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Development of a refuge-kairomone device for monitoring and control of the vine weevil, *Otiorhynchus sulcatus*, by lure-and-kill and lure-and-infect

Robert W.H.M. van Tol [a,*], Ivonne A.M. Elberse [b], Denny J. Bruck [c,1]

[a] Wageningen University and Research, P.O. Box 69, 6700, AB, Wageningen, the Netherlands
[b] The Netherlands Food and Consumer Product Safety Authority (NVWA), P.O. Box 9102, 6700, HC, Wageningen, the Netherlands
[c] USDA-ARS, Horticultural Crops Research Laboratory, Corvallis, USA

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ABSTRACT

Root weevils in the genus *Otiorhynchus* are an important pest in the nursery and small fruit production worldwide. The night-activity of the adult weevils obstruct timely monitoring and oviposition often starts before effective control measures are taken. The primary objective of this research goal was to develop an effective trap for monitoring that can be used in conjunction with the kairomone (*Z*)-2-pentenol and an effective means to kill the insects that enter the trap.

A novel ruffle refuge trap (WeevilGrip) caught on average 4 to 5 times more weevils than a grooved board refuge in a field trial. Addition of the kairomone to the WeevilGrip further increased catches 52%. Linseed oil increased mortality to 59% and addition of Botanigard (ai *Beauveria bassiana*, strain GHA, Certis, BotaniGard WP 10–25%) increased mortality to 79%.

The lure-refuge device consists of a flexible ruffle that can be wrapped around trees or placed on the soil within ground covers. This flexible shape maximizes contact with weevils compared to other available weevil trap designs. The WeevilGrip is an improved monitoring tool to support growers in integrated control strategies.

1. Introduction

The vine weevil, *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae), is a major pest in nursery and small fruit production throughout temperate climate zones around the world (Lundmark, 2010; Moorhouse et al., 1992). Control with insecticides targeted at adults is becoming increasingly difficult as the more effective chemistries (especially in Europe) are banned by legislation and new alternatives are not available. Growers are struggling with the control of this pest because of the invisibility of the two life-stages of the weevil, namely 1) adults are night-active feeding on leaves, 2) larvae live in soil eating plant roots. Although biological control of this pest with entomopathogenic fungi and nematodes in nurseries is possible and applied by some growers, high costs prevent large-scale introduction and acceptance of biological control (van der Horst and van Tol, 1995; van Tol, 1996). Monitoring of the hot spots of adult infestation and removal or killing of adult weevils is essential to make biological control effective and economically affordable (Cram, 1970, 1980; Cram and Daubeny, 1982; Fisher, 2006; Georgis et al., 2006; Shanks and Doss, 1986; van Tol et al., 2004; van Tol and Raupp, 2005). Currently growers lack good monitoring and trapping systems to remove the weevils in substantial numbers. Since freshly emerged weevils have a pre-oviposition period of 4–8 weeks depending on the host-plant species available (Fisher, 2006; Maier, 1981; Nielsen and Dunlap, 1981), growers have the opportunity to remove weevils before the onset of egg-laying. For both organic and IPM growers mass-trapping or lure and kill with entomopathogenic fungi against the adult weevils could provide a solution as shown for the vine weevil (Pope et al., 2018).

There are numerous trap or refuge types tested and available such as corrugated cardboard, grooved board, pitfall trap, roguard trap and ChemTica cone trap (Buxton, 2003; Casteels et al., 1995; Gordon et al., 1995; Li et al., 1995; Phillips, 1989; Reineke et al., 2011), but efficacy of these devices is low and variable. Roberts et al. (2019) tested different commercial trap and refuge types in small tents with plants and found only the Vine weevil trap of ChemTica (Heredia, Costa Rica) to be significantly better than any of the other traps tested (26.7% recapture). In an open field with rhododendron, however, we found numerous weevils in the dried folded leaves on the soil but near zero weevils in

