Almighty Cover Crops

In the BIOVINE project, cover crops are tested in vineyards for several plant protection purposes.

Fungal pathogens (e.g. *Plasmopara viticola*, *Botrytis cinerea*, *Phaeoacremonium* spp.) are able to produce inoculum (spores) on plant debris present on the soil surface of vineyards. These spores can then reach plant surfaces and cause severe grapevine infections when environmental conditions are favorable. The capacity of plant diversity to increase the resistance of crops towards pests and invasive species is very well-known. For instance, *Brassica* spp. have been already investigated for their capacity to effectively suppress soil-borne inoculum of *Cylindrocarpon*-like asexual morphs (causal agents of Black-foot disease in grapevines) in vineyard soils. It may also have positive effect on the dagger nematodes of *Xiphinema* genus, in particular the species *Xiphinema index*. Cover crops also stimulate the development of microbial communities such as arbuscular mycorrhizal fungi (AMF). Many management strategies have been developed against these important grapevine pathogens, but the effects of soil cover vegetation or organic mulching against spore dispersal, acting as a barrier, have been scarcely explored.

Thus, in the BIOVINE project (www.biovine.eu) specific experiments were planned in order to verify the possibility of using cover crops: i) to control some relevant pathogens producing inoculum (spores) on plant debris present on the soil surface of vineyards; ii) to determine the presence of *Cylindrocarpon*-like asexual morphs and also of *Cadophora* spp., *Phaeoacremonium* spp., and *Phaeomoniella chlamydospora* (causal agents of Petri disease of grapevines) on the roots of cover crops; iii) to promote AMF communities associated with grapevine roots; iv) to control arthropod pests (repellent of arthropods or attracting beneficials); v) to investigate *Brassica* plants effect on soil-borne pest nematode *Xiphinema index;*

Splash and air dispersal small plot experiment

An experiment at the campus of the Università Cattolica del Sacro Cuore of Piacenza, was set up in small plots, either grown or not with cover crop plants. The main goal was to verify the possibility of reducing the amount of splash- and air-borne spores escaping from the soil surface in order to reduce the disease risk. Different plots were created sowing horseradish (*Armoracia rusticana*) at different timing in order to have simultaneously different cover crop heights (none on bare soil, plants developed in about 10, 40 and 80 cm in heights; see Figure 1). In two separate experiments the percentage of soil directly reached by rainfall drops and the dispersal of rain splashes on leaves were assessed.

In a first experiment, small strips of blotting paper were randomly placed on the ground in the different plots, then artificial rainfall was provided using a blue coloured water. Although, rainfall drops were able to reach the soil, the average value was significantly lower in the plot where the cover crop was growing. The most relevant difference was observed in the plot where the horseradish was at the beginning of its development and by expanding its leaves increased the “shield” effect on the soil (about 10 cm in height, Figure 1B).

In a second experiment, the soil was uniformly covered with a coloured powder, highly hygroscopic, in order to mark raindrops when they touch the soil (Caffi and Rossi, 2012). Blotting paper strips were placed at different heights (i.e. 40, 80 and 120 cm) in each plot in order to catch the coloured splashes (Figure 2). The highest number of splashes colouring the artificial leaves were observed on bare soil,

while the presence of the horseradish was able to significantly reduce the number of potential dispersals of soil-borne or soil-transient pathogen propagules (Figure 3).

Trunk pathogens detection on cover crops' roots

Cover crop plants were carefully dug out from the soil to keep the root system intact, introduced in plastic bags and stored in a fridge at 4 ºC until the moment to be processed at the UPV. In total, 44 cover crops samples were evaluated in 2019 from experimental plots established in Italy (n=22), Romania (n=12), Slovenia (n=4) and Switzerland (n=6). Fungal isolations were performed on Malt Extract Agar + Streptomycin Sulphate (MEAS). Fourteen roots fragments and seven internal crown fragments were plated per plant, incubated in darkness at 25ºC for 10–15 days, and fungal colonies transferred to Potato-Dextrose Agar (PDA) for sporulation and identification. For fungal identification, microscope observation of fungal colonies was performed aiming to a tentative grapevine trunk pathogens selection. Then, single spore cultures were obtained from selected colonies, which identity was confirmed by using gene sequencing (ITS and HIS-3 regions were studied).

