Integrated Protection in Field Vegetables
IOBC-WPRS Bulletin Vol. 153, 2020
pp. 1-4

Characterization of a rare sugar against *Phytophthora* spp.

Abdessalem Chahed[1,2,3], Andrea Nesler[1,2], Ilaria Pertot[1,4], Gerardo Puopolo[1,4], Michele Perazzolli[1,4]
[1]Department of Sustainable Agro-ecosystems and Bioresources, Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38010 San Michele all'Adige, Italy; [2]Biological Products for Agriculture, Bi-PA, Technologielaan 7, 1840 Londerzeel, Belgium; [3]Department of Plant Induced Resistance and Bioprotection, University of Reims, Moulin de la Housse, 51687 Reims, France; [4]Center Agriculture Food Environment (C3A), University of Trento, via E. Mach 1, 38010 San Michele all'Adige, Italy
E-mail: michele.perazzolli@unitn.it

Abstract: *Phytophthora* spp. are one of the most aggressive and widespread plant pathogens responsible for important crop losses and it is controlled by fungicides. However, the overuse of chemical fungicides is a threat to human health and the environment. Eco-friendly fungicides are needed to improve sustainable plant protection strategies. The aim of this study was to assess the efficacy and to understand the mode of action of a rare sugar (tagatose) against *Phytophthora infestans* and *Phytophthora cinnamomi*. Tagatose inhibited *P. infestans* growth, but not *P. cinnamomi* growth, and it altered respiration-related activities. We demonstrated that tagatose is a promising compound that could be used as innovative biopesticide for the control of *P. infestans*.

Key words: rare sugar, *Phytophthora* spp., biological control, antioomycete

Introduction

Rare sugars are defined as monosaccharides and their derivatives that rarely exist in nature (Granström et al., 2004). Among them tagatose inhibited the growth of phytopathogens and it was patented for the control of important crop diseases, such as tomato and potato late blight (*Phytophthora infestans*) (Ohara et al., 2010). Tagatose could be a promising alternative to synthetic chemical fungicides, thanks to the absence of deleterious effect for human health (Levin, 2002; Vastenavond et al., 2011). However, the mode of action of tagatose on plant-associated microorganisms is unknown. The aim of this study was to elucidate the mode of action of tagatose in two phytopathogenic *Phytophthora* spp. *in vitro* and to provide deeper knowledge for the further development of eco-friendly fungicides.

Material and methods

Assessment of tagatose impact on Phytophthora spp. growth
Phytophthora infestans and *P. cinnamomi* were grown in Petri dishes on pea agar medium (PAM, 12.5% frozen peas and 1.2% agar in distilled water) at 18 °C and 25 °C, respectively (Puopolo et al., 2014). The radial growth of *P. infestans* and *P. cinnamomi* was assessed four and ten days after incubation (DAI) at 18 °C and 25 °C, respectively, as average of the two perpendicular diameters subtracted by the plug diameter and divided by two.

Assessment of the oxygen consumption rate and reactive oxygen species accumulation
The *P. infestans* and *P. cinnamomi* mycelial suspension was incubated in the absence (control) and presence of 5 g/l tagatose in pea broth in 96-well microplate under orbital shaking at 80 rpm at 18 °C and 25 °C, respectively. The oxygen consumption rate (OCR) was measured using the MitoXpress Xtra Oxygen Consumption Assay (Luxcel Biosciences) and Intracellular ROS were quantified with H_2DCF-DA (Molecular Probes, Thermo Fisher Scientific) according to the manufacturer's instructions.

Results and discussion

Phytophthora infestans growth was inhibited by tagatose at 4 and 10 DAI and the inhibitory effect was comparable between 5 g/l and 10 g/l tagatose (Figure 1 A). Conversely, *P. cinnamomi* growth was slightly inhibited by only 10 g/l tagatose at 4 DAI (Figure 1 B), indicating that tagatose exhibited differential activities against species belonging the same genus.

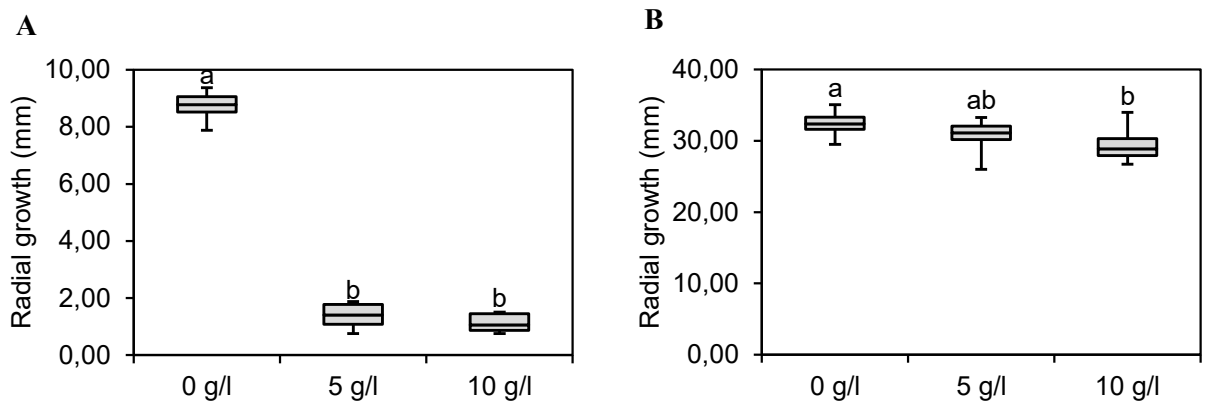
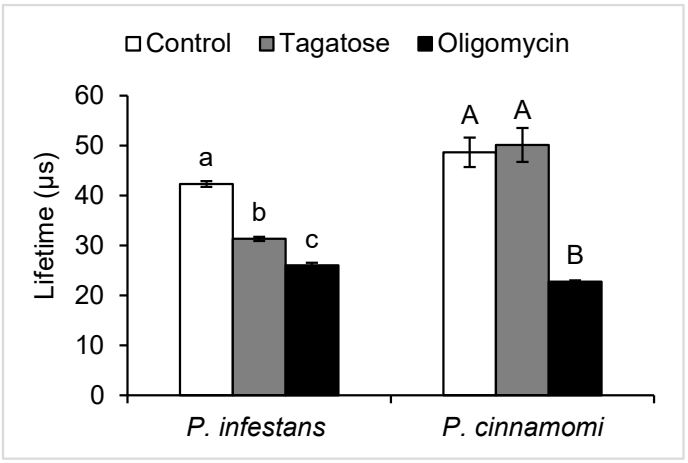


