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Rhizina undulata on stem and roots of 84-year-old Scots pine trees

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Apothecia of *Rhizina undulata* Fr. were found on and around 84-year-old Scots pine trees in fire-free managed forest. The trees were weakened: crowns were strongly transparent, needles were yellowish or red in part of the crown, the 1997 height increment of shoots decreased by about 60. One apothecium developed on the stem of tree, 7 cm above the ground level. The other 8 were associated with the roots. Three weeks after ascocarps had been removed 8 new ones were found. Some details referring to apothecia are given: distances from the root collar, dimensions, fresh weight, moisture and "index of undulativity" (Fw).

Key words: *Rhizina undulata*, Scots pine.

INTRODUCTION

Rhizina undulata Fr. is recognized as a serious cause of damage in young coniferous plantations established on burned forest site. In Poland, after the large scale forest fires in August 1992 (in 4 Forest Regions 24 thousand hectares of stands were burned) *R. undulata* apothecia were found commonly on and around Scots pine seedlings. However, many dying seedlings were noted. Mańka (1993) and Sierota and Małecka (1997) suggested that their mortality was attributed rather to a hot and dry period after planting than to the abundant presence of the pathogen. *R. undulata* was found to be a dangerous pathogen on germinants of *Pinus concorta* in *in vivo* tests (Egger and Poden 1986) and on seedlings of many coniferous species both in greenhouse studies and in the field conditions (Morgan and Driver 1974). Seaby (1977) reported that *R. undulata* was the cause of death of planted spruce trees within 5 years after fire; no more deaths were recorded during the subsequent 3 years. Gremmen (1976) recommended

the replanting of conifers not earlier than 2 years after fire or sowing as an alternative. Z a k and H o (1994) found that mycorrhized seedlings used for replanting burned stands reduced the damages caused by *R. undulata.*

Apothecia of *R. undulata* develop even 3 months after the fire and are commonly found 1–3 years after the fire (S a t o, Y o k o z a w a and S h o j i 1974; S i e r o t a 1992). G i n n s (1974) found a serious mortality of *Pseudotsuga menziesii* seedlings in the first year after planting on burned sites. However, three years after the fire apothecia were rarely found and no more than 24% deaths occurred in the area. In the 10-year-old and older *Pinus thunbergii, P. densiflora* stands apothecia were rarely found (S a t o et al. 1974). T h o m p s o n and T a t t a r (1973) found apothecia of *R. undulata* adjacent to roots of dying 80-year-old red spruce (*Picea rubens* Sarg.) in Green Mountain National Forest (USA). The authors found that the fungus was the cause of the disease and dying of trees, even though the attemps to isolate the mycelium from the roots were unsuccessful. It was the first report referring to the association of *R. undulata* with roots of such old trees in the United States.

In the present paper the case of *R. undulata* in fire-free 80-year-old Scots pine (*Pinus sylvestris*) managed stand is described. The parameters of apothecia formed in the stem and roots of trees are given and the index of undulativity of ascocarps (Fw) is discussed.

MATERIALS AND METHODS

The trees investigated were found in a managed stand in Forest District Choczewo – Biała Góra 24k (northern Poland, the Baltic Sea region) in July 1997 during phytopathological monitoring procedures (S i e r o t a and L e c h 1996). The 84-year-old Scots pine stand was established on dune sandy soil. The forest floor was partially covered by *Vaccinium myrtillus, V. vitis-idaea, Calluna vulgaris* and *Melampyrum pratense.* The mean diameter breath high (dbh) of pine stand was 38 cm and height 23 m. The typical moderate thinning had been performed 10 years earlier. No fire had ever been observed in the stand.

Since the crowns of some trees were strongly thinned and yellowish, and differed clearly from the other ones – the crown, stem, root collar and roots were subject to detailed routine inspection. It was stated that last year's (1997) height increment of shoots was equal 1/3 of that in 1996; the latter was rarely noticed on the other trees (Fig. 1).

