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Editorial

# Biological Control in Forests Protection

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**Citation:** Nowakowska, J.A.; Belbahri, L.; Oszako, T. Biological Control in Forests Protection. *Forests* **2023**, *14*, 446. https://doi.org/10.3390/f14030446

Received: 15 February 2023
Accepted: 15 February 2023
Published: 21 February 2023



Forests are a vital component of the natural environment as they support biodiversity, sequester carbon, play a key role in biogeochemical cycles, and produce the organic matter necessary for terrestrial organisms. Forests currently face threats ranging from natural phenomena such as lightning-caused fires, storms and some forms of pollution to man-made threats such as land use change (deforestation or intensive agriculture). In recent years, threats from pests and pathogens, especially non-native species [1], have increased in forests. Damage, decline and mortality caused by insects, fungi, pathogens and combinations of pests can lead to significant ecological, economic and social losses. In the fight against forest pests and pathogens, biocontrol can be an effective alternative to chemical pesticides and fertilisers. This review of forest pests and potential adversaries in the natural world highlights microbial inoculants and research efforts to further develop biological control agents against forest pests and pathogens. Recent studies have shown promising results for the use of microbial inoculants as preventive measures. Other studies suggest that these species have potential as fertilisers.

The possibilities of biological plant protection in Europe are now gaining particular importance in light of the Integrated Pest Management Directive and concern from both the agricultural (including forestry) and the horticultural sectors (fruit production or ornamental plants). There has been a change in approach, with a focus on environmentally friendly methods. This Special Issue shows several possibilities with the use of fungi of the genus *Trichoderma* against new invasive organisms of the genus *Phytophthora* [2], the bacteria *Aneurinibacillus migulanus* [3] and *Bacillus velezensis* [3], as well as the method of nanotechnology [4] or the use of organic substances [5].

The so-called ink disease is devastating to chestnut trees (*Castanea sativa*) worldwide and is caused by *Phytophthora* species. The only methods available to counteract it are chemical and agronomic treatments [2]. The authors of the study in the Tuscan Apennine (San Godenzo, Italy) focused on evaluating the in vitro antagonistic capacity of 20 Trichoderma isolates to reduce *Phytophthora xcambivora*. Each *Trichoderma* isolate was tested for its ability to inhibit the pathogen through antagonism in dual culture and antibiosis through the production of secondary metabolites (diffusible and volatile organic compounds). The six most productive isolates of *Trichoderma* spp. were evaluated for their ability to synthesise chitinase, glucanase and cellulase and to act as a mycoparasite. All six selected isolates showed the ability to control the pathogen in vitro through a synergistic coupling of antibiotics and mycoparasitism at different levels, regardless of the species to which they belong, but rather in relation to specific traits of single genotypes. In particular, *T. hamatum* SG18 and *T. koningiopsis* SG6 have shown the most promising results in pathogen inhibition, so further studies will be conducted to confirm their efficacy in vivo [2].

In addition to fungi, bioprotection can also use bacteria, e.g., *Bacillus velezensis*, a ubiquitous, non-pathogenic endospore-forming bacterium often isolated from soil, water, plant roots and fermented foods. It has the ability to promote plant growth under a variety of biotic and abiotic stress conditions and additionally inhibits many plant diseases, including bacterial, fungal and oomycetes. Once the root is colonised, host plants respond more quickly and/or effectively to biotic and abiotic stresses. The analysis of the pangenome and subsequent identification of primary, accessory and unique genomes is of great importance for developing more effective biocontrol agents and fertilisers [6].

Another soil bacterium with great potential is *Aneurinibacillus migulanus*, whose Nagano and NCTC 7096 strains have the ability to actively suppress several plant diseases in agricultural and forest ecosystems [3]. Both strains produce gramicidin (GS), a biosurfactant, and inhibit the growth of certain bacteria and fungal-like organisms through haemolysis. In the present study, the *A. migulanus* Nagano strain was found to inhibit all 13 species of *Phytophthora* pathogens. In apple infection tests, *P. rosacearum* MKDF-148 and *P. cryptogea* E2 were found to be the most aggressive, and poractivation of *A. migulanus* Nagano apples (which had clear infection halos) significantly reduced the severity of disease symptoms, indicating that GS is a major factor affecting biocontrol capabilities [3].

Attempts have also been made to use extracts from wood-decomposing plants [7] and wood-decomposing fungi [8] to control a harmful insect and *Fusarium oxysporum*, the cause of seedling death, respectively.

Active extracts were obtained from *Parthenium hysterophorus*, whose aqueous extracts killed the harmful mealybug on another plant, *Dalbergia sissoo* [7]. At doses of 200 µg/mL and 500 µg/mL, high (80%) insect mortality was detected after 72 h in the contact method. The results suggest that aqueous *Parthenium* extracts could be an environmentally friendly insecticide for large-scale use in forestry.

