Olfactometer screening of repellent essential oils against the pollen beetle (*Meligethes spp.)*

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Key words: *Meligethes aeneus*, *Meligethes viridescens*, essential oil, lavender, cornmint, lemongrass

Abstract

*Essential oils can have an impact on pollen beetle (*Meligethes spp.) *host plant location behaviour. We compared the effects of essential oils of* Mentha arvensis, Eucalyptus globulus, Melaleuca alternifolia, Citrus sinensis, Citrus paradisi, Citrus limon, Juniperus mexicana, Abies sibirica, Illicium verum, Gaultheria procumbens, Cymbopogon flexuosus, Syzygium aromaticum, *and* Litsea cubeba *using a Y-tube-olfactometer. Essential oils were diluted 1:10 in acetone and 40 µl of the dilution were applied on a 3.1cm² filter paper. Filter papers were placed in the odour containers of the olfactometer together with a flower cluster of spring oilseed rape with 5 open flowers and 10-15 buds. The control treatment involved filter papers treated only with acetone. Hungry pollen beetles were released individually into the olfactometer. Six replicates with six beetles each were conducted. Highest repellency values were obtained for* Mentha arvensis*,* Cymbopogon flexuosus*, and* Litsea cubeba*.*

Introduction

Organic agricultural methods to control pollen beetle (*Meligethes spp*.) are limited. Although effective insecticides are available for organic producers (Spinosad), their use is often restricted by guidelines of producers associations. In Swiss organic production (Bio Suisse) the use of insecticides in oilseed rape is interdicted. Therefore, alternative non-insecticidal methods to control pollen beetles are needed. Experiments in UK showed that essential oils can have an impact on pollen beetle host plant location behaviour (Mauchline et al., 2005; Mauchline et al., 2008; Mauchline et al., 2013). In a laboratory study that compared five different essential oils, lavender oil (*Lavendula angustifolia*) showed the highest repellency value (Mauchline et al. 2005). Lavender oil is relatively expensive, and cost is an impediment for farms to adopt it as a repellent. We compared 15 different essential oils using a Y-tube-olfactometer to see if any of lower cost would have similar efficacy.

Material and methods

The pollen beetles used in all experiments were collected in an untreated winter oilseed rape field in north-western Switzerland. Beetles were denied food for 40 hours before starting the experiment.

Spring oilseed rape **flowers** (variety Hero) used as odour source were produced in a nearby greenhouse. Flower clusters with 5 open flowers and approximately 10-15 closed buds were cut immediately before starting the experiments and were permanently supplied with water.

The experiments were conducted using a **Y-tube-olfactometer** (Belz et al., 2013). Odour sources were placed in the odour containers of the olfactometer. Pollen beetles were released individually into the olfactometer using flexible forceps. The measurement started as soon as the beetle crossed the first line (1.5 cm from the release point; 10 cm distance to the Y-junction) and stopped as soon as the beetle crossed the finish line (4 cm behind the Y-junction). The choice of the beetle was recorded. The beetle was removed from the olfactometer for determination of species (*M. aeneus, M. viridescens*) and sex. Beetles that failed to cross the finish line within 90s after crossing the first line, were removed from the olfactometer and were not included in the analysis. Each replicate consisted of six responding pollen beetles. Odour sources (=flowers and repellent odours) were replaced after each replicate. Six replicates (= total 36 beetles) were conducted per treatment. The sequence of the tested substances was randomized between each replicate to avoid influence of the time of day. Experiments were conducted between 7:30 and 16:30 h. The experimental room was kept at 22±3°C, 50±10% rh relative humidity.

The following **essential oils**, supplied by qualiessentials gmbh (Germany), were used in the experiments: cornmint oil (*Mentha arvensis*), orange oil sweet (*Citrus sinensis*), wintergreen oil (*Gaultheria procumbens*), lemongrass oil (*Cymbopogon flexuosus*), eucalyptus oil (*Eucalyptus globulus*), fir needle oil (*Abies sibirica*), lemon oil (*Citrus limon*), tea-tree oil (*Melaleuca alternifolia*), clove oil (*Syzygium aromaticum*), star anise oil

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(*Illicium verrum*), grapefruit oil white (*Citrus paradisi*), Texas cedarwood oil (*Juniperus mexicana*), Litsea cubeba oil (*Litsea cubeba*), lavender oil (*Lavendula angustifolia*). In addition to the essential oils, the product Heliosol (Omya Agro, Switzerland) was tested. Heliosol is a pine oil based product used as wettening and sticking agent for plant protection purpose. Essential oils and Heliosol were diluted 1:10 in acetone and 40 µl of the dilution were applied on a 3.1 cm^2 filter paper (MN713, Macherey-Nagel, Germany). After 30 minutes, when the acetone had evaporated, the filter papers were placed together with a flower cluster in the odour containers of the olfactometer. The control used filter papers treated only with acetone with a flower cluster.

Statistical analysis: Repellency values (RV) were calculated per replicate according to Mauchline et al. (2005): [RV = number of beetles on the untreated flower / (number of beetles on the untreated flower + number of beetles on the treated flower)]. In order to test if the essential oils had a significant effect compared to the “untreated” control, a Wilcoxon signed rank test was performed to test whether mean RV was significantly different from 0.5. In order to compare the efficacy of different essential oils, the RV were [arcsin√(x)] transformed. Normality of data and homogeneity of variance were tested. RV were compared by an ANOVA followed by a Tukey HSD post hoc tests (α=0.05).