From these samples, 12 *Cylindrocarpon*-like asexual morphs isolates were obtained, all belonging to the species *Dactylonectria torresensis* (Figure 4). This species is a pathogen associated with black-foot disease of grapevines worldwide. In fact, among the causal agents of this disease, *D. torresensis* has been reported as the most frequent species. Moreover, *D. torresensis* showed some preference to colonize *Trifolium repens*, because 5 out of 12 isolates were obtained from this cover crop species from samples collected in Italy, Switzerland and Romania. Regarding other grapevine trunk pathogens such as *Cadophora* spp., *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora*, they were not detected in any cover crop sample. These results showed that some cover crop species can act as alternative hosts for the species *D. torresensis*.

Detection of AMF communities

In the first part of the BIOVINE project, 59 plant species were selected; both cover crops generally used in agriculture and common weeds. Their ability to form AM symbiosis was evaluated under controlled conditions using one AMF. First results show that the level of AMF root colonization depends on plant species and plant families. Interestingly, some common weeds are better colonized compared to cover crops, suggesting these plants could be a reservoir of AMF diversity in the vineyard and with which the grapevine could interact during its developmental cycle and benefit from their services.

Biovine partners sampled cover crops and grapevine roots (Fig 5.) at different stages of grapevine development. Cover crop root system was carefully dug out from the soil to keep it intact, washed, and stored in plastic bags at 4 ºC until DNA is extracted. In 2020, root colonization of 39 cover crops and 22 grapevine root samples was estimated. DNA from all samples was extracted and AMF diversity was assessed by sequencing a fragment of the large ribosomal sub-unit.

Detection of arthropod pests and beneficials

An extensive systematic literature search was performed to identify plant species suitable for repelling the grapevine moth (*Lobesia botrana*) and for conserving and promoting beneficials.

i) Repel grapevine moth: *Allium sativum*, *Armoracia rusticana*, *Artemisia absinthium*, *Lavandula angustifolia*, *Tagetes* sp. and *Tanacetum cinerariifolium* were identified as potential candidates to repel *L. botrana* from grapes. Extracts of these plant species were prepared and tested in the

laboratory. The tested extracts had neither a strong effect on the survival of *L. botrana* larvae, nor did they repel larvae from feeding. However, nearly all extracts repelled females from egg laying (Fig. 6). Thus, the extracts from *Allium sativum*, *Artemisia absinthium* and *Tagetes* sp. were retrieved and tested against *L. botrana* under field conditions. *Lobesia botrana* infestation was lowest on grapes protected by a mixture of these three extracts. Thus, this mixture of extracts might have potential to protect vines against grapevine moths.

ii) Attract and conserve beneficials: Many beneficials such as predatory mites, spiders, carabids, ladybirds, lacewings, hoverflies and parasitoids feed on pollen and nectar. Their activity can therefore be increased by the provision of nectar- and pollen-rich plant species (e.g. plants from the families Apiaceae, Asteraceae or Fabaceae). Selected cover crops were sown within vineyards in France, Italy, Romania, Slovenia, Spain and Switzerland and were sampled for the presence of arthropods according to a common protocol. Although there were large differences in the arthropod communities among the six countries in 2019, cover crops favoured the abundance of arthropods and predators overall. Moreover, predatory beetles were more abundant in vegetated vineyards than in vineyards with bare soil. However, this augmentation in beneficials did not lower the abundance of pests such as grapevine moths, spider mites or the two leafhoppers *Emposca vitis and Scaphoideus titanus* on grapevines.

Determination of Brassica plants effect on nematodes

The experimental vineyard was set up on a *X. index*-infested plot in Slovenia. The nematode population size was assessed by soil sampling to the depth of 30 cm, nematode extraction from the soil samples and *X. index* quantification using stereo microscope. Cover crops were sawn in April into the inter-row space and grown until the end of June when they were mulched and incorporated into the soil. The biofumigation** took place during the hot summer months of July and August. After biofumigation period *X. index* population was assessed again.

The results showed significant reduction of *X. index* nematode population on biofumigated plots compared to initial population. *Sinapis alba* reduced the *X. index* population for approximately 50 percent compared to the initial population size while biofumigation with *Brassica napus* and *Brassica nigra* decreased the nematode population to 57 percent and 61 percent of the initial population size, respectively. On the control plot (without cover crops and biofumigation), the population of the *X. index* increased to 117 percent compared to the initial population.