Extracts that inhibit phytopathogenic fungi, e.g., the *F. oxysporum* complex (*sensu lato*), may use active compounds produced by other wood-degrading fungi commonly found in forests [8]. Such effective activity in preventing and treating infested seedlings has been observed with methanolic extracts of *Fomitopsis pinicola*, *Ganoderma applanatum* and *Trametes versicolor*. The latter two species have shown particular biological activity in increasing plant resistance. Field trials are currently needed to confirm the results obtained in laboratory studies on plant protection.

In plant protection, not only two-organism tests on Petri dishes [2] and morphological analyses [8] but also molecular biological techniques, e.g., the expression of HSP genes responsible for the formation of resistance proteins [9], are being used with increasing success. These are particularly useful for studying entomopathogenic fungi of the genera *Beauveria*, *Metarhizium*, *Purpureocillium*, *Lecanicillium* and *Paecilomyces* [10] or biodiversity in soils as specific environmental resistance to fungal pathogens [5].

To uncover the adaptation mechanism in response to high-temperature stress, genes (DEGs) in the parasitic beetle *Dastarcus helophoroides* were studied in breeding (commercial) and natural populations [9]. A high-throughput sequencing technique was used to sequence the transcriptome of the two *D. helophoroides* populations. A total of 47,763 non-redundant transcripts with an average length of 989.31 bp and an N50 of 1607 bp were obtained. Under high-temperature stress conditions, 1108 DEGs were found in the commercial population, while 3946 DEGs were found in the natural population, which was 3.56-fold higher than in the commercial population. The high-temperature stress of *D. helophoroides* promoted the expression of heat shock proteins (HSP) and metabolism-related genes in both populations, but the synthesis and hydrolysis metabolism of the natural population was much higher, allowing it to produce more resistant substances (such as HSP, superoxide dismutase (SOD), peroxiredoxin (Prx), etc.). These results show that it is possible to select and domesticate a specific ecotype of *D. helophoroides* with better efficacy as a biocontrol agent.

So far, the presence of entomopathogenic fungi (EPF) in soil [10] has not been investigated in Croatian forests. In 2018, 2019 and 2020, their occurrence, diversity and distribution were studied, and their abundance was assessed in soil samples collected throughout the country and analysed by fungal isolation on selective culture media. To assess EPF density in soils, colonies of each fungal species were counted, and the results were expressed as the number of colony-forming units (CFU) per gramme of dry soil. After morphological and molecular analysis, five genera of entomopathogenic fungi were identified: *Beauveria* spp., *Metarhizium* spp., *Purpureocillium* spp., *Lecanicillium* spp. and *Paecilomyces* spp. The results showed that the range of total EPF colony density in soil was between $4 \times 103$ and $27.4 \times 103$ CFU $g^{-1}$. EPFs of the genus *Beauveria* were the most common, detected at four of the five sites and at 16 of the 25 sampling sites, but the highest average number (density) of colonies belonged to the genus *Metarhizium*. As this type of study has never been conducted in Croatia, this is the first evidence that insect pathogenic fungi are present in the soils of different natural forest sites. Such studies can help in the selection and use of entomopathogens suitable for biological control in specific target areas [10].

Other environmentally friendly technological solutions are also constantly being sought in forest conservation. It seems that nanotechnology can be an environmentally friendly alternative [4]. Experiments have been conducted to confirm the antifungal effect of silver and copper nanoparticles against the white rot fungus *Fomes fomentarius* in vitro and for the protection of *Fagus sylvatica* wood. Silver nanoparticles inhibited fungal colony growth at the highest concentration of 50 ppm [4].

A 20-year study of a pine stand on post-agricultural land in Poland [5] has shown that woody debris in the form of organic matter (wood residues, sawdust, bark compost) can be successfully used to restore symbiotic mycorrhizal communities, as is the case with forest soils. The restoration of organic matter in soils that had been altered by long-term agricultural use had a cohesive effect on the composition of mycobiota antagonistic to pathogens, especially *Heterobasidion annosum*. The natural formation of the forest habitat was accelerated, e.g., by numerous fungi of the genera *Amanita* and *Russula* or hyperpathogens of the genus *Trichoderma*, which shape the resistance of the soil environment to pathogens. Mycorrhizal fungi not only increased the absorption surface of the roots many times over but also protected them (mechanically and chemically) from pathogens.

**Funding:** The study was partly carried out within the framework: WZ/WB-INL/3/2021 and financed from the science funds from Ministry of Science and Higher Education in Poland.

**Conflicts of Interest:** The authors declare no conflict of interest.

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