* Corresponding author.
E-mail addresses: rob.vantol@wur.nl (R.W.H.M. van Tol), i.elberse@nvwa.nl (I.A.M. Elberse), denny.bruck@gmail.com (D.J. Bruck).
[1] Current address: Corteva Agriscience, 7300 NW 62nd Ave, Johnston IA 50131, USA. denny.bruck@gmail.com

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refuge/trap types such as the grooved board and the ChemTica Vine weevil trap. This demonstrates the importance of testing traps in an open field more than in cages where they cannot avoid the traps and no other refuges are available (Bruck DJ, unpublished). We performed a similar experimental set-up as Roberts et al., (2019) with 'tent' cages and different designs of refuges. From these experiments we found only the WeevilGrip to be effective and we therefore decided to test the WeevilGrip compared to grooved board (standard grower practice for weevil monitoring) in a field trial. Grooved boards are used successfully to monitor vine weevils in Dutch nurseries (Li et al., 1995). We tested the new refuge for the vine weevil (WeevilGrip, Agri Gripping, the Netherlands, https://agri-gripping.com/, https://www.brimex.nl/plaagbestrijding/taxus-kever-bestrijden, http://www.innogreen.nl/weevilgrip) in a field experiment in conjunction with the patented vine weevil kairomone (Bruck et al., 2018). The kairomone (Weevil Lure, Agri Gripping, the Netherlands, https://agri-gripping.com/, www.pherobank.com) attracts adult vine weevil in strawberry and several other ornamentals (van Tol et al., 2012). Next to determining the number of weevils captured we also tested how effective the best refuge was to deliver a kill option, the entomopathogenic fungus *Beauveria bassiana* (product Botanigard®, ai *Beauveria bassiana*, strain GHA, Certis, BotaniGard® WP 10–25%) that needs to contact the weevils, as well as a non-deterrent oil capable of killing the weevils through spiracle blocking. Before initiating field trials, we tested several oils (glycerol, sesame oil, linseed oil, Tween80 and Bayer 11E® oil - no data available) on weevil behaviour. Only three oils (glycerol, linseed oil and Tween80) did not deter weevils entering the WeevilGrip (van Tol, unpublished). Ranger et al., (2009) found a product based on sesame oil (84.5%) (product Armorex, Soil Technologies, USA) to effectively kill grubs after dipping. It was superior to seven other commercial botanicals tested. Linseed or flaxseed oil and sesame oil have quite similar composition (de Cássia Avellaneda Guimarães et al., 2013). Oleic acid and linoleic acid as mono-unsaturated compounds in both oils and the poly-unsaturated compound α-linolenic acid in linseed oil only. We tested linseed oil as an alternative for sesame oil as it did not inhibit weevil entering the WeevilGrip.

2. Experimental methods

2.1. Refuge devices (Fig. 1)

Refuge trap types tested were: (A) *Grooved board* (handmade boards by Bruck, USDA, Oregon, USA) – wooden board 40 × 20 cm and 20 mm thick, with five 10 mm deep and 10 mm wide grooves in the length of the board, (B) *WeevilGrip* (Agri Gripping, the Netherlands, https://agri-gripping.com/) (Bruck et al., 2018) - ruffle made of folded fabric (~4 cm diameter folded fabric) consisting of 100% polyester (Micro Mesh # 1280, Nick of Time Textiles, Allentown PA, USA). The fabric was evenly perforated with 1 mm holes. Distance between the holes was ~2 mm (60 cm long, 4 cm wide).

2.2. Vine weevil kairomone

The compound (*Z*)-2-pentenol was obtained from Bedoukian (Danbury, CT, USA). The chemical was used without further purification (95%). The compound was charged in polylactic acid granules or applied as pure liquid in a dispenser (Weevil Lure, Agri Gripping, the Netherlands) (Bruck et al., 2018). The compound consisted of the pure compound and was not diluted with water or any other compound.