The experiments showed that Brassica cover crops used as biofumigant crops could reduce *X. index* nematode pests in the upper soil layer (up to 30 cm depth) (Fig.7), however, more than half of the initial nematode population remained in the soil. This approach can be advised to the farmers in addition to the management options of crop rotation and fallow periods which may shorten the period needed before vineyard replanting. However, the soil should be tested to confirm absence of the *Xiphinema* spp. nematodes before replanting.

Figure 1. Cover crops tested in Piacenza (Italy) at different development stages. Photo credit, dr. Tito Caffi.

Figure 2. Experiment of rain splashes dispersal on bare soil: artificial rain drops are coloured when touch the ground and the splashes are absorbed by the blotting paper positioned at different heights. Photo credit, dr. Tito Caffi

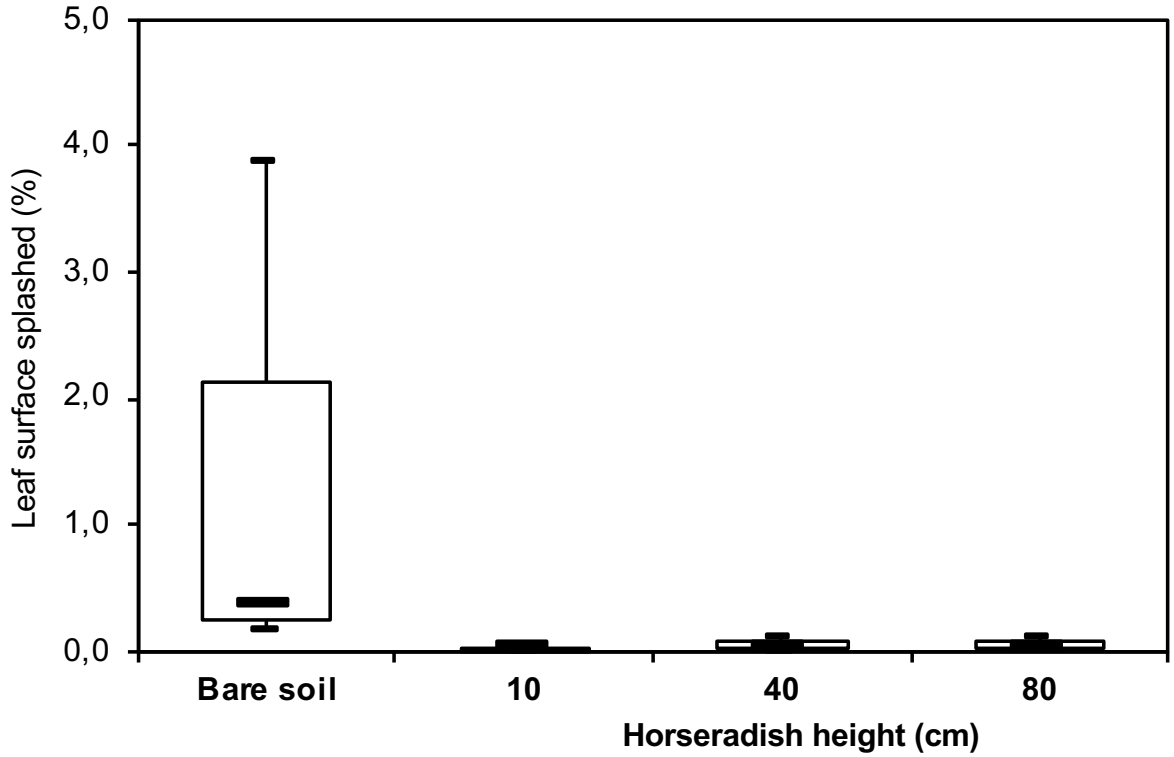

Figure 3. Boxplots of rain splashes observed on artificial leaves in the different experimental plots.

