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Plant Pathology with a Purpose

Programme
and
Abstracts

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failed to cause infection on chilli pepper, cowpea, cotton and tomato. Furthermore, the detection of *C. eragrostidis* and *C. clavata* in yam tubers in this study, confirms the ability of these fungi to invade and survive in the tubers under natural conditions, underlining the risk these infected tubers could pose to yam cultivations and alternate host crops from season to season.

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***In vitro* interaction of *Amanita muscaria* and *Phytophthora cinnamomi*: possible biocontrol effect**

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Ink disease, caused by the Oomycetes *Phytophthora cinnamomi* Rands. and *Phytophthora cambivora* (Petri) Buism., is a major soilborne problem in European chestnut (*Castanea sativa*) stands all over South Europe. Chemical treatments are not effective and comprise serious environmental and economic costs, so biological control is under study as a possible solution for this problem. Ectomycorrhizal (ECM) fungi are generally considered as potential biocontrol agents, for several of them have shown a positive effect on growth and survival of infected plants. The mechanisms underlying these effects are, however, still unknown. It is possible that they result from a chemical antagonism or a physical barrier of the ECM fungus over the pathogen, but other hypotheses involve active responses by the plant. *Amanita muscaria* is an important ECM fungus of *C. sativa* in the Northeast of Portugal. The present study intended to investigate a possible antibiosis effect of *A. muscaria* (Am) over *P. cinnamomi* (Pc). For this purpose, we used isolates of Am and Pc collected from a local chestnut orchard. The interaction between the organisms was tested by the dual culture technique on Petri dish, using mycelial inoculum, in two different culture media: MMN and PDA. The following dual cultures were tested: Am + Am, Pc + Pc, Am + Pc (inoculated simultaneously) and Am → Pc (Pc inoculated 7 days after the inoculation of Am). Interaction was analysed daily for a period of seven days (given the rapid growth of Pc), and was based on radial growth and morphological features of both organisms. The growth of *P. cinnamomi* was heavily constrained by the presence of *A. muscaria* (in both Am + Pc and Am → Pc dual cultures), and more spores were formed, when compared with Pc + Pc cultures. The results suggest a heavy antagonistic effect of the ECM fungus over the pathogen. Other ECM fungi are currently under study for the analysis and comparison of possible different effects over *P. cinnamomi*.

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IN VITRO CULTURE CONDITIONS OF COLLETOTRICHUM GLOEOSPORIOIDES FOR SPORE PRODUCTION: a) EFFECT OF MEDIUM COMPOSITION AND LIGHT ON SPORE PRODUCTION

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Colletotrichum gloeosporioides, the imperfect stage of the ascomycete *Glomerella singulata*, is responsible for anthracnose in a wide variety of plant species. *Hypericum perforatum*, which is responsible for the synthesis of hypericin, a metabolite with important pharmaceutical applications, is one of the species sensitive to *C. gloeosporioides*. Since *H. perforatum* tolerance to *C. gloeosporioides* is dependent on plant variety, we intend to select tolerant varieties *in vitro* for metabolite production without fungicide application. *C. gloeosporioides* spores are needed to inoculate and induce a selective pressure on plants, but *in vitro* sporulation is particularly difficult to achieve. The establishment of *in vitro* conditions for sporulation was the main goal of the trials here presented. Two variables were tested for *C. gloeosporioides* spore production: medium composition and light. The effect of medium composition was tested by growing the fungus in PDA (Potato Dextrose Agar) and MMN (modified Melin & Norkrans). We also tested the influence of moving the fungus from PDA to MMN and vice-versa. Growth and spore formation was recorded for a period of three months. Light influence was tested by submitting the fungus to light and dark conditions, for both media. Growth and spore formation under these conditions was recorded for a period of two months. Preliminary results showed PDA to be a better medium for spore production in *C. gloeosporioides*. The presence or absence of light did not influence spore production.

In vitro interaction of *Amanita muscaria* and *Phytophthora cinnamomi*: possible biocontrol effect



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INTRODUCTION

Ink disease, caused by the Oomycetes *Phytophthora cinnamomi* Rands. and *Phytophthora cambivora* (Petri) Buism., is a major soilborne problem of European chestnut (*Castanea sativa*) stands all over South Europe. Chemical treatments are not effective and comprise serious environmental and economic costs, so biological control is under study as a possible solution for this problem. Ectomycorrhizal (ECM) fungi are generally considered as potential biocontrol agents, as several of them have shown a positive effect on growth and survival of plants infected with different pathogens.

