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The Rare Polypore *Antrodiella citrinella* and Its Special Phenology in the Black Forest National Park (Germany)

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Abstract

In the old growth spruce-fir forest "Wilder See", a long-term protected zone of the Black Forest National Park (Baden-Wuerttemberg, Germany), the phenology of two associated polypore fungus species on *Picea abies* logs, *Fomitopsis pinicola* and the rare red-data list species *Antrodiella citrinella* were investigated. We combined detailed field studies and temperature-related in-vitro growth studies. Whereas perennial fruitbodies of *F. pinicola* developed from late spring to autumn, the annual *A. citrinella* fruitbodies were formed mainly in late autumn (October, November) and again in spring (April and May). Growth studies on agar media confirm that *A. citrinella* grows faster at lower temperatures (5-10 °C), whereas *F. pinicola* is clearly superior in growth rate between 15 °C and 25 °C. This indicates that *A. citrinella* is a generally rare, but locally common species that may have been overlooked because of its special phenology in colder periods. The "Wilder See" area in the National Park seems to be an important refuge for the species. Its existence seems to depend on high humidity, high amount of woody debris (*P. abies*) and the occurrence of *F. pinicola*. In the presence of living *A. citrinella* fruitbodies, fruitbodies of *F. pinicola* were found to be always dead. This supports earlier speculations that the relation may be parasitic.

Keywords: Fungi; *Picea abies*; *Fomitopsis pinicola*; Mycoparasitism; Temperature preferences; Old growth forest; Dead wood density; Indicator species

Introduction

Antrodiella citrinella Niemelä & Ryvarden (*Flaviporus citrinellus* (Niemela & Ryvarden) Ginns; Lemon-colored Antrodiella) is a rare wood-inhabiting annual polypore causing white rot mainly on *Picea abies* preferring wet swampy localities [1]. Fruitbodies have a pale to bright citrine yellow pore surface forming small spores and develop resupinate to very narrowly reflex on wood. Its distribution in Europe follows that of a very common perennial polypore, *Fomitopsis pinicola* (Sw.) P. Karst. (Red-Belt Conk), causing brown rot on a wide range of trees, preferably on conifers but also on deciduous tree species. Known localities of *A. citrinella* are from natural spruce forests in Scandinavia and mountains of central Europe, France, Poland and the Czech Republic [1-4]. In Germany, the species is only known from old-growth forests in Bavaria and listed as "extremely rare" (category R) in the red-data list of macrofungi in Germany [5]. *A. citrinella* usually grows on old *P. abies* logs on or close to the basidiocarps of *F. pinicola*.

The former Bannwald (closed protected forest) "Wilder See" in the Black Forest National Park [8] measuring 150 ha is an old growth European spruce (*P. abies*) and Silver fir (*A. alba*) forest with *P. abies* as dominant species (Figure 1). Within the framework of a (still ongoing) mycological inventory of the Wilder See area [8] we specifically looked for *A. citrinella* and finally found it for the first time on 29 September 2014 and again in November in the same year. It was not found (no fruitbodies were formed) in the warmer months. In contrast, growing and sporulating perennial fruitbodies of *F. pinicola* were found throughout the snow-free periods of the year.

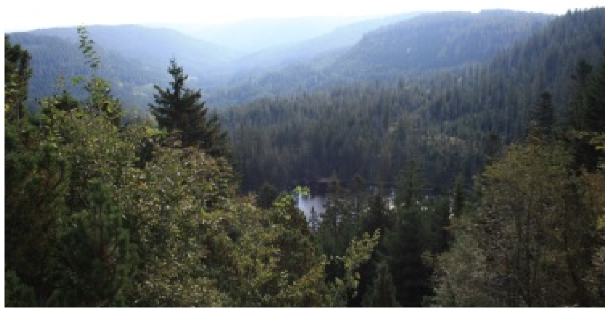

Figure 1: The cirque of the "Wilder See" study area seen from the western plateau with *Picea abies* as dominant tree species (photo M. Scholler).

The objective of this study is to find out whether *Antrodiella citrinella's* phenology of fruitbody formation is different from the one of the associated *F. pinicola* and whether this corresponds with temperature preferences.