Figure 4. Colony of a Dactylonectria torresensis strain obtained from the roots of Trifolium repens in Switzerland. Photo credit, dr. Josep Armengol

Figure 5. Grapevine root sampled in France

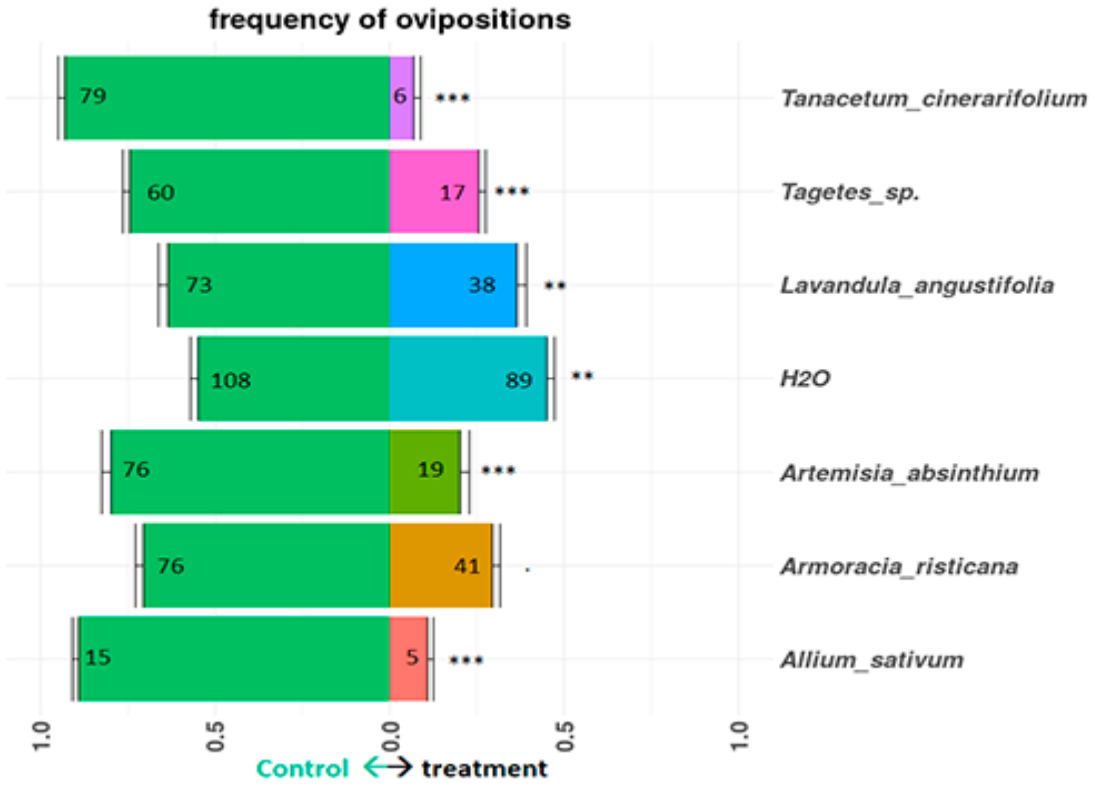

Figure 6. Effect of plant extracts on oviposition by Lobesia botrana females under laboratory conditions.

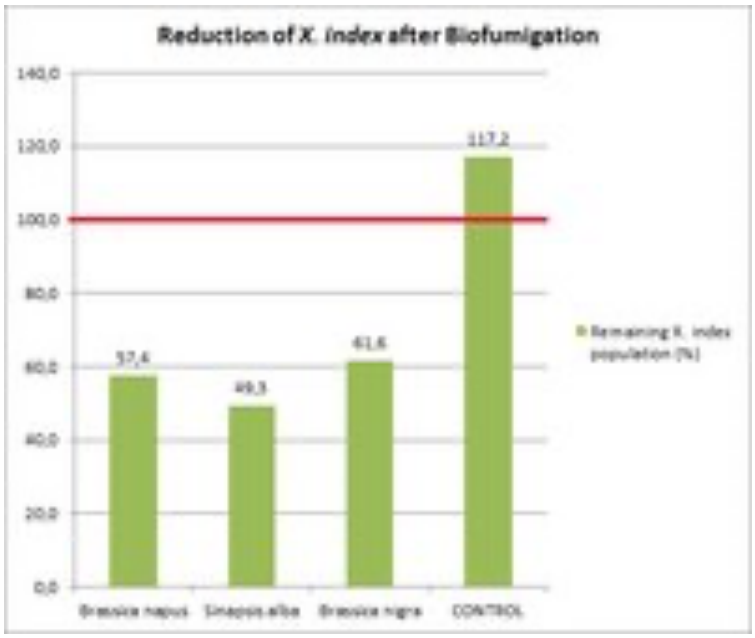

Figure 7. Population of X. index nematodes after biofumigation compared to the initial population size