Figure 1. Effect of tagatose on *Phytophthora* spp. growth. *Phytophthora infestans* (A) and *P. cinnamomi* (B) growth (mm) was assessed four days after incubation (DAI) on pea agar medium in the absence (0 g/l) and presence of 5 g/l or 10 g/l tagatose. Different letters indicate significant differences according to the Kruskal-Wallis's test ($P \leq 0.05$).

The OCR was inhibited and ROS production was increased in tagatose-incubated *P. infestans* (Figure 2 A), suggesting inhibition of mitochondrial processes. On the other hand, the OCR and ROS generation were not affected by tagatose in *P. cinnamomi* (Figure 2 B).

Tagatose inhibited *P. infestans* growth and caused a reduction in the OCR and an increase of the ROS level, indicating severe deficiencies in tagatose-incubated *P. infestans*. On the other hand, *P. cinnamomi* growth, OCR and ROS accumulation were not affected by tagatose, indicating species-specific responses to this rare sugar. Further studies are required to better understand tagatose impacts on mitochondrial processes and gene expression regulations in *P. infestans* and *P. cinnamomi*.

A



B

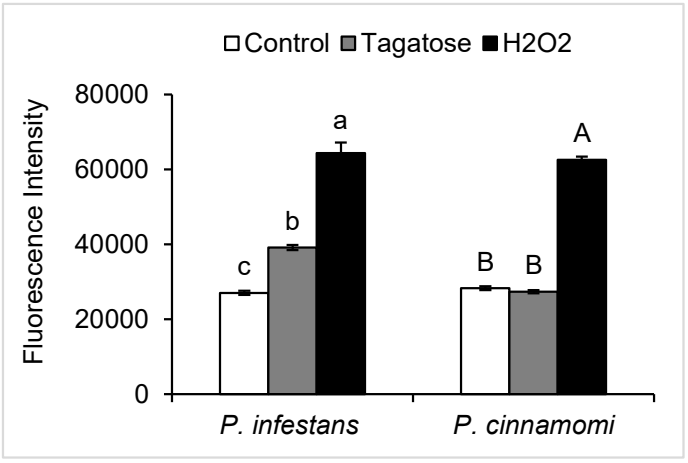


Figure 2. Effect of tagatose on *Phytophthora* spp. respiration processes and accumulation of reactive oxygen species**.** The oxygen consumption rate (A) and the generation of reactive oxygen species (B) of *P. infestans* and *P. cinnamomi* mycelial suspension was assessed 16 h after incubation in pea broth in the absence (white) and presence of 5 g/l tagatose (grey). Oligomycin and hydrogen peroxide (H_2O_2) were used as control treatment of the OCR and ROS assay (black), respectively. Different letters indicate significant differences according to the Kruskal-Wallis's test ($P \leq 0.05$).

Acknowledgements

This project has received funding from the European Union´s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement no. 722642 (project INTERFUTURE).

References

Granström, T. B., Takata, G., Tokuda, M., and Izumori, K. 2004. Izumoring. J. Biosci. Bioeng. 97: 89-94.

Levin, G. V. 2002. Tagatose, the new GRAS sweetener and health product. J. Med. Food 5: 23-36.

Ohara, T., Ishida, Y., Kudou, R., Kakibuchi, K., Akimitsu, K., Izumori, K., and Tajima, S. 2010. Plant disease control agent comprising d-tagatose as active ingredient, and plant disease control method. EP 2 329 713 A1**:** 1-46.

Puopolo, G., Cimmino, A., Palmieri, M. C., Giovannini, O., Evidente, A., and Pertot, I. 2014. *Lysobacter capsici* AZ78 produces cyclo(L-Pro-L-Tyr), a 2,5-diketopiperazine with toxic activity against sporangia of *Phytophthora infestans* and *Plasmopara viticola*. J. Appl. Microbiol. 117: 1168-1180.

Vastenavond, C., Bertelsen, H., Hansen, S. R., Laursen, R., Saunders, J., and Eriknauer, K. 2011. Tagatose (D-tagatose). In: Alternative Sweeteners (ed. O'Brien-Nabors, L.): 197-222. 4. ed., CRC Press, Boca Raton, Florida.