IHPS Inštitut za hmeljarstvo in pivovarstvo Slovenija

Hmeljar

1-12/2024
ISSN za tiskano izdajo: 1318-6183
ISSN za spletno izdajo: 2536-1570
Januar - december 2024, letnik 86, strani 1-92

English translation of the publication of Dr. Sebastjan Radisek's group on pages 76-78 of the Magazine ***Hmeljar*** (Hops) published by Slovenian Institute of Hop Research and Brewing (IHPS). The described work has been developed in the context of SUSTEMICROP Project (PRIMA 2021 Section 2)

Development of new approaches for the prevention of Verticillium wilt, downy mildew, and powdery mildew in hops in the framework of the European project SUSTEMICROP

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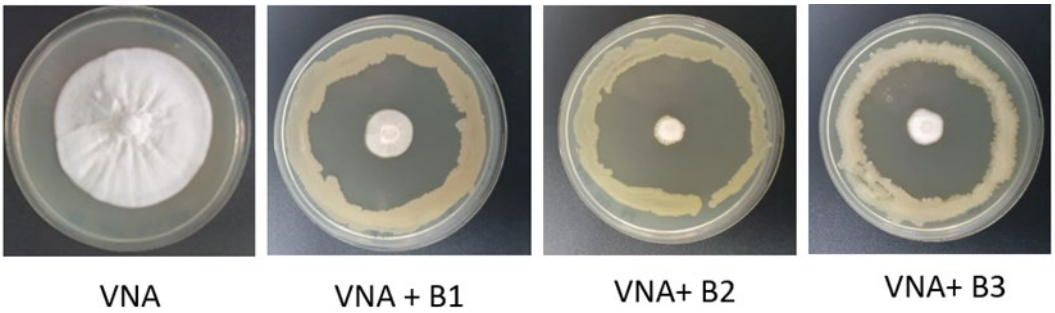
The **SUSTEMICROP research project** (**https://sustemicrop.eu/sl/**) is carried out by a consortium of 10 institutions from seven countries and aims at developing new sustainable low-risk plant protection methods on vines, hops and date palm. The project is divided into four technological areas: (1) discovery of new beneficial micro-organisms for the prevention of soil pathogens, (2) development of new essential oil-based fungicides for the prevention of foliar diseases, (3) resistance testing and introduction of resistant varieties for planting, and (4) on-farm evaluation of the effectiveness of the innovations introduced. IHPS collaborators, together with Spanish colleagues from the University of Leon, are carrying out activities in hops with a focus on the prevention of soil-borne diseases such as Verticillium wilt and fusarium wilt of hops, as well as the protection of plants against infections by downy mildew and powdery mildew.

Preventing Verticillium wilt with microbiologically enriched compost

Verticillium wilt of hops is caused by the soil fungi *V. nonalfalfae* and *V. dahliae*, which can parasitise many plants other than hops, especially broad-leaved plants. In hops, the most serious infections are caused by *V. nonalfalfae* in the form of the lethal pathotype (PV), with infections developing to the disease stage of plant death on susceptible varieties. The fungus forms persistent organs on infected plants which allow it to survive in the soil for several years. This leads to the accumulation of permanent organs in the soil over time and, consequently, to the progression of the infection in the plantation. As the infection is carried out via the roots, the plants can also defend themselves by the rich and symbiotic microbiota on the roots, which can prevent infection through competition and antifungal production. In the case of perennial monocultures with intensive tillage, the presence of certain beneficial micro-organisms is often depleted, which can be mitigated by adding certain substrates to the soil, such as green manures (monocultures) and compost, and by using microbiological preparations (biofertilizers or biofungicides).

In order to detect beneficial bacteria and fungi directly from the hops, the first part of the project involved a comparative metataxonomic analysis of the rhizosphere of infected and non-infected plants to identify significant differences in the composition of the microbiota and to gain insight into the microorganisms present on the roots of hops. We have published a dedicated paper on this topic in the reputable scientific journal *Microorganisms* (accessed from https://doi.org/10.3390/microorganisms11071819). In addition, several isolates of beneficial bacteria and streptomycetes have been isolated from hops in Slovenia and Spain and tested to show that they can completely kill or inhibit the growth of *V. nonalfalfae* in cultivation and, in the case of hops tested by artificial infection, significantly slow down the development of Verticillium wilt. The overall

selection was highly selective, as out of more than 700 initial isolates, only two isolates of *Streptomyces* and one of *Pseudomonas* were selected for further field trials.



Inhibited colony development of *Verticillium nonalfalfae* (VNA) on microbiological medium in the presence of beneficial bacteria (B1, B2, B3) (Photo: C.Calvo-Peña)

Testing the effectiveness of beneficial bacteria in combination with hop compost to prevent hop wilt in the growth chamber (Photo: S. Radišek)

For the beneficial bacteria to work effectively in the field, it is necessary to provide sufficiently high doses and to create the conditions for their development and colonisation on the roots. This can be achieved by pre-multiplication on substrates such as composts, which are then used for fertilisation.