While looking for the reason of the tree dieback, the apothecia of *Rhizina undulata* were found. One of them [No. 1] was formed on the stem of tree A about 7 cm above the ground level. The top of the crown was broken by

Fig. 1. Transparent crown of the evaluated tree (on left)

Fig. 2. Apothecia of *R. undulata* in August (see arrows)

Fig. 5. Apothecium of *R. undulata* on root collar

Fig. 6. Asci and ascospores of *R. undulata* from apothecium No. 1
Scale bare = 10 μm

wind many years ago; the dbh was 27 cm. The other apothecia grew on litter between 3 neighbouring trees (Fig. 2).

More detailed observations of the ascocarps were performed (3 times) in the middle of July and 4 and 7 weeks later. The apothecia found were carefully collected from the ground (sand was removed), measured (maximum length and width and area of the outline of fresh apothecia) and weighed. The asci and ascospores from hymenial layer were observed microscopically in the laboratory. The apothecia were dried (105°C during 24 hours) and loss of weight and actual moisture were evaluated. The index of fresh weight of apothecium related to area: Fw/g (g/cm^2) was calculated, elucidating thus indirectly both the "undulativity" of apothecia and the "activity" of mycelium to form the ascocarp body (Fig. 3). Small samples of wood were taken from roots associated with the apothecia and from root collar and subsequently fungi were reisolated on MEA.

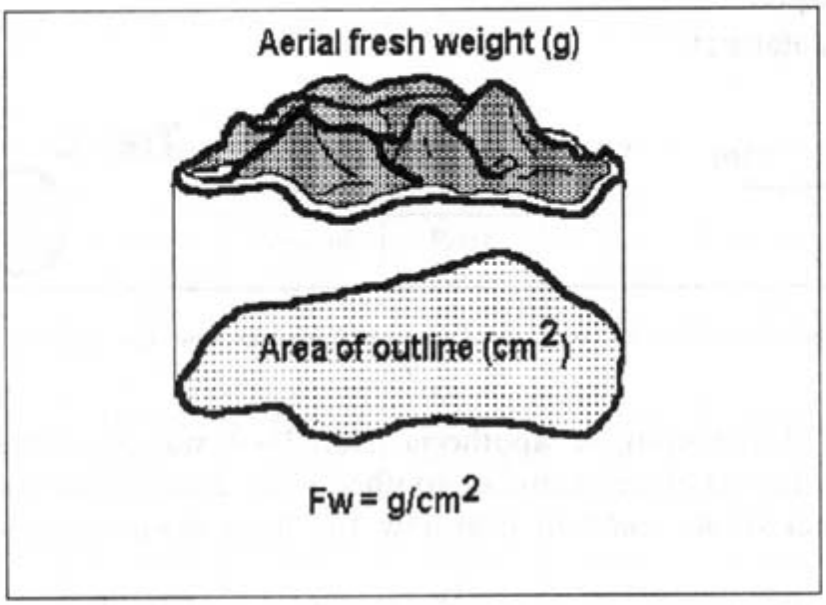

Fig. 3. Calculation of *Fw* index

RESULTS

The first assay of presence of *R. undulata* ascocarps, regardless the apothecium No. 1 found on the stem of one of the trees (A) investigated, enabled the identification of 6 other ascocarps [No. 2 and 4–8] (Fig. 4). Apothecium No. 1 was formed on debarked part of the stem (old wound?). The wood necrosis was about 5 cm deep and 32 cm above the apothecium; the brown decay of tissues refers to about 20% of the area of root collar. Apothecia No. 2–8 developed at different distances from the tree A (Table 1A).

