THE INFLUENCE OF A COPPER-CONTAINING FUNGICIDE ON THE GAMETOPHYTE OF SOME NON-TARGET PTERIDOPHYTE SPECIES

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POPESCU Anca Georgiana¹

Abstract: The aim of this study was to assess the effects of a fungicide with 20% metallic copper content on spore germination and gametophyte development in two non-target pteridophyte species: Asplenium scolopendrium and Athyrium filix-femina. The experimental variants were: V1 - 0.1% fungicide in Knop solution, V2 - 0.5% fungicide in Knop solution, V3 - 0.7% fungicide in Knop solution, and the Control variant (C) – Knop solution. The fungicide used affected spore germination in all the tested variants. The lowest germination percentages were registered in the species Asplenium scolopendrium: V1 - 69.33%, V2 - 65.66%, V3 - 51.33%. In terms of gametophyte differentiation, the experiments led to delays in developmental stages, absence of rhizoids and necrosis of prothallic cells. The results of the study can be used to assess the impact of the fungicide on the ecosystems in which it is applied, ecosystems where pteridophytes are also present or ecosystems adjacent to them.

Key words: Asplenium scolopendrium, Athyrium filix-femina, copper, gametophyte, germination, fungicide, pteridophytes

Received 25 October 2013                                          Revision accepted 8 November 2013

Introduction
Pesticides have been cited among the factors that influence spore germination, gametophyte and sporophyte differentiation (Keary et al. 2000, Sheffield 2002, Luo & Ikeda 2007, Cassanego et al. 2010, Droste et al. 2010). The gametophytic generation or the gametophyte phase of pteridophyte species begins with the spores (usually haploid), produced in sporangia and ends with the formation of the zygote. During its differentiation, the gametophyte follows the stages of prothallial filament, prothallial blade and cordate prothallus with gametangia on which the zygote is formed. (Ehrendorfer 1999, Fernández & Revilla 2003). The gametophyte, independent from the sporophyte with a short lifespan, has been used in experiments designed to study the response of plants to the influence of different stress factors present in their environment, such as temperature (Pangua et al. 1994), light (Mohr 1963, Tomizawa et al. 1983, Sugai et al. 1984), nutrient elements (Fernández et al. 1999), salinity (Pangua et al. 2009), acid rain (Evans & Bozzone 1978) etc.. Gametophyte differentiation begins

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with spore germination. Gametophytes are that generation in the life cycle which is sensitive to stress factors in their environment. In the case of terrestrial plants, germination tests are among those used to assess the ecological risks caused by pesticides or other pollutants in ecosystems (Catalá et al. 2011). Pteridophytes are non-target organisms currently used in toxicity tests needed to assess the ecological risks associated with pollutants.

The aim of this study was to assess the effects of a fungicide with metallic copper content on spore germination and gametophyte development in two non-target pteridophyte species: *Asplenium scolopendrium* L. and *Athyrium filix-femina* (L.) Schott.

**Material and methods**

**Biological material:** spores collected from *Asplenium scolopendrium* L. and *Athyrium filix-femina* (L.) Schott. Spores were collected from individuals in the Vâlșan Valley (Argeș county, Romania).

**The fungicide used** contains 20% metallic copper; it is supplied as wettable powder and included in toxicity class IV. The experimental variants are presented in Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Experimental variants</th>
<th>Fungicide concentration</th>
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</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>Knop solution</td>
</tr>
<tr>
<td>V1</td>
<td>0.1% fungicide in Knop solution</td>
</tr>
<tr>
<td>V2</td>
<td>0.5% fungicide in Knop solution</td>
</tr>
<tr>
<td>V3</td>
<td>0.7% fungicide in Knop solution</td>
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</tbody>
</table>

Fungicide concentrations applied during the experiment were those indicated for phytosanitary treatments. The Knop solution (1865) used had the following composition: 1.00 g l\(^{-1}\) Ca(NO\(_3\)\(_2\)), 0.25 g l\(^{-1}\) MgSO\(_4\), 0.25 g l\(^{-1}\) KH\(_2\)PO\(_4\), 0.25 g l\(^{-1}\) KNO\(_3\). Spores were cultivated in 50 ml of prepared solutions, in culture vessels sealed with Parafilm and kept in the Sanyo growth chamber under controlled temperature (25/15°C day/night), humidity and light conditions (16-hour photoperiod, 16:8 h L:D). After a week the spore germination percentage was determined by counting under the microscope 100 spores for each experimental variant. For each experimental variant we calculated the average and the standard deviation. After 3, 6 and 14 weeks, respectively from the cultivation of spores, we performed microscopic observations on gametophyte development. The photos were taken through an Optika B-275 microscope, using a Canon PowerShot A630 camera. The experiment was conducted in triplicate.