Links & References

BIOVINE project website, www.biovine.eu

Rossi V. and Caffi T, 2012. The Role of Rain in Dispersal of the Primary Inoculum of Plasmopara viticola. Phytopathology, 102(2): 158-165. https://doi.org/10.1094/PHYTO-08-11-0223

Agustí-Brisach C. and Armengol J. 2013. Black-foot disease of grapevine: an update on taxonomy, epidemiology and management strategies. Phytopathologia Mediterranea, 52: 245-261.

Berlanas C., Andrés-Sodupe M., López-Manzanares B., Maldonado-González M.M. and Gramaje D. 2018. Effect of white mustard cover crop residue, soil chemical fumigation and *Trichoderma* spp. root treatment on black-foot disease control in grapevine. Pest Management Science, 74: 2864-2873. doi: 10.1002/ps.5078

Gramaje D., Urbez-Torres J.R. and Sosnowski M.R., 2018. Managing grapevine trunk diseases with respect to etiology and epidemiology: current strategies and future prospects. Plant Disease, 102: 12–39. doi: 10.1094/P DIS-04-17-0512-FE

Vukicevich E., D. Lowery T., Bennett J.A. and Hart M., 2019. Influence of groundcover vegetation, soil physicochemical properties, and irrigation practices on soil fungi in semi-arid vineyards. Frontiers in Ecology and Evolution. doi: 10.3389/fevo.2019.00118

Gianinazzi S., Gollotte A., Binet MN., van Tuinen D., Redecker D. and Wipf D. 2010. Agroecology: the key role of arbuscular mycorrhiza in ecosystem services. Mycorrhiza. doi.org/10.1007/s00572-010-0333-3.

Trouvelot S., Bonneau L., Redecker D., van Tuinen D., Adrian M. and Wipf D. 2015. Arbuscular mycorrhiza symbiosis in viticulture: a review. Agronomy for Sustainable Development. doi.org/10.1007/s13593-015-0329-7

Drain A., Bonneau L., Recorbet G., van Tuinen D., Wipf D. and Courty PE. 2019. Characterization of Arbuscular Mycorrhizal Communities in Roots of Vineyard Plants. Methods in Rhizosphere Biology Research. doi.org/10.1007/978-981-13-5767-1_3

Wipf D., Krajinski F., van Tuinen D., Recorbet G. and Courty PE. 2019. Trading on the arbuscular mycorrhiza market: from arbuscules to common mycorrhizal networks. New Phytologist. doi.org/10.1111/nph.15775

Noceto PA., Hériché M., Fromentin J., Rossi V., Širca S., Ranca A., Kehrli P., Armengol J., Trouvelot S., van Tuinen D., Courty PE. and Wipf D. 2020. Les couverts végétaux : un atout majeur pour réduire les intrants de synthèse et augmenter les services écosystémiques au vignoble. La Revue des Œnologues n°175

Bello, A., Arias, M., Lopez-Perez, J. A., Garcia-Alvarez, A., Fresno, J., Escuer, M., Arcos, S. C., Lacasa, A., Sanz, R., Gomez, P., Diez-Rojo, M. A., Buena, A. P., Goitia, C., de la Horra, J. L., and Martinez, C. (2004). Biofumigation, fallow, and nematode management in vineyard replant. Nematropica 34:53-64.

Ferris, H. & McKenry, M.V., (1976). Nematode community structure in a vineyard soil. J. Nematol. 8 131-137.

Monfort, W.S., Csinos, A.S., Desaeger, J., Seebold, K., Webster, T.M. & Diaz-Perez, J.C., (2007). Evaluating brassica species as an alternative control measure for root-knot nematode (M. incognita) in Georgia vegetable plasticulture. Crop Prot. 26, 1359-1368.

Zasada, I.A. & Ferris, H.,(2004). Nematode suppression with brassicaceous amendments. Soil Biol. Biochem. 36, 1017-1024.

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