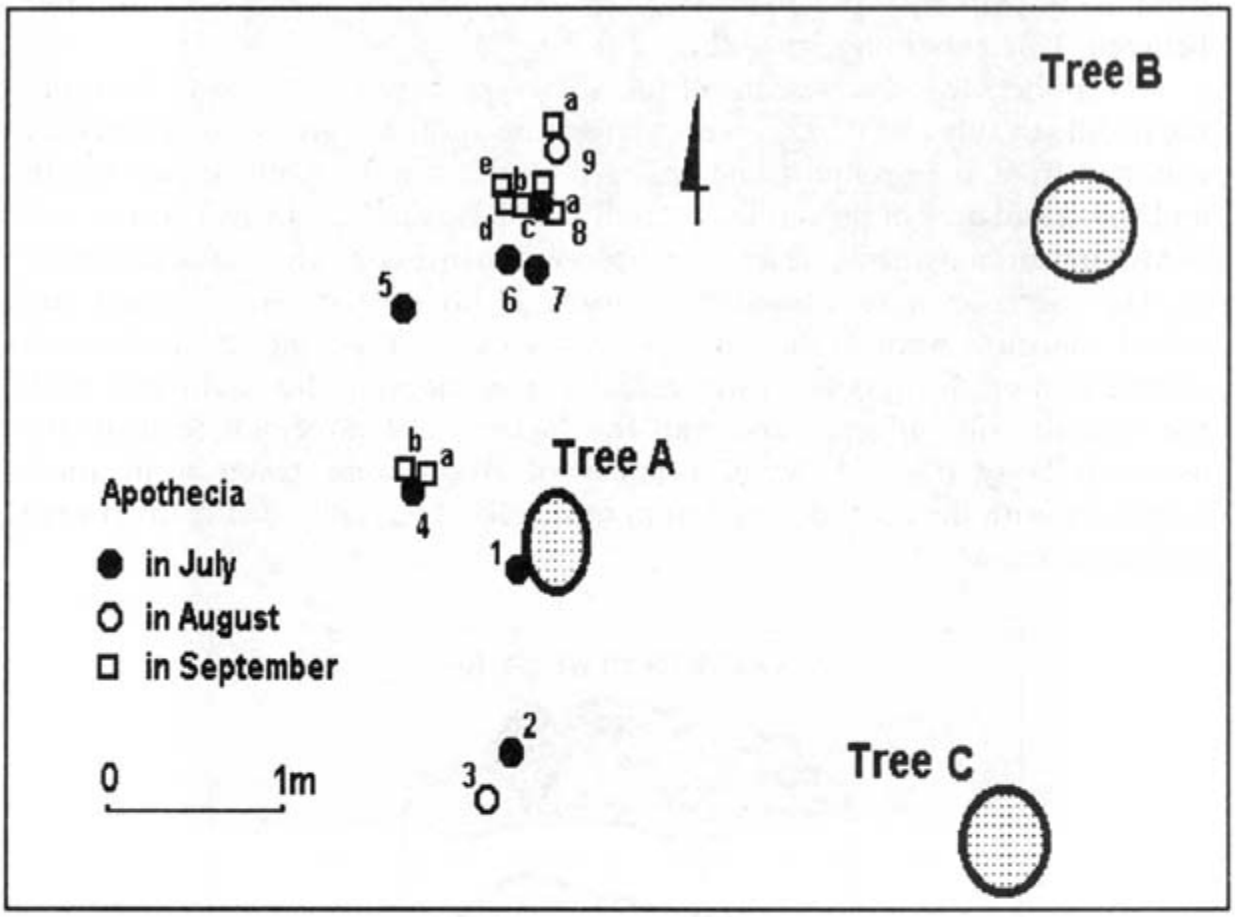

Fig. 4. Spatial distribution of *R. undulata* apothecia around the trees A, B and C

The spatial distribution of apothecia No. 3–9 was connected with the distribution of roots of tree A and of apothecia No. 2 and 3 with roots of tree B. The mycelium of *R. undulata* overgrew the litter layer between roots and ascocarps.

The apothecia No. 1–2 and 4–8 were found in the middle of July. During the next 4 weeks new ascocarps No. 3 and 9 developed and the surface of apothecia No. 1, 2 and 6 became dry. Since mid-July and during August no rainfall was noted; the air temperature was rather high (26–30°C). The apothecia were carefully removed and measured.