Thus, in 2024, we started three-year field trials using composted hops in which we mixed a mixture of three selected isolates prepared by the University of Leon and Agrogenia Biotech (Spain). The enriched compost experiment is being carried out in two infected hop fields of the cultivars Celeia and Aurora, where the test field in each hop field was divided in a block into a composted and a non-composted part. The choice of the test fields was based on a comparable level of infestation. The application of compost was carried out after de-soiling at a rate of about 15 t/ha, followed by cultivation to apply the compost close to the hop roots. As a parallel experiment, in both plantations,

plants were replanted on the sites of the diseased plants that had died, part of them in the compost and part of them directly in the soil.

Application of enriched compost in a test section of a hop field infested with hop wilt (Photo: S. Radišek)

Planting seedlings in enriched compost in places where plants have died from hop wilt in the previous year (Photo: S. Prislan)

The results of the first year of the trial showed a quarter lower incidence of diseased plants in the compost-applied section in the case of the Celeia hop field, while the incidence in the case of the Aurora variety was comparable between the two fields, but limited to single diseased plants.

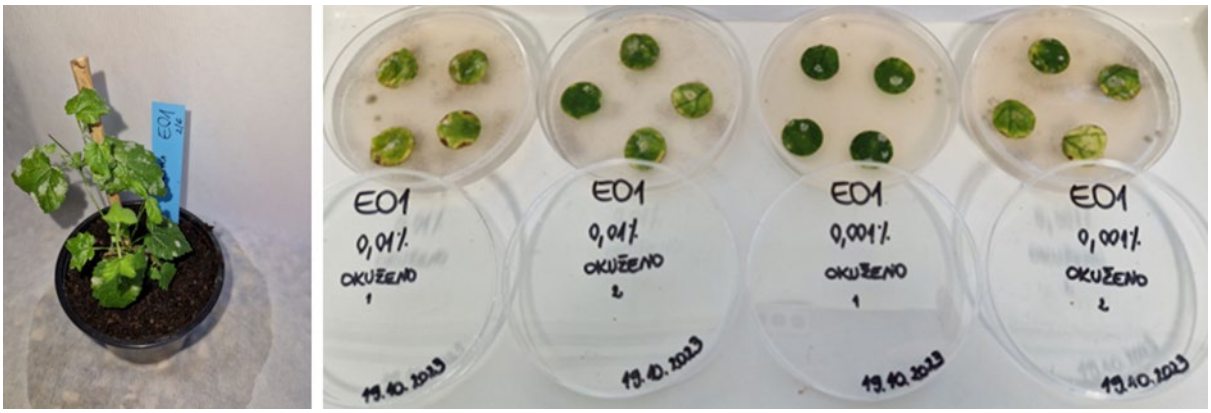
The application of compost and observations on disease development will be continued for the next two years, which should be sufficient to determine **whether the application of enriched compost in hop fields does indeed have an effect on reducing the incidence of hop wilt.** In addition to monitoring disease incidence, we have also started a series of measurements of other factors. Thus, in order to monitor the uptake of nutrients and organic matter, chemical analyses of the composts, chemical and pedological analyses of the soils in the test plots before and at the end of the growing season, measurements of the quantity and quality of the crop on representative plants, and samples to monitor the microbiological activity of the soil were carried out. In the first year, differences were detected mainly at yield level, as the compost-treated plants developed higher yields (up to 15%) in both varieties, while no differences were detected in cone quality parameters.

Development of new low-risk fungicides for the control of downy mildew, and powdery mildew in hops

In the second technology strand, we have started testing the efficacy of essential oils that could form the basis for the development of new fungicides to prevent infections by downy mildew, and powdery mildew in hops.

The essential oils tested included rosemary, lavender, lemongrass, eucalyptus and motherwort, which were prepared at the National Research Institute of Rural Engineering, Water and Forestry (INRGREF) in Tunisia. In a first step, a phytotoxicity test was carried out on hop leaf discs and concentrations below 0.01% were found to be suitable for further work. Efficacy tests were carried out using the leaf disc method and by growing the plants in a growth chamber, exposing them to hop ash borer and hop peronospores and to ideal conditions for disease development. The plants were sprayed weekly with an essential oil emulsion, using a chemical preparation with the active substance metrafenone (Vivando ®) as standard in the case of hop powdery mildew and Cuprablau Z 35 WP in the case of downy mildew. The results of the tests showed a preventive action of lavender essential oil in the case of hop powdery mildew, while in the case of downy mildew we found a very good action of lemongrass and eucalyptus essential oils.

Similar trials are being carried out in France on vines by the project partner, the French Institute of Vine and Viticulture (IFV), focusing on testing the prevention of downy mildew on vines. **Based on the results obtained by IHPS and IFV, formulated preparations for selected essential oils will be produced, which will also be suitable for testing in plantations, with the final objective of developing a new fungicide preparation.**



Testing the efficacy of essential oils to prevent hop powdery mildew using the leaf disc method (right) and plants grown in a growth chamber (left) (Photo: S. Radišek).

Acknowledgements: hop growers Jure Stepišnik, Sebastjan Prislan, Jaka Orožim and Karli Orožim are actively involved in the SUSTEMICROP project by conducting field trials, for which we would like to thank them.

This Project is part of the PRIMA programme supported by the European Union