The influence of pyroligneous acid (lat. *acetum pyro-lignosum*) application on the growth of *Botrytis cinerea* pythopathogen

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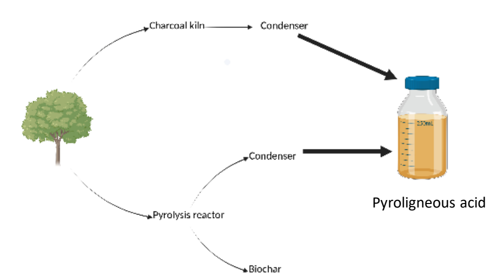
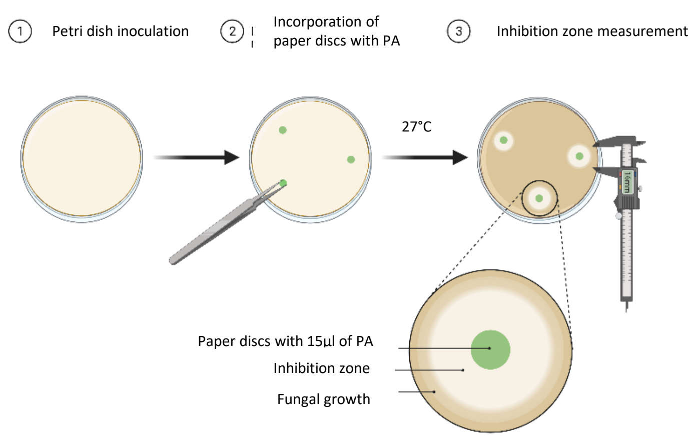
INTRODUCTION

In recent years, climate change has been challenging the achievement of high product quality and quantity to meet growing global demand. These phenomena adversely affect crops by bringing long periods of drought alternating with short periods of rain, creating favourable environments for the proliferation of pathogenic fungi. To protect the environment, the EU has drafted new decrees to safeguard the environment around us, encouraging the production of organic products without the use of pesticides or chemicals, leading to the origin of organic farming. In recent years, Poland has focused heavily on berry production, contributing 40% of the harvested area and yield in European strawberry production (FAOSTAT, 2022). Lately, due to climate change, farmers have found problems in stopping the proliferation of pathogenic fungi that lead to serious losses in the quality and quantity of the final product. *Botrytis cinerea* has considered the second most important phytopathogen. It is an economically important fungus, as it settles in the flower and does not appear until the fruit is fully ripe. In this project, we want to analyse a natural substance to slow down or stop the growth of *B. cinerea*. Recently, attention has begun to shift to the use of pyroligneous acid (PA, lat. *acetum pyro-lignosum*) because of its antimicrobial and antifungal properties (Grewal A. et al 2018). PA is a substance that can be obtained from the distillation of vapours obtained from the pyrolysis of wood (to obtain charcoal or biochar). This substance has already been tested against other fungi, such as *Alternaria mali*, *Trametes versicolor, Aspergillus niger* and *A. fumigatus* (Jung K.-H., 2007 and Suresh G. et al., 2019).
Our research aims to analyse six different concentrations of pyroligneous acid within an inhibition test towards three different *Botrytis cinerea* strains isolated from strawberry plants.