The results of measurements of apothecia growing in August are presented in Table 1A. The lowest parameters characterised apothecium No. 9 and apothecium No. 1 formed on decayed stem above the root collar (Fig. 5), whereas the greatest size was estimated for apothecia No. 5 and 8. The "undulating" structure which developed on the surface of *R. undulata* apothecia influenced the actual differentiation of the area and the weight of the latter: there were flat apothecia No. 6 and 8 and undulating ones No. 9 and 2 (see index Fw/area). The mean moisture of the apothecia was 89.17%

(at $\sigma_{(n-1)} = 2.60$ and coefficient of variation $v = 2.92\%$) and it ranged from 83.85% (No. 9) to 93.52% (No. 6). The dimensions and moisture of apothecia No. 5, 7 and 8 were almost identical.

The ascospores and asci typical for *Rhizina undulata* (following the example of apothecium No. 1) are shown in Fig. 6.

The last observations were performed three weeks later (in September) and 8 newly formed ascocarps were found. Three of them ("a") were strictly related to the position of "maternal" apothecia No. 4, 8 and 9. The other ones ("b, c, d, e") grew in the nearest neighbourhood of the latter. The apothecia were removed and measured as previously. The parameters of the freshly formed apothecia (Tab. 1B) were similar although lower than those of their predecessors. The mean moisture was 78.00% (at $\sigma_{(n-1)} = 7.39$ and coefficient of variation $v = 9.48\%$) and it ranged from 68.79% (No. 8e) to 85.39% (No. 9a). For apothecia No. 4a, 8a and 9a the parameters obtained, weight and moisture were almost the same as the respective values for the "maternal". The values of the Fw/area index were lower than those for the apothecia found in August (see Tab. 1).

Table 1

Characteristics of *Rhizina undulata* apothecia found on stem (tree A) and around the trees in August (A) and in September (B)

No.	Distance from tree (m)	Maximal dimension (cm)	Area of outline (cm^2)	Fresh weight (g)	Dry weight (g)	Moisture (%)	Index Fw* (g/cm^2)
A							
1	0	4.6 × 3.8	13.32	5.1132	0.5989	88.29	0.384
2	2.10	6.0 × 3.5	15.21	15.3216	1.3645	91.09	1.007
3	2.30	5.6 × 3.4	15.32	9.8602	1.1882	87.95	0.644
4	**0.82**	**8.2 × 7.1**	**45.15**	**23.7182**	**2.3409**	**90.13**	**0.525**
5	1.61	10.7 × 8.0	60.05	26.2707	2.8925	88.99	0.437
6	1.46	9.5 × 7.8	53.11	6.2394	0.4043	93.52	0.117
7	1.37	9.7 × 6.3	57.37	30.3882	3.2106	89.43	0.530
8	**1.67**	**9.5 × 8.2**	**59.64**	**16.4312**	**1.7552**	**89.32**	**0.276**
9	**1.80**	**3.1 × 2.6**	**6.65**	**13.5966**	**2.1955**	**83.85**	**2.045**
B							
4a	0.83	3.4 × 3.9	8.26	3.9580	0.6800	82.82	0.479
4b	8.86	1.2 × 0.8	0.72	0.1183	0.0325	72.53	0.164
8a	1.65	7.6 × 3.5	13.38	5.5872	0.8370	85.02	0.418
8b	1.72	2.8 × 3.6	7.32	2.7705	0.8611	68.92	0.378
8c	1.75	3.6 × 3.4	7.60	2.4547	0.3638	85.18	0.323
8d	1.77	2.7 × 3.4	5.50	1.8476	0.4559	75.32	0.336
8e	1.79	2.8 × 1.8	2.92	1.0658	0.3326	68.79	0.365
9a	1.88	9.5 × 8.2	15.57	5.5459	0.8100	85.39	0.356