Methods

Study area

The study area is the western part (Figure 2) of the nature reserve „Wilder See - Hornisgrinde“, a core zone of the Black Forest National Park (Baden-Wuerttemberg, Germany), with a size of 98 ha. Logging in the study area was banned for more than 100 years. The eastern part was not studied because it is hardly accessible. The altitude of the study area is 840-1.050 m. Roughly the area consists of a more or less even southwestern belt above 1020 m and a cirque below 1.020 m. The plateau is dry and open with *P. abies*, *A. alba* and *P. mugo* as dominant tree species (Figure 3). Below 1020 m is the cirque with a glacial lake at 910 m altitude. The headwall of the cirque is steep in the south and west and flatter in the north. The form of abrasion is in the east. The cirque vegetation is characterized by *P. abies* as dominant tree species, by high humidity and by dense woody debris (decaying logs) of *P. abies* (Figure 4). The high density of *P. abies* logs is a consequence of bark beetle (*Ips typographicus*) infestation. 60 % of the attacked trees were killed within two decades after 1991 [9].

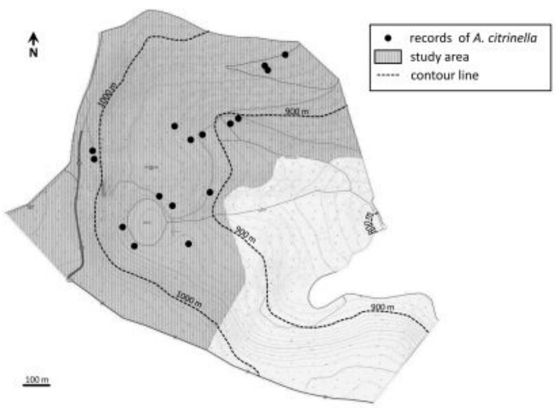

Figure 2: Distribution of *Antrodiella citrinella* in the “Wilder See” area. The species was found only in the cirque below 1020 m altitude (source: Open Street Maps, 4 October 2016, strongly modified).

The climate is humid-montane and (humid) high-montane [10]. The temperature is 5-6 °C on annual average, the annual precipitation is 2.000-2.200 mm [11]. The average monthly temperatures shown in Figure 5 where obtained from DWD Climate Data Center (CDC) (station-ID: 1468; 797 mNN; ftp://ftp-cdc.dwd.de/pub/CDC/observations_germany/climate/daily/kl/recent/) and hight-adjusted (0, 65 °C per 100 m).

Figure 3: Western plateau with dry and open stands above 1020 m (photo M. Wieners).

Figure 4: Dense dead logs of Picea abies below 1020 m characterize the cirque (photo M. Wieners).

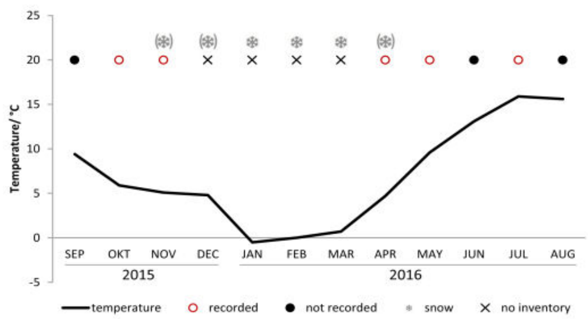

Figure 5: Chronology of fruitbody formation of *Antrodiella citrinella* and hight-adjusted mean temperature in the study area.

Field studies: distribution and phenology

In a pre-study at below 0 °C January 18, 2015 we looked for both fungus species and only found the perennial fruitbodies of *F. pinicola*. Therefore, a survey on phenology and distribution of *A. citrinella* was performed in the mainly frost-free periods from September 2015 to August 2016, once or twice a month for one day.

On each day different areas including geographical parts, the higher elevation plateau area and the cirque area below 1020 m was searched for *A. citrinella* on woody debris. Fruitbodies of *A. citrinella* on one piece of wood or fungal fruitbody were treated as one record [12]. In addition, the co-existence of *F. pinicola* was recorded by measuring the distance to *A. citrinella*. Also, it was recorded whether fruitbodies were living or dead.