**Results and discussion**

**Spore germination under the influence of the tested fungicide.** The results obtained in the spore germination test show that the fungicide used had influenced the germination process. The species *Asplenium scolopendrium* was more affected than *Athyrium filix-femina* (Fig. 1, 2). Thus, in the species *Athyrium filix-femina* there is
a17.33% difference between the germination percentages obtained for the C variant and for the V3 variant, while in *Asplenium scolopendrium* the difference is 37.66%. The tests also revealed that there is a negative correlation between the germination percentage and the fungicide concentration. The calculated correlation index was -0.8955 in *Asplenium scolopendrium* and -0.9264 in *Athyrium filix-femina*.

**The influence of the pesticide on gametophyte differentiation.** In leptosporangiate pteridophytes (as gametophyte differentiation begins from spores) the formation of the prothallic filament, of the prothallic blade and of the cordate prothallus on which the embryo is formed takes place in approximately 3 months. This period varies, depending on the species (Fernandez & Revilla 2003).
Periodic microscopic analyses of the gametophytes obtained from spores showed that, unlike the C variant, the differentiation process was affected in the experimental variants with fungicide treatment (Table 2). Thus, after 3 weeks, most gametophytes in the C variant of *Asplenium scolopendrium* were at the stage of forming prothallic blades (Plate I.1), while the V2 and V3 variants contained only germinated spores. After 6 weeks from the initiation of the experiment, the cordate prothallus was about to be formed in the C variant (Plate I.4). The V1-V3 variants had formed prothallic filaments. We noticed unelongated rhizoids or even the absence of rhizoids (Plate I.5-6). After 14 weeks, cordate prothalli were present in the C, V1 and V2 variants (Plate I.7). Needle-like crystals were also located at the tip of the prothallic trichomes in V1 (Plate I.8). V3 remained at the stage of prothallic filaments with necrotic cells (Plate I.9).

Table 2

<table>
<thead>
<tr>
<th>Experimental variants</th>
<th>&quot;Stage of gametophyte differentiation&quot;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td><strong>Asplenium scolopendrium</strong></td>
</tr>
<tr>
<td></td>
<td>3 weeks</td>
</tr>
<tr>
<td>C</td>
<td>formation of prothallic blade</td>
</tr>
<tr>
<td>V1</td>
<td>filaments $\rightarrow$ prothallic blade</td>
</tr>
<tr>
<td>V2</td>
<td>germinated spores</td>
</tr>
<tr>
<td>V3</td>
<td>germinated spores</td>
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|                       | **Athyrium filix-femina**             |
|                       | C                                     |
|                       | prothalllic blades $\rightarrow$ cordate prothallus | young cordate prothallus | cordate prothallus with archegonia and anteridia |
| V1                    | formation of prothallic blade, unelongated rhizoids | prothalllic blades | cordate prothallus with anteridia |
| V2                    | filament $\rightarrow$ prothallic blade | filaments and prothallic blades with unelongated rhizoids | necrotic filaments and blades |
| V3                    | germinated spores (99%), filaments | prothallic filaments and prothallic blades | necrotic filaments and blades |

* Stage for most gametophytes analysed.
A delay in gametophyte differentiation was also registered in the species *Athyrium filix-femina* during the 14 weeks of culture (Table 2, Plate II.1-5). Thus, after 3 weeks, the cordate prothallus was about to be formed in the C variant (Plate II.1), while in the V3 variant most gametophytes were only at the stage of germinated spores (Plate II.3). After 6 weeks from the cultivation of spores, the cordate prothallus was present in the C variant. Prothallic blades and filaments had developed in the V1-V3 variants.

Different stages in gametophyte differentiation were also observed after 14 weeks from the initiation of the experiment (Table 2). In addition to slower gametophyte differentiation, mention should be made of the inhibition of the elongation process of rhizoids as well as the necrosis of prothallic cells.

The results obtained may be linked to the different capacity of plants to tolerate Cu and other metals in their environment. Some fern species are known as hyperaccumulators of metals (Nishizono et al. 1987a, Sela et al. 1989). Gametophytes of *Pteris vittata* and *Athyrium yokoscense* were shown to tolerate and accumulate lead (Kamachi et al. 2005). Gametophytes of *Pteris vittata* were also tolerant of high levels of arsenic and showed arsenic hyperaccumulation (Gumaelius et al. 2004, Kamachi et al. 2005). Copper had a greater affinity for the cell wall and was prevented from entering the cytoplasm. A large proportion of these heavy metals in the cell wall were
exchanged as ions (Nishizono et al. 1987b). Other compounds containing copper affected spore germination as well. Copper bromide induced changes in spore germination, growth and ultrastructure of *Polypodium cambricum* gametophytes (Muccifora 2008). The germination percentage was only 25% and the ultrastructure of gametophytes showed the absence of a vacuolar compartment.

**Conclusions**

The fungicide used in the experiment influenced the process of spore germination in the two non-target species tested, *Asplenium scolopendrium* and *Athyrium filix-femina*. As far as gametophyte differentiation is concerned, we registered not only a delay in developmental stages, but also the absence of rhizoids, negative effects on the elongation process of rhizoids and necrosis of prothallic cells. These changes may be used to assess the impact produced by the fungicide in the ecosystems where it is applied, ecosystems in which pteridophytes are also present or ecosystems adjacent to them.

**References**