2.3. Kairomone release systems

2.3.1. Pasteur pipette dispenser

Plant volatile dispensers were made of 1.5 ml LDPE Pasteur pipettes (Labo Scientific, Ede, the Netherlands). Test compounds were introduced into the pipette, the tip of which was then sealed by heat. Prior to use, the tip of the pipette was cut off at 1 cm above the reservoir portion. The open tip of the dispenser had an internal diameter of 3.5 mm. Release rate of the pipettes (N = 6) was determined by placing 0.4 ml of (*Z*)-2-pentenol in a laminar airflow cabinet (DLF 460 EC, Clean Air Techniek B.V., Woerden, the Netherlands) at 24 °C and measuring the weight loss of the pipettes for 12 days (Fig. 3).

2.3.2. Polylactic acid granules

Biodegradable granules (2–3 mm diameter) consisting of polylactic acid (PLA) (ACCUREL® XP 951B, Evonik, Essen, Germany) were charged with (*Z*)-2-pentenol by adding equal weight proportions of the compound and granules in a rotating flask for approximately 1 h until all liquid (*Z*)-2-pentenol was absorbed. Charged granules (2 g/bag) were packed in polyethylene-aluminium coated paper bags (size 13 × 9 cm) to prevent evaporation (Pherobank B.V.), sealed and stored at −20 °C until use. Release rate of the granules was determined by placing 2 g of charged granules in a Petri-dish in a laminar airflow cabinet (N = 3) (DLF 460 EC, Clean Air Techniek B.V., Woerden, the Netherlands) at 24 °C and measuring the weight loss of the granules daily until more than 95% of the odour was evaporated (Fig. 3).

2.4. Vine weevil populations

The field experiment in the USA performed had a natural infestation of weevils in a field of *Malus* M-9 (RN-29) apple-rootstock. At the start of the experiment the weevils were approximately 1 week old as before the 30th of May there were no weevils recorded. A population of weevils had been present in this field for many years. Weevils for the lure and kill trial were collected from the previous year with eggs inoculated *Astilbe* sp. In 3 L pots in the Netherlands and kept at 20 °C in a climate controlled room at long-day light conditions (16 h light, 8 h dark; RH ~60%). Adult weevils were fed with *Euonymus fortunei* (Turcz.) Hand.-Mazz. and *Taxus baccata* L. leaves approximately one month before the start of the experiment.

2.5. Field-refuge-kairomone experiment

The experiment was performed in a commercial apple-rootstock field in Dayton, Oregon (Malus 'M-9 (RN-29)', Dayton, Oregon 97114, USA: 44° 09′ 24.3" N, 123° 03′ 28.2" W) in 2012. On May 30th, 2012 the field was monitored for weevil presence. Preliminary monitoring (with WeevilGrip refuge traps) was performed to determine the density distribution of the weevils in the field. As a test area we chose part of the field with higher densities. Due to differences within the plot and the size of each block, we adapted the lay-out of the block design in such a way that the local differences in density of weevils present at the start of the experiment were more equal in each block (higher density first block to lower density last block). The following four treatments were tested (5 replicates), (1) *Grooved board*, (2) *WeevilGrip*, (3) *WeevilGrip* + *Weevil Lure granules*, (4) *WeevilGrip* + *Weevil Lure vial*. The granule lure was formulated as 2 g PLA slow-release granules charged with 50% (Z)-2-pentenol and placed on the soil adjacent to the WeevilGrip. The lure dispenser (Weevil Lure vial), containing 0.4 ml (Z)-2-pentenol, was placed approximately 30 cm above the WeevilGrip at the top of the canopy. Distance between each treatment was 20 m. Dispensers were refreshed once a week and slow-release granules twice a week. WeevilGrip refuges were placed in full length on the soil in the middle of the row with apple rootstock plants (Fig. 1b). Grooved board traps were placed adjacent to the plant row in direct contact with the plants (Fig. 1a). WeevilGrip refuge traps, dispensers and slow-release granules were placed in the field on May 31st, 2012 and monitored for weevil presence twice-weekly between 1 and 4 pm between June 4th and June 26th, 2012 (seven monitoring dates). Plants within the treatment row up to 30 cm distance from the center of the WeevilGrip or grooved board in either direction was checked for weevil presence at the same day/time as the WeevilGrip was checked.