In-vitro growth and temperature

Pure cultures of *A. citrinella* and *F. pinicola* were obtained from fresh fruitbody trama collected in the study area. Mycelium was grown on 8.5 cm malt agar plates (25 g malt extract (powder), 20g Agar and 1.000 mL aqua dest.) at 18 °C. From these preliminary cultures circular 1 cm pieces where cut off and placed in the middle of 8.5 cm malt agar plates. Cultures were incubated at 5, 10, 15 and 25 °C at 62 ± 5 % relative humidity. Three (and at 10 °C four) replicates per species/temperature were used. The colony diameter was measured after 7 (in the 10 °C replicates after 6, 8), 14 and after 21 days (if possible). The growth rate was defined as mean radial increment at two sides divided by the number of days at the last possible measurement.

Voucher specimens and cultures

Dried specimens of some records of *A. citrinella* and *F. pinicola* were placed in the fungus collections of the Natural History Museum Karlsruhe (Karlsruhe, Germany). (KR; see http://www.smnk.de/sammlungen/botanik/pilze/digitaler-katalog/). Living cultures were deposited in the fungus collections of the Department of Bacterial Physiology SBUG, University of Greifswald (Greifswald, Germany). Accession numbers: *A. citrinella* SBUG-M 1723 (this culture corresponds with herbarium specimen KR-M-0037905), *F. pinicola* SBUG-M 1724.

Results

Distribution

Living fruitbodies of *Antrodiella citrinella* were found 18 times and always in association with dead fruitbodies of *F. pinicola*. They were either found in close neighborhood of *F. pinicola* (Figure 7) or on fruitbodies of *F. pinicola* directly (Figure 6). Whilst the closest fruitbodies (up to 30 cm distant) were always dead, fruitbodies further away on the same log were often alive.

Figure 6: *Antrodiella citrinella* on dead fruitbodies of *Fomitopsis pinicola* on *Picea abies* (photo M. Wieners).

Figure 7: *Antrodiella citronella* on *Picea abies* stem below and in close neighborhood to dead *Fomitopsis pinicola* fruitbody in October 2015 (Photo M. Wieners).

A. citrinella was found scattered throughout the cirque area up to 1010 m altitude. It was not recorded on the plateau above 1.020 m altitude (Figure 2). In contrast, *F. pinicola* was found all over the study area including the plateau (Figure 3).

Phenology

Antrodiella citrinella was found eighteen times in five months, namely in late autumn 2015 (October, November), in spring 2016 (April, May) and in summer 2016 (July; Figure 5). The number of records (days per month) are listed in Table 1. Perennial living and growing fruitbodies of *F. pinicola* were observed all over the snow-free periods.

Records/investigation days								
	2015			2016				
	Sep	Oct	Nov	Apr	May	Jun	Jul	Aug
Number of Investigation days	2	2	2	1	2	1	2	1
Number of records of *A. citrinella*	0	4	7	5	1	0	1	0

Table 1: Number of investigation days and number of *Antrodiella citrinella* records observed in the snow-free months in the Wilder See study area.

Growth rate in pure culture

The growth rates are shown in Figure 8. Both species grew most slowly at 5 °C and fastest at 25 °C. *A. citrinella* grew significantly faster than *F. pinicola* at 5 °C and at 10 °C, whereas *F. pinicola* grew slightly faster at 15 °C and much (two times) faster at 25 °C ($p < 0{,}001$). The growth rate of *A. citrinella* clearly increases from 5 to 15 °C but at 25 °C it is hardly higher than at 15 °C. In contrast, the growth rate of *F. pinicola* continuously increases from 10 to 25 °C.

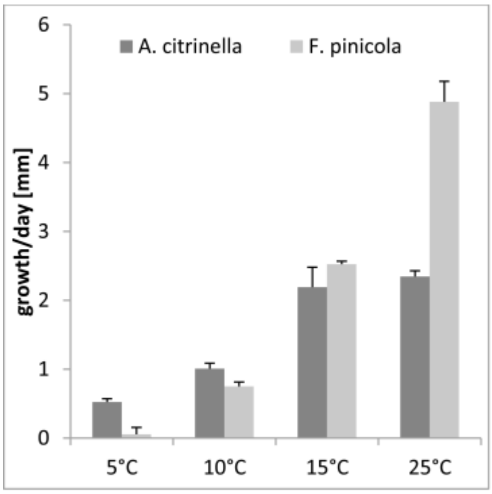

Figure 8: *In-vitro* growth rates of *Antrodiella citrinella* and *Fomitopsis pinicola* on malt agar at different temperatures.

