wall ornamentations of ripe spores are very probably coated by the permanent, thin cell wall of the sporogenous hyphae. In reticulate *Tilletia* spores (e.g. *T. controversa*) the ridges appear under SEM mostly as broadly obtusate bars, whereas under LM (in a mounting fluid) they appear as sharp blades especially in median view (Zogg and Schwinn, 1971, fig. 15-17). Some other examples are given in plate 2.

The violent opening of the corona of the spores of *T. sabaudiae* was demonstrated in a series of other *Tilletia* species and can be best observed in *Tilletia* species which possess high ornamentations from about (1.5)2 μm up to 9 μm (admittedly with some excep-

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Plate 2. Tilletia spp. parasitizing the ovaries of different species of Gramineae (type of ornamention and the opening of the corona are given in table 1). Spores on the left and in the middle: LM, median and surface views (x 1100 each); on the right: SEM. – Fig. 6: *T. elymi* Diet. et Holw. on *Elymus glaucus* Buckl. (SEM: x 5000); fig.7: *T. sphaerococca* (Wallr.) Fischer-Waldh. (= *T. decipiens* Schröter) on *Agrostis schraderiana* Becherer (SEM: x 5000); fig. 8: *T. pallida* G.W. Fischer on *Agrostis palustris* Huds. (SEM: x 3000); fig. 9: *T. texana* Long on *Hordeum nodosum* L. (SEM: x 3000; outermost spore membrane was ruptured accidentally).

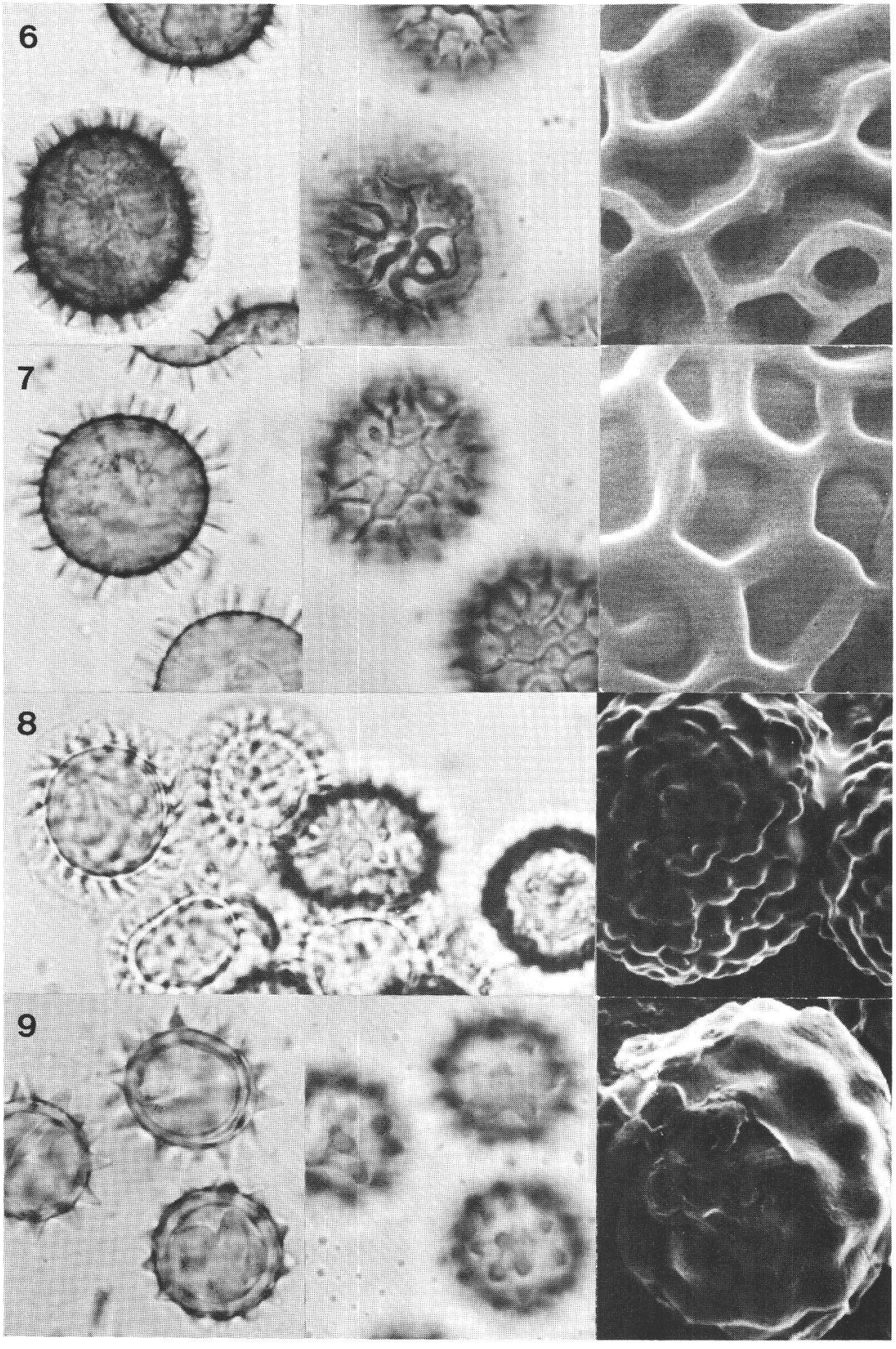
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Table 1. Type of the opening of the corona of the spores of different *Tilletia* species in water; observations by LM.

