USING COVER CROPS TO CONTROL LOBESIA BOTRANA IN ORGANIC VINEYARDS

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Abstract. This work aim to develop new viticultural systems based on increased plant diversity within vineyards intending to plant cover crops species with repulsiv or insecticide effects for the control of the grapevine moth (*Lobesia botrana*). *Lobesia botrana* is a major pest in European vineyards that can cause economic damages. SCV identified six potential candidates to repel *L. botrana* from grapes. In autumn 2018, SCV prepared extracts of these plant species and provided them to Agroscope. Over the winter, Agroscope tested the efficacy of these plant extracts 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, three of these extracts repelled L. botrana females from egg laying. Considering these results obtained by Agroscope, these plant extracts were prepared and tested by SCV in 2019. In an experimental vineyard in Murfatlar (Romania), the three extracts were applied either on their own or in a mixture of all three together at the ripening phase (BBCH 83-85). Pheromone traps were used to observe the impact on the adult pest population of *L. botrana*. Treatments in which the *Artemisia absinthium* extract was applied on its own or in the mixture recorded the smallest number of adults. Moreover, only 0.25% of grapes where infested by *L. botrana* in the mixture treatment compared to 3.8% of clusters in the treatment with the *Tagetes* sp. extract. We therefore conclude that the mixture of *Artemisia absinthium, Tagetes* sp. and *Allium sativum* might have an interesting potential to protect grapes against *L. botrana* infestation.

Keywords: viticulture, pest control, ecological system, repellency, organic farming.

INTRODUCTION

The capability of plants for increasing the resistance of ecosystems to pests and invasive species is a well-known ecosystem service. However, monocultures (including vineyards) do not exploit the potential of plant diversity (Gurr etl all., 2007, Tomoiaga, 2015). The BIOVINE project aims to favor this ecosystem services and to identify plant species able to repel and control arthropod pests.

Soil borne pathogens are responsible for 50% diseases that affect major crops, including grapevine (Ivanova et all., 2018). Besides that, in warm climate the grapevine moth *Lobesia botrana* is a major pest in European vineyards, its economic importance consists of a quantitative reduction of the harvest in spring due to the consumption of inflorescences and the infestation of berries over the summer, which enhances the risk of the development of pathogens such as *Botrytis cinerea* and in consequence decreases must and wine quality. Thus, an extensive systematic literature search was performed to identify plant species suitable for repelling *L. botrana* and for conserving and promoting beneficials.

MATERIAL AND METHODS

Plant extracts

SCV prepared plant extracts from dried aerial plant material in autumn 2018. Dried plant material of wormwood (Artemisia absinthium), horseradish (Armoracia rusticana), lavender (Lavandula angustilfolia), garlic (Allium sativum), chrysanthemum (Tanacetum cinerariifolium) and marigold (Tagetes sp.) were infused in 1 liter of rainwater (Table 1). In December 2018, Agroscope received these extracts in plastic bottles. Extracts were thereafter stored at 6°C in the fridge until the beginning of the laboratory trials.

Table 1

Summary table of the different plant extracts

Plant species	Concentrations used in trial	Dried plant material in 1 l of rainwater [g]
Artemisia absinthium	1	100
Armoracia rusticana	1	30
Lavandula angustilfolia	1	100
Allium sativum	1	12h in 3 tablespoon of linseed oil
Tanacetum cinerariifolium	1 (adult repellency) Dilution of 0.8 of the initial extract (larval toxicity)	100
Tagetes sp.	1	250

Artificial diet

An artificial diet for larval rearing of *L. botrana* was prepared with 25% of the Manduca - Heliothis Premix (Stonefly Industries, USA) and 75% plant extract or distilled water (=untreated control). Except for *T. cinerariifolium*, plant extracts were used pure and undiluted (Table 1). The seven different diets were prepared all at the same time and stored at 6°C until their application in the experimental trials.