*Fw – Fresh weight of apothecium/area of outline

Bold – "maternal" apothecia for those found in September

After 3 weeks of incubation of 30 wood samples taken in September from wood of roots and tree base some fungi were reisolated: *Trichoderma viride* (79% of isolates), *Penicillium* sp. (11%), *Hormoconis resinae* (Lindau) Arx and Vries (2%), *Ophiostoma* sp. (2%), *Sporothrix schenckii* Hektoen and Perkins (2%), *Mortierella* sp. (1%), *Sclerophoma pythiophila* (1%), *Phialocephala* sp. (1%), *Mycelium radicis atrovirens* (1%); mycelium of *R. undulata* was not obtained. It was established that this manner of reisolation was not favourable for detecting the *R. undulata* mycelium. Many nematodes were found in the decayed wood.

DISCUSSION

T h o m p s o n and T a t t a r (1973) reported associations of *R. undulata* apothecia with roots of 80-year-old trees, although their case referred to *Picea rubens* trees in natural forests, previously burned. G r e m m e n (1991) described also possibilities of the development of the *Rhizina undulata* root rot in 50-60-year-old *Pinus nigra* stand as a result of the so called "coffee fires". The presence of *R. undulata* apothecia on stem and roots of the old Scots pine trees in fire-free managed stand has been reported for the first time.

The trees probably weakened by other reason were attacked by *R. undulata*. It is difficult to forejudge now if the infection process started through the old wound in the root collar of tree A, where the fungus found favourable conditions for growth or through some roots. Many of the arising apothecia grew just above the decayed roots and contacts with rhizoctonias and mycelium under the bark of roots were macroscopically found. The infection processes in the roots influenced the crown. Needles on some branches were yellow or red – without any symptoms of fructification of needle pine cast fungi; the 1997 height increment of shoots decreased – even by 60% in comparison to that in 1996.

The parameters of apothecia, asci, ascospores and paraphyses were similar to those described by B r e i t e n b a c h and K r ä n z l i n (1984).

It is interesting to note that 3 weeks after removing the apothecia which had formed in June-August, new ones were found in the same places, just under the roots of the weakened tree. The number, parameters and moisture of newly formed apothecia were similar to those obtained for maternal ones. However they were less undulate than in the latter. No relation was found between mycelium activity and mass production of ascocarps (values of the index Fw of maternal apothecia) and position or number of newly formed ones. The most numerous apothecia were found in September after

removing the maternal ascocarp No. 8, for which linear dimensions and Fw index were not characterized by extra activity of mycelium.

The results may indicate a high activity of the mycelium in decayed roots and persistence of the inoculum in the root system. The pathogen may cause a serious threat to the neighbouring trees in the stand (secondary infections via root contacts).

The results obtained indicate that *Rhizina undulata*, the root pathogen of young coniferous trees in reforested sites after fire, is able to develop in roots of mature Scots pine trees growing on sandy soils. The fungus can cause the necrosis of the inner bark, cambial zones and wood of weakened trees growing also in fire-free areas. In the future *R. undulata* may play an important role as a root rot pathogen, particularly in connection with expected climate changes in boreal forest zone.

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Rhizina undulata na strzale i korzeniach 84-letnich drzew sosny zwyczajnej

Streszczenie

Apotecja *Rhizina undulata* (Fr.) zostały znalezione w lipcu na strzale i na glebie wokół 84-letnich sosen w drzewostanie gospodarczym, w którym nigdy przedtem nie notowano pożaru. Oceniane drzewa wykazywały symptomy osłabienia – korony były silnie przerzedzone, igły żółkniejące lub rude. Jedno apotecjum było uformowane na strzale, 7 cm ponad powierzchnią gleby, zaś wśród 8 innych stwierdzono makroskopowo bezpośredni związek grzybni z korzeniami drzewa. Trzy tygodnie po usunięciu owocników z podłoża (wrzesień) stwierdzono utworzenie się nowych 8 owocników. W pracy podano szczegóły dotyczące apetecjów – odległości ich formowania od szyi korzeniowej drzewa, wymiary, masę, wilgotność i wartości wskaźnika ich „fałdowatości" (Fw).