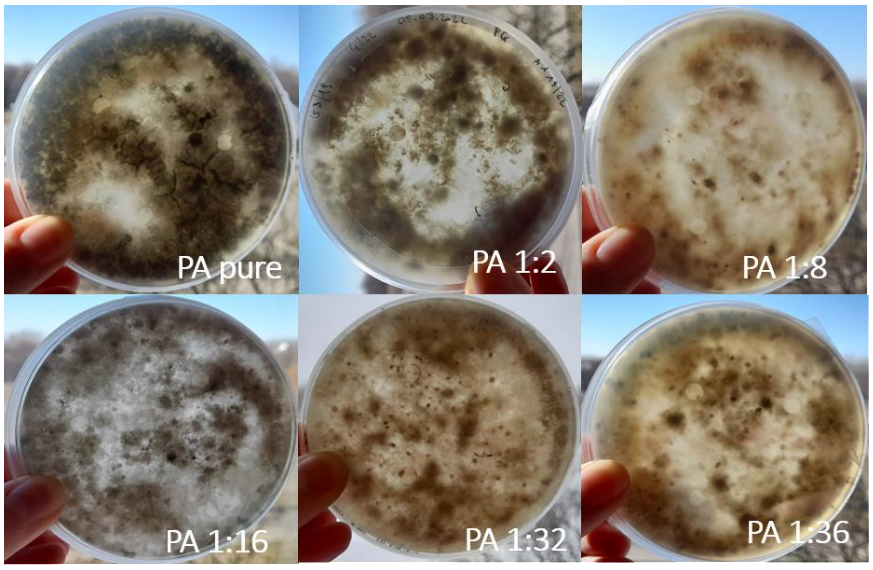
MATERIALS AND METHODS

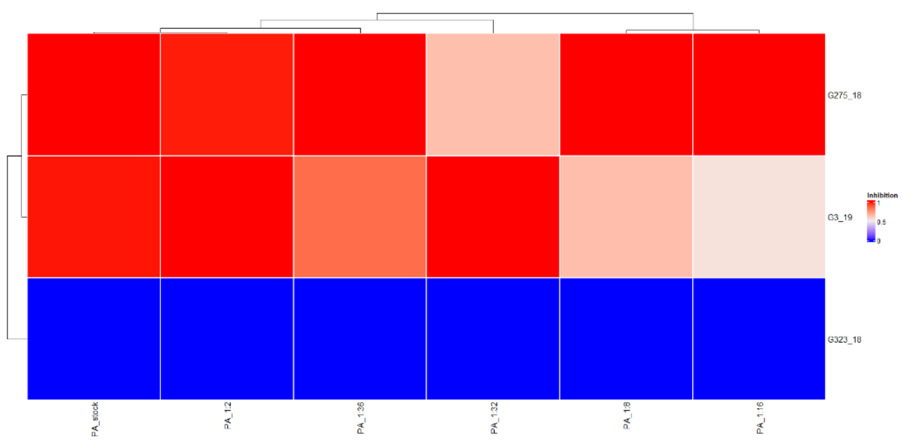
We pre-cultured in Potato Dextrose Agar medium (PDA) the three different strains of *B. cinerea* (G275/18, G323/18, and G3/19) at 27°C. After 5 days, we transferd a quarter of the fungal mycelium from the Petri dish into FF-inoculating fluid and measured the transmittance at 75% by turbinimeter (BIOLOG, USA). In each Petri dish, we spreaded 300 µl of the homogenised mycelium (at 75% of transmittance) and later applied 15 µl of the different PA concentrations (pure PA, 1:2, 1:8, 1:16, 1:32, and 1:36) to the culture media on three sterile paper disc.
Pyroligneous acid (PA) dilutions were prepared by using PA previously filtered through a 0.2 µm filter and then preparing the various dilutions with denaturalised sterile water. All plates were incubated at 27°C and every 24 hours the inhibtion zone was measured and photos taken.



RESULTS

After 7 days of sampling, we could see how the application of pyroligneous acid can influence the growth of *Botrytis cinerea*. From the photos, we can see that this substance did not lead to clear creation of inhibition zones without mycelium growth around the paper disc. Starting with the application of the highest concentration, it can be observed that at any concentration applied, minimal inhibition of fungal mycelium growth can be observed.
A zone of inhibition can be considered as such when a fungus fails to grow in the area adjacent to the application of the test substance, but an inhibiting effect can also be the manifestation of a low-density mycelium, as this too can be considered a negative aspect on fungal growth. In fact, in our case at the end of the sampling, we noticed the formation of large areas where the mycelium growth was less dense, compared to normal growth. This can be seen very well by comparing the fungus exposed to a dilution of 1:8 versus pure PA.
By representing the measurements of the zones of inhibition by heatmaps, we can observe the separation of the dilution analysed into two large groups. The first group consists of pure PA, PA 1:2, and PA 1:36 where the greatest inhibition can be observed while the second group consists of two dilutions of PA at 1:8 and 1:16 where only one fungal strain is highly affected by the application of this substance. Then there is a final dilution (PA at 1:32) that is somewhere in between the two groups describe above. During sampling, we observed that a strain of *B. cinerea* G323/18 presented fast growth and no strong inhibition compared to the other two strains. In fact, this strain showed a zone di inhibition for pure PA (8.06 mm) and PA 1:2 (8.89 mm). In comparison, the other two strains presented inhibition zones from a maximum of 15 mm to a minimum of 7 mm.
Another thing that can be observed in the photos is that PA diluted with water can lead to a better inhibiting effect on mycelium growth as the aqueous solvent can allow for better dispersion leading to an effect not only at the point of application but also at other points. This observation is very noticeable when looking at photos comparing pure PA to any diluted PA.





CONCLUSION

This preliminary experiment confirmed the antifungal effects of pyroligneous acid. Analysing these 6 different dilutions of PA, we observed not a strong inhibition leading to destabilisation of the pathogenic fungus responding with no growth of the mycelium. In our case, we noticed a response from the fungus by changing the density of the mycelium, thus thinking of an interference of the substance on the normal metabolism of *B. cinerea*.

FAOSTAT (https://www.fao.org/faostat/en/#compare)
Grewal A., Abbey L., Rao Gunupuru L. (2018) Production, prospects and potential application of pyroligneous acid in agriculture. J Anal Appl Pyrolysis 135: 152-159
Jung K.-H. (2007) Growth inhibition effect of pyroligneous acid on pathogenic fungus, *Alternaria mali*, the agent of Alternaria blotch of apple. Biotechnol Bioprocess Eng 12: 318-322.
Suresh G., Pakdel H., Rouissi T., Kaur Brar S., Fliss I., Roy C. (2019) In vitro evaluation of antimicrobial efficacy of pyroligneous acid from softwood mixture. Biotechnol Res Innov 3: 47-53.

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