Tilletia spp. Hosts	Spore wall ornamentation	Opening of the corona in water; height of the corona in μm, measured in Shear/Graham-medium	
		violent opening	no or indistinct opening
T. anthoxanthi Blytt (*Anthoxanthum*, ovaries)	reticulate-cerebriform	(2.5)3-4(5)	—
T. asperifolia Ell. et Ev. (*Sporobolus*, ovaries)	reticulate	1.5-2(2.5)	—
T. bolayi Zogg (*Bromus*, leaves)	reticulate-cerebriform	—	0.5-1.5(2)
T. brachypodii-ramosi Zogg (*Brachypodium*, leaves)	reticulate	(0.5)1.5-2.5(3)	—
T. caries (DC) Tul. (*Triticum*, ovaries)	reticulate	—	0.5-1(1.5)
T. cerebrina Ell. et Ev. (*Deschampsia*, ovaries)	reticulate-cerebriform	(2)3-4(5)	—
T. controversa Kühn (*Triticum*, ovaries)	reticulate	1.5-3(4)	—
T. corona Scribn. (*Pennisetum*, ovaries)	reticulate	(1.5)2-3	—
T. elymi Diet. et Holv. (*Elymus*, ovaries)	cerebriform	2-3(4)	—
T. flectens Lagh. (*Deschampsia*, leaves)	reticulate	—	1-2*
T. foetida (Wallr.) Liro (*Triticum*, ovaries)	none, spore wall smooth	—	0
T. fusca Ell. et Ev. (*Festuca*, ovaries)	reticulate	2-3(4)	—
T. holci (West.) Schröter (*Holcus*, ovaries)	reticulate-cerebriform	(4)5-6(8)	—
T. indica Mitra (*Oryza*, ovaries)	verrucous	1.5-5	—
T. lolii Auersw. (*Lolium* ovaries)	reticulate	2-3	—
T. olida (Riess) Schröter (*Brachypodium*, *T.* leaves)	reticulate	—	0.5-1(1.5)
T. pallida G.W. Fischer (*Agrostis*, ovaries)	indistinctly reticulate-spiny	3-5	—
T. sabaudiae Zogg (*Poa*, ovaries)	indistinctly reticulate-spiny	6-9	—
T. scrobiculata G.W. Fischer (*Poa*, leaves)	reticulate	—	(1)2(3)*
T. secalis (Cda.) Körn. (*Secale*, ovaries)	reticulate	(1.5)2-3(3.5)	—
T. sesleriae Juel (*Sesleria*, leaves)	cerebriform	—	1.5-3(4)*
T. sphaerococca (Wallr.) Fischer-Waldh. (*T. decipiens* [Pers.] Körn.) (*Agrostis*, ovaries)	reticulate	(2)3-5	—
T. sterilis Ule (*Festuca*, leaves)	reticulate	—	2-4.5*
T. texana Long (*Hordeum*, ovaries)	verrucous	(1.5)2-3(4.5)	—

* exceptions (reasons not yet explicable)

tions). This may be demonstrated in the three smuts *T. foetida, T. caries* and *T. controversa* parasitizing the ovaries of *Triticum* as well as in the two smuts *T. olida* and *T. brachypodii-ramosi* developing their sori in the leaf blades of *Brachypodium.*

T. foetida: spore wall smooth, no ornamentations, no opening; the outermost membrane can be detected only rarely.

T. caries: spore wall reticulate, low ornamentations, 0,5-1(1.5) μm high, no opening or only a hardly detectable, indistinct opening of the corona; the outermost membrane is sometimes visible.

T. controversa: spore wall reticulate, high ornamentations, 1.5-3(4) μm high, violent opening of the corona as in *T. sabaudiae;* the outermost membrane is visible frequently and without difficulty. – Graham (1959) studied in detail the influence of a series of different mounting media (as well as boiling and not boiling the spores resp.) on the «mucilaginous» sheath of *T. controversa,* which tends to over-expand under alkaline conditions and to contract variably under acid conditions, and the sheath can even be dissolved by alcoholic potash, for example.

T. olida: spore wall reticulate, low ornamentations, 0.5-1(1.5) μm high, no opening or only a hardly detectable, indistinct opening of the corona; the outermost membrane is only hardly visible.

T. brachypodii-ramosi: spore wall reticulate, hihg ornamentations, (0.5)1.5-2.5(3) μm high, violent opening of the corona; the outermost membrane is visible frequently without diffuculty.

In table 1 a series of such examples is enumerated.

Zusammenfassung

Ein neuer Brandpilz, *Tilletia sabaudiae* (in den Ovarien von *Poa nemoralis*) wurde beschrieben. Die auffallenden Unterschiede, die sich bei der Betrachtung der Sporen im gewöhnlichen Lichtmikroskop bezw. im Rasterelektronenmikroskop ergaben, führten zu einigen Überlegungen und Hinweisen bezüglich der «Schleimhülle» und der äußersten Membran von reifen *Tilletia*-Sporen (Wand der Sporenmutterzelle ?). Die Sporenhülle (Ornamentierung der Wandoberfläche der Sporen, «Korona») entfaltete sich schlagartig, sobald die trockenen Sporen von Wasser (oder von anderen flüssigen Einschlußmedien) vollständig umgeben waren. Nach dem Verdunsten des Wassers schmiegte sich die Korona dicht an den Sporenkörper, entfaltete sich bei erneuter Wasserzugabe wiederum schlagartig. Dieser wiederholbare Vorgang, dessen Mechanismus noch nicht bekannt ist, konnte auch bei verschiedenen anderen *Tilletia*-Arten beobachtet werden.

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