Larval toxicity in the laboratory

40 plastic cubes (2x2x2cm) per treatment were half-filled with 2.5g of the corresponding artificial diet and infected with a newly hatched 1st stage larvae using a fine paintbrush. Infections were completed over two days. Cubes were incubated in a growing chamber (25°C, 70% RH, 16:8 photoperiod). After about a month, the first emergences were observed. Two weeks later, the final count of adults (presence/absence) was realised to calculate Abbott's efficacy. A generalized linear model with a bionomial distribution of the response variable (presence/absence of adults) was used to test the effect of the plant extracts compared to the control treatment using the package lmerTest (Kuznetsova et all, 2017, Core Team, 2018).

Larval repellency in the laboratory

Two rectangular plastic dishes (5x3.5x1cm) were disposed on each side of a plastic box (20x10x10cm). One rectangular dish was filled with 9 gram of a plant extract treated diet and compared to another dish of the same amount with only the pure artificial diet (=control treatment). Thereafter a 2x2cm piece of plastic film with mature *L. botrana* eggs was placed in between the two diet dishes. Then the plastic box was closed and stored for two days under controlled conditions in a climate chamber

(25°C, 70% RH, 16:8 photoperiod). After these two days, both diet dishes were taken out of the box and closed definitely with a lid. They were returned to the same climate chamber so that the hatched *L. botrana* larvae that crawled into the diet dishes could complete their development. Six weeks after the beginning of the trial, the number of emerged adults was counted in the diet dishes. Within each big plastic boxes, the proportion of adult in the two diet dishes was used as an indication of preference. Overall, each plant extract was tested 5-times against the control treatment. Due to its limited amount, *T. cinerariifolium* was not tested in this trial. A mixed effect linear regression was applied within each plant extract type with the count of adults as response variable. The treatment (plant treated media or control media) was used as fixed factor and the boxes as random factor.

Adult repellency in the laboratory

A transparent plastic yoghurt cup was divided into 4 equal sized zones on the outside with a pen. With a paintbrush, plant extract were applied on the inside every second zone and the other two zones were left untouched as a control. A total of five replicates per treatment were prepared. Additionally, 4 cups were set up to compare zones painted with tap water and control zones. Once the treated zones were dry, five couples of mated *L. botrana* were placed in each cup. A small plastic container with a cotton strand and filled with tap water was inserted in the cups to provide insects with water. After 2.5 days of exposure in the growing chamber (25°C, 70% RH, 16:8 photoperiod), the proportion of eggs per zone type within each cup was assessed. The effect of each treatment was evaluated separately with a mixed effect linear regression with the package lmerTest in R3,4. The number of eggs was used as a response variable, the treatment (treated zones or control zones) as a fixed factor and the cup as a random factor.

Adult repellency in the field

The extracts from *Allium sativum*, *Artemisia absinthium* and *Tagetes* sp. were retrieved and tested against *L. botrana* under natural field conditions in the experimental organic vineyard of SCV at Murfatlar during the ripening phase of grapes (BBCH 83-85). The three extracts were applied both individually and in a mixture where all three were combined. Pheromone traps were used to observe the population dynamics of *L. botrana* adults. In addition, grape infestation was observed to calculate the level of attack. For statistical calculations SPSS Statistics 17.0 software package was used and the Duncan test was applied to compare treatments.

RESULTS AND DISCUSSIONS

Larval toxicity in the laboratory

Only the diet treated with the *Artemisia absinthium* extract showed a statistically significant efficacy of 36.1% compared to the control diet (Figure 1). However, an efficacy of 36% under our laboratory conditions with the present *A. absinthium* extract can be considered to be insufficiently toxic and effective under field conditions. In the past, an active ingredient had to reach an efficacy of about 90% under our laboratory conditions to show a substantial effect in a commercial vineyard.

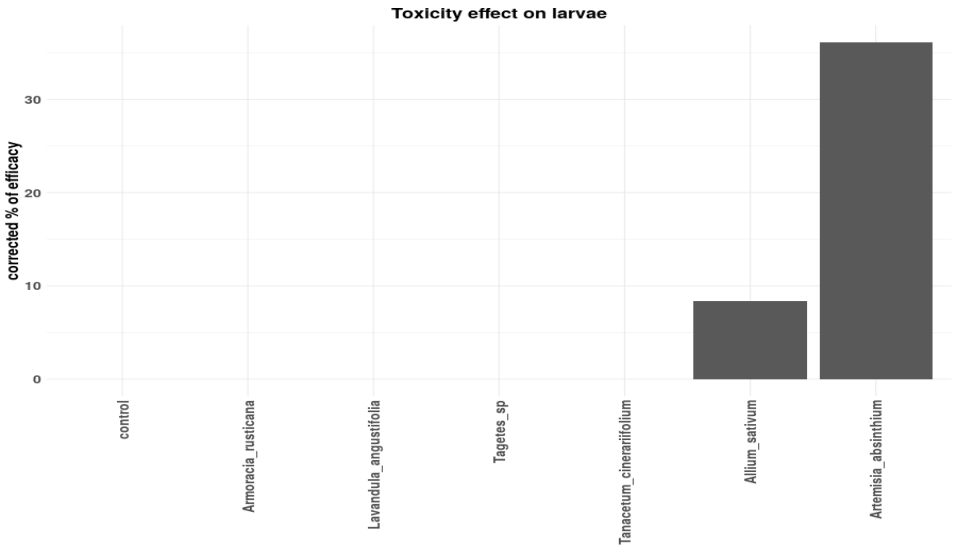

Fig. 1. Abbott's efficacy for each plant extract treatment. Negative efficacy was set to 0

Larval repellency in the laboratory

None of the tested plant extracts showed a significant lower or higher proportion of emerged adults than the control treatment (Figure 2). The diet treated with *Allium sativum* had a tendency towards less adults than the control. However, this repellent effect cannot be disentangled from a potential toxicity effect of *A. sativum* on *L. botrana* larvae.

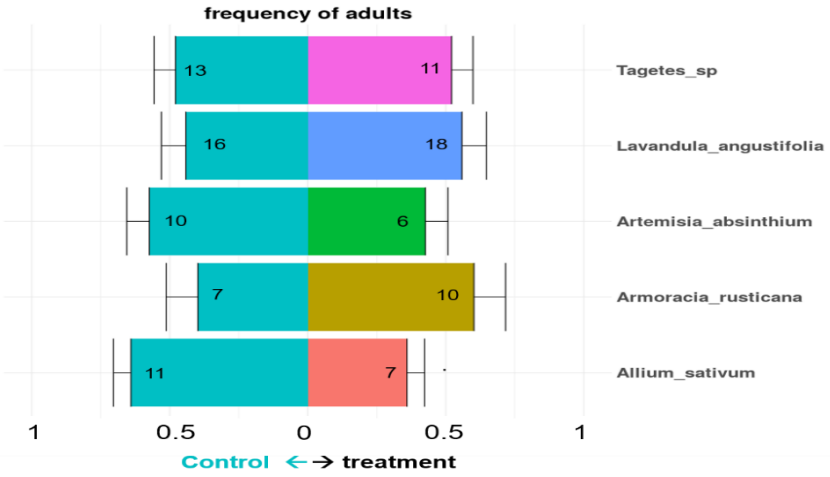

Fig. 2. Mean frequency of adults in the control media (green bars towards left) compared to the plant extract treated media (colored bars according to treatment towards right). N=total number of adults emerged

Adult repellency in the laboratory

All zones where a plant extract was applied had significantly less eggs than the control zones (Figure 3). Applying tap water also reduced significantly the proportion

of oviposition. The effect size is however smaller than when plant extracts were applied.

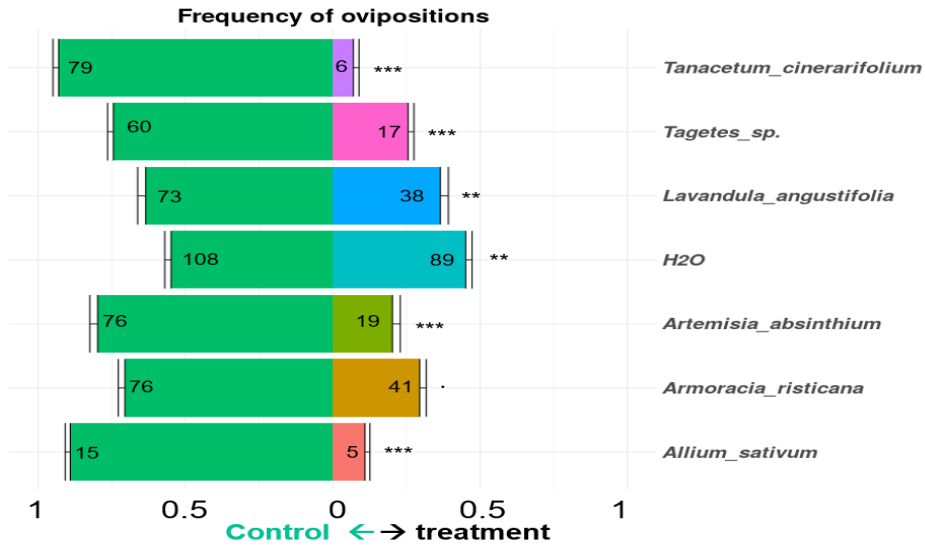

Fig. 3. Mean frequency of ovipositions in control zones (green bars towards left) compared to the plant extract treated zones (colored bars according to treatment towards right). N=total number of eggs laid, * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Adult repellency in the field

The lowest number of adults were captured in the variants in which the *Artemisia absinthium* extract was applied on its own or in the mixture. Moreover, only 0.25% of grape clusters were infested with *L. botrana* larvae in the mixture treatment of the three extracts compared to 3.8% in the *Tagetes* sp. treatment (Figure 4).

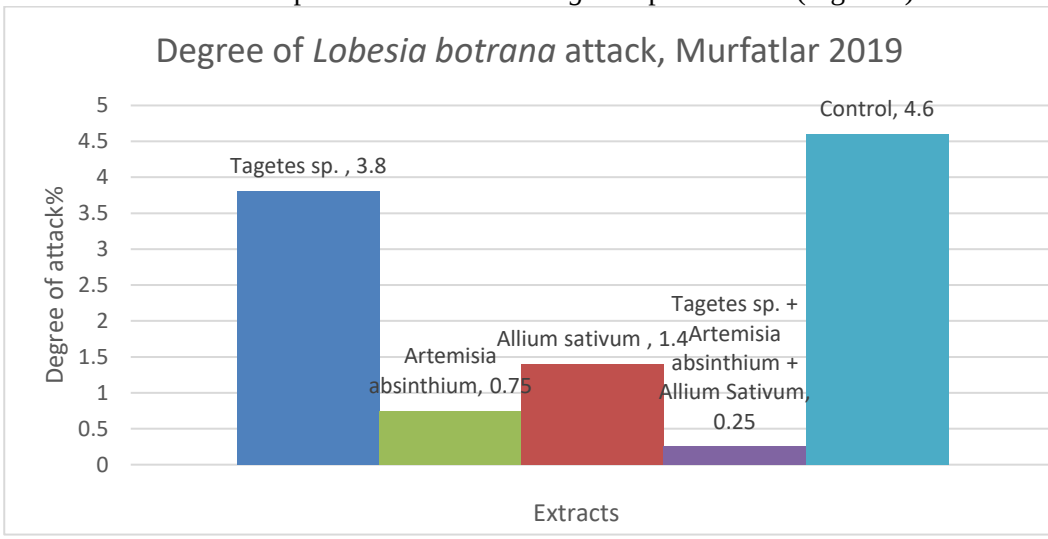

Fig. 4. Percentage of grape clusters attacked by *L. botrana* larvae in the vineyard in 2019. Treatments with different letters are significantly different at $P \leq 0.05$

CONCLUSIONS

We conclude that the mixture of *Artemisia absinthium*, *Tagetes* sp. and *Allium sativum* might have an interesting potential for protecting grapes against *L. botrana* infestation. We therefore recommend to continue testing these plant extracts. If effects can be observed in the vineyard, laboratory experiments can be performed to explore the mechanism of *L. botrana* repellency.

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