The mechanisms underlying these effects are, however, still unknown. It is possible that they result from a chemical antagonism or a physical barrier of the ECM fungus over the pathogen, but other hypotheses involve active responses by the plant.

Amanita muscaria is an important ECM fungus of *C. sativa* in the Northeast of Portugal. The present study intended to investigate a possible antibiosis effect of *A. muscaria* (Am) over *P. cinnamomi* (Pc). For this purpose, we used isolates of Am and Pc collected from a local chestnut orchard.

MATERIAL & METHODS

Co-cultures of *Amanita muscaria* and *Phytophthora cinnamomi* mycelium were developed for the following conditions: Pc-Pc, Am-Pc (simultaneous inoculation) and Am $\xrightarrow{7}$ Pc (Pc inoculation 7 days after Am inoculation) in MMN (pH 6,6) and PDA (pH 5,4) media (6 replicas).



The radial growth of Pc towards Am (internal radius, Ri), was measured for a period of seven days of co-culture.



Pc was characterized macro and microscopically, after 2 and 15 days of co-culture.



RESULTS

Growth of *Phytophthora cinnamomi* on PDA

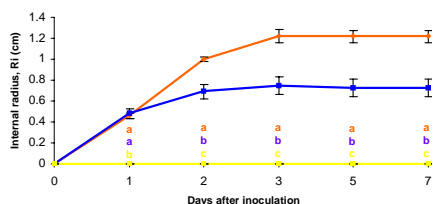
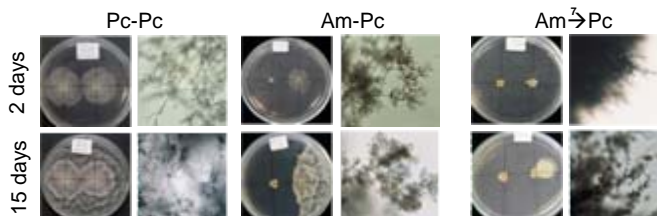


Figure 1 – Average values (\pm SE, n=6) of the internal radius (Ri) of Pc for the systems Pc-Pc (—), Am-Pc (—) e Am $\xrightarrow{7}$ Pc (—) in PDA medium. For each day, values marked with different letters are statistically different, as determined by the Student-Newman-Keuls test (p<0,05).

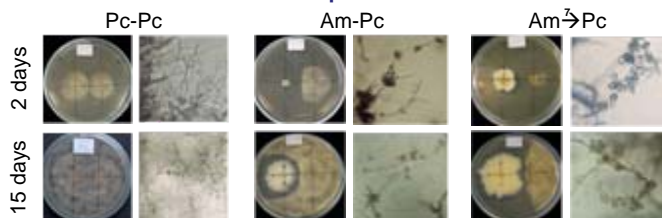
Macro and microscopic characteristics of Pc



Even though *A. muscaria* does not grow on PDA, it seems to exert a strong antagonistic effect over *P. cinnamomi*.

Growth of *Phytophthora cinnamomi* on MMN

Macro and microscopic characteristics of Pc



A. muscaria grows better on MMN than on PDA, but its antagonistic effect over *P. cinnamomi* seems to be less marked.

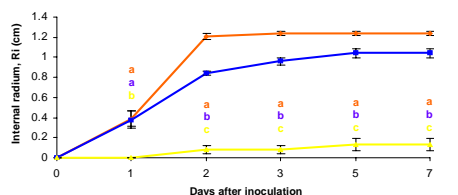


Figure 2 – Average values (\pm SE, n=6) of the internal radius (Ri) of Pc for the systems Pc-Pc (—), Am-Pc (—) e Am $\xrightarrow{7}$ Pc (—) in MMN medium. For each day, values marked with different letters are statistically different, as determined by the Student-Newman-Keuls test (p<0,05).

CONCLUSIONS

1. The growth of *P. cinnamomi* was heavily constrained by the presence of *A. muscaria* (in both Am-Pc and Am $\xrightarrow{7}$ Pc dual cultures).
2. More spores were formed, when compared with Pc-Pc cultures.
3. The results suggest a heavy antagonistic effect of the ECM fungus over the pathogen.
4. Other ECM fungi are currently under study for the analysis and comparison of possible different effects over *P. cinnamomi*.