Discussion

Antrodiella citrinella is a rare species in Germany and records in the Black Forest National Park were the first for the federal state of Baden-Wuerttemberg. We could show that the species is fairly common in moist and near-natural stands of European spruce within the cirque of the "Wilder See" (steep slopes between 930 and 1020 m altitude) but was not observed in the more open and dryer regions in the western and southwestern plateau zone, between 1020 and 1050 m altitude (Figure 4). For sure, the major fructification periods of *A. citrinella* in the study area will be the snow-free late autumn and spring periods. But we do not exclude that the species may also grow and form fruitbodies in mild winter weeks.

Numerous (17) *A. citrinella* fruitbodies were found in late autumn from October to November and again in spring from April to May. One record was obtained in summer (20 July 2016; Table 1); it was a very small and poorly developed single fruitbody found on a wet log. Fruiting may then have been supported by an intense rain on 13 July which caused a cold snap from 19.4 °C to 9.2 °C within 3 days. Mycologists usually do not explore macrofungi at higher elevations in the cold months from October to May. Therefore, the generally rare species may easily have been overlooked. In addition, it seems to be limited to forests with considerable and dense dead *P. abies* wood (as already indicated by Ryvarden & Gilbertson) [6] and continuous high air humidity. Concluding from the overall conditions in the study area, there should be more suitable habitats in the Black Forest. *A. citrinella* was common in the study area in April 2016 with 5 recorded stands. We looked for it and *F. pinicola* in the same month in other similar stands with woody *P. abies* debris, but more open and drier climatic conditions within the Black Forest National Park ("Huzenbacher See" area, "Lotharpfad" and "Pommertswald"). We found living *F. pinicola* fruitbodies in high quantity but *A. citrinella* only once at "Lotharpfad", a windthrow area with even higher dead wood density than in the "Wilder See" area. This indicates that the "Wilder See" protected area with its moist habitats, wet climate and high proportion of dead wood within the cirque is an important retreat area for *A. citrinella*. The species is an indicator species for montane old-growth forests like this. In the future, when authorities and conservationists discuss and have to decide whether areas like this should be protected or not, we suggest considering the Lemon-colored Antrodiella in late autumn as a major indicator.

With our *in vitro* study results we confirm an observation in Scandinavia that *A. citrinella* replaces *F. pinicola* [13]. This replacement obviously takes place at low temperatures in late autumn and spring when average temperatures are around 10 °C or below. This corresponds well with our in-vitro growth observations that show *A. citrinella* growing faster than *F. pinicola* between 5 and 10 °C. Our results support that *A. citrinella* replaces *F. pinicola*. Rayner and Boddy emphasized in 1988 [14] that replacement may not only take place by decayers but also by parasites of the pioneer species. Holmer et al. 1997 [12] concluded from *in-vitro* studies that *A. citrinella* is a secondary wood decayer. But their studies were carried out at room temperature, which is substantially too warm to simulate the situation in nature. We rather think that the association is parasitic based on the following observations: First, we found that *A. citrinella* is always associated specifically with *F. pinicola* - but not vice versa. Furthermore, *A. citrinella* fruitbodies are never associated with living but always with dead *F. pinicola* fruitbodies. These perennial fruitbodies - in contrast to *A. citrinella* - were often found small and young. Also, we could not confirm fresh and living *F. pinicola* in 2016 at sites where we had found *A. citrinella* in 2015 but not in 2016. This may also be a consequence of mycoparasitism. Although these fruitbodies are often overgrown with *A. citrinella*, it need not necessarily be fruitbody colonization but mycelial parasitism in the substrate as we know it from e.g. many Tremellales [15]. Mycelial parasitism may explain why fruitbodies of *A. citrinella* can be formed at distances of up to 30 cm from its putative host.

Vampola already assumed in 1991 that there are mycoparasitic relations between *A. parasitica* Vampola and *A. semisupina* (Berk. & M.A. Curtis) Ryvarden and their associated polypores [16]. This is also supported by a recent phylogenetic study showing that all fungicolous and possibly mycoparasitic species of *Antrodiella*, including *A. citrinella* and *A. parasitica*, are closely related [17]. To provide final evidence for a parasitic mode of interaction, however, more growth and micromorphological studies are required.

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