Weevils found were returned to their location in the field.

2.6. Lure-and-kill experiment

The experiment was performed in gauze cages (60 x 60 × 90 cm) in July/August 2014. Cages were placed outside with a soil layer (10 cm) of peat soil inside and three *E. fortunei* 'Dart's Blanket' (Turcz.) Hand.-Mazz. planted close together and treated WeevilGrip around them (Fig. 2). The treatments applied are shown in Table 1. WeevilGrip ruffles were dipped in water (treatment A) with dilutions of BotaniGard® (*B. bassiana* GHA, WP 10–25% WP, Certis) (treatments B, F, H), glycerol (treatment E, F), linseed oil (treatment G, H) and Tween80 (treatment E, F, G, H to emulsify water and oil after shaking and before dipping the WeevilGrip) added. After treatment, the WeevilGrip was hung to dry for 2 h (treatment A, B, E, F, G, H). WeevilGrip were dry treated with spores of *B. bassiana* (BotaniGard®) (treatment D) with kaolinite and diatomaceous earth (treatment C, D). The treatments with linseed oil contained 46% α-linolenic acid (Vitaal, Teutoburger Ölmühle). To confirm viability, Botanigard® spores were plated. For this we prepared a *Beauveria* SDAY agar of which we spread a thin layer of approximately 1 ml of agar on a microscope slide. Three separate droplets of 10 μl suspension of *Beauveria* spores (~10^9 spores/ml) were incubated next to each other on the dried SDAY agar (adapted after Faria et al., 2010). The plates with spores were incubated at 25 °C in a dark box with wet paper to keep the humidity high. After 24 h incubation the germinated and ungerminated spores were counted (minimal 200 spores per droplet). After placement of the treated WeevilGrip around the base of the plants (Fig. 2) all cages received 30 weevils per cage that were placed in the cage during the day as a group in a small black box (5x5x5 cm) with a single opening allowing the weevils to leave in the evening. Treatments were replicated 4 times. Weekly for 5 consecutive weeks after weevil release, dead individuals were counted and removed. The number of living weevils was counted and placed back in the cage.

2.7. Analysis

The field test was set-up as block design where the blocks consisted of four plots. The total number of vine weevils per trap were analyzed using ANOVA on the ^{10}log transformed values using the 12th version of the statistical package GenStat (Payne et al., 2009). All data were pooled

Fig. 1. Grooved board refuge trap (a) and WeevilGrip ruffle refuge trap (b) tested in a field trial (a) on apple-rootstock plants for trapping of *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae).

Fig. 2. *Euonymus fortunei* 'Dart's Blanket (Turcz) Hand.-Mazz. plants in a cage with a treated WeevilGrip refuge trap surrounding the plants.

and analyzed for differences. The model consists of the additive effects of block/plot, date and odour. Estimates of the means of the weevils per trap were back transformed to the original scale with approximate standard errors. A Fisher's protected least significant difference test was performed on transformed data for Treatment. The results in Fig. 4 present average results and standard errors. The statistical analysis result (presented by numbers above the graphs) are based on the actual statistical test performed. The results in Fig. 5 present average data over time with standard error. The individual data cannot be analyzed due to the Fisher's protected test performed but the total effect of Date*-Treatment was significant with a F-probability value of 0.032. The graph is shown to illustrate the variation over time in catch between the treatments.

For the lure-and-kill experiment on the weekly total number of dead weevils per cage a model with binomial distribution was used and a regression analysis on logit transformed data was performed (GenStat) with fitted terms of block and treatment. Means separation was determined by pair-wise comparisons using *t*-tests. Thereafter, estimates of the means of the dead weevils per trap were back transformed to the original scale with approximate standard errors. The standard errors are appropriate for interpretation of the predictions as summaries of the data rather than as forecasts of new observations.

3. Results

3.1. Kairomone release systems

The release rate of (*Z*)-2-pentenol from the LPA granules (Fig. 3) indicate a fast release within the first four days after exposure (~3–5 mg/day) to the open air after which the release per day slows down quickly with no further release after 6 days when 95% of the compound has evaporated from the granules. The release from the LDPE pipette tips was much lower (~1 mg per day during 12 days) to that of the granules with the difference that 400 mg of (*Z*)-2-pentenol per dispenser providing a linear release rate for at least 3–4 weeks compared to 3–4 days non-linear release for the granules. Based on this result we decided to refresh the granules with (*Z*)-2-pentenol twice a week and the LDPE pipette tips weekly in the field refuge-lure experiment to have kairomone odour continuously present in the field.

3.2. Field-refuge-kairomone experiment

In the 2012 field experiment, we counted the number of weevils in the WeevilGrip refuge and the weevils in the plants in the plant row next

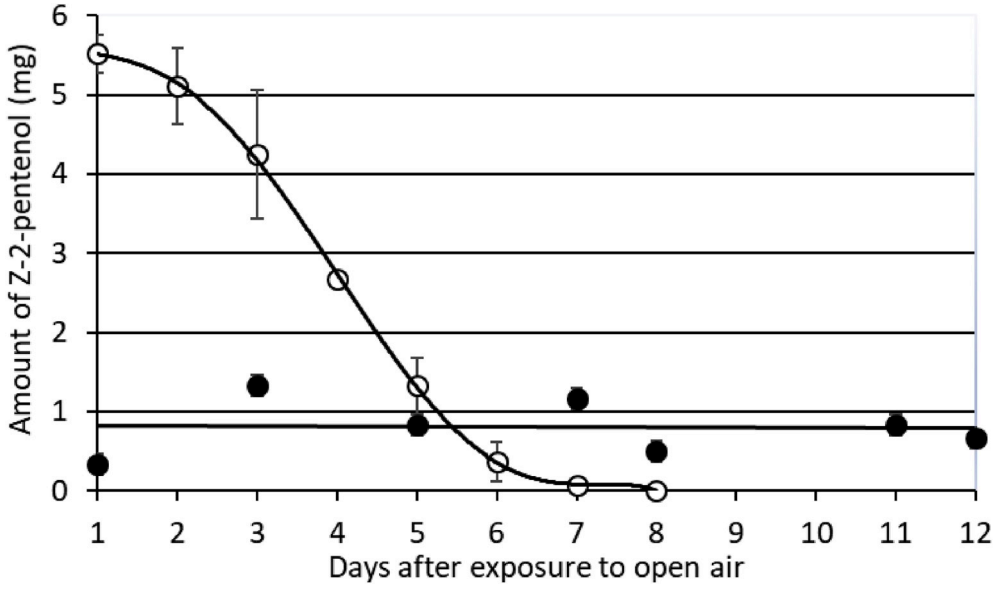

Fig. 3. Weight loss of 20 mg (N = 3) biodegradable polylactic acid (PLA) granules charged for 50% with (*Z*)-2-pentenol over time (open dot line) and weight loss of 0.4 ml (~400 mg, N = 6) pure (*Z*)-2-pentenol over time from an open pipette tip dispenser (closed dot line). Dots represent day of measurement. The standard errors are based on the true values of the release data.

Table 1
Treatments of the WeevilGrip in the lure-and-kill experiment.

Added to WeevilGrip	Name product	Company product	Treatments							
			A	B	C	D	E	F	G	H
Water (control)[$]	–	–	63	63	–	–	56.6	56.6	56.6	56.6
Beauveria bassiana GHA[a]	Botanigard®	Certis	–	10^7	–	10^7	–	10^7	–	10^7
Kaolinite[#]	–	