Results and discussion

Behaviour of beetles in the olfactometer: When released into the olfactometer, the pollen beetles needed a few seconds to get on their feet and to orient themselves towards the airflow. Once they started to walk, they moved forward until they reached the junction of the two arms. Irritated either by the light, which was placed directly above the junction or by the disturbance in the airflow, most beetles stopped or walked a vertical looping in the junction. After a few seconds, they started walking into one arm of the olfactometer, usually at a rapid pace. On average, it took the beetles 39.8±0.6 s to cover the distance (14 cm) from the first line to finish line. Beetles rarely moved back into the other olfactometer arm. A total of 772 beetles were released into the olfactometer. Out of these beetles, 232 individuals (=30%) failed to cross the finish line within 90s after crossing the first line. They were removed and not included in the analysis. A total of 540 beetles were responsive. Beetles showed best reaction early in the morning. This might be due to the daily activity pattern of pollen beetles: food foraging behaviour is probably stronger in the morning. Field collected beetles were used for the experiments. Determination of sex and species was done after the beetles had passed the olfactometer. Out of the 540 responding beetles, 474 individuals were *Meligethes aeneus*, 66 individuals (=12.2%) were *Meligethes viridescens*. The sex ratio (M:F) of 0.47 was identical for both species.

Pollen beetle choice in the olfactometer: Ten out of the 15 tested essential oils in a 1:10 dilution significantly repelled the pollen beetles and showed a repellency value (RV) significantly greater than 0.5 (Table 1; Wilcoxon signed rank test, $p<0.05$). The essential oils from cedarwood, orange, wintergreen, eucalyptus and lemon did not have a significant effect on pollen beetle choice. The RV was not significantly different from 0.5 (Wilcoxon signed rank test, $p>0.05$). However, all tested essential oils had a mean RV > 0.5. Thus, none of the tested essential oils was attractive for the pollen beetles. Cornmint had the highest RV (1.00): none of the beetles chose the olfactometer arm with cornmint essential oil. Lemongrass and *Litsea* essential oils, as well as the pinolene based plant protection product Heliosol also showed high repellency values of 0.92. Lavender oil was less repellent: RV was 0.81. These results are in accordance with the literature: Maucheline et al (2005) compared different essential oils at 10% dilution and observed the following mean RV: 0.97 for peppermint (*Mentha piperita*), 0.97 for lavender, 0.95 for Tea tree, and 0.9 for eucalyptus.

Prices of different essential oils: In addition to a high repellency value, the price of an essential oil is a major factor to choose candidates for field application strategies. Prices of essential oils fluctuate during the year, depending on origin and harvesting time of plants. Prices given in Table 1 are rough estimations by qualiessentials gmbh. The cheapest essential oil in our experiments was grapefruit oil. Lemongrass and Litsea oil are also reasonably priced. Cornmint oil is considerably more expensive and lavender oil was by far the most expensive oil in our experiments.

Conclusions

Based on the results of the experiments and on the prices of the essential oils, the development of a field application strategy will focus on cornmint oil, lemongrass oil and *Litsea cubeba* oil.

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Table 1: Repellency values (± se) of different essential oils tested in the Y-tube-olfactometer containing "flower clusters + filter paper + acetone" and "flower clusters + filter paper +essential oil diluted 1:10 in acetone" and estimation of price per kg of the essential oils.

Essential oil	RV±se	Wilcoxon Test	Tukey HSD-Test	Prices of essential oils
Cornmint	1.00±0.00	*	D	31.50 € / kg
Lemongrass	0.92±0.06	*	CD	17.50 € / kg
Litsea	0.92±0.06	*	CD	18.00 € / kg
Heliosol	0.92±0.04	*	CD	18.00 € / kg
Tea tree	0.89±0.06	*	BCD	32.00 € / kg
Grapefruit	0.86±0.03	*	ABC	14.00 € / kg
Fir needle	0.83±0.06	*	ABCD	28.50 € / kg
Star anise	0.83±0.06	*	ABCD	22.50 € / kg
Lavender	0.81±0.05	*	ABCD	104.00 € / kg
Clove	0.81±0.08	*	ABC	30.00 € / kg
Lemon	0.75±0.08	n.s.	ABC	
Eucalyptus	0.69±0.07	n.s.	ABC	
Wintergreen	0.69±0.11	n.s.	ABC	
Orange	0.67±0.06	n.s.	AB	
Cedarwood	0.64±0.05	n.s.	A	

Statistical analysis: Wilcoxon signed rank test testing if RV is different from 0.5 with $p<0.05$; Tukey: Data transformed [arcsin$\sqrt{(x)}$], Four-way-ANOVA: essential oil: $F_{14,72}=5.03$, $p<0.0001$; temperature: $F_{1,72}=21.57$, $p<0.0001$; humidity: $F_{1,72}=0.12$, $p<0.13$; position of olfactometer arms: $F_{1,72}=7.73$, $p=0.007$; Tukey HSD post hoc tests $\alpha=0.05$, different letters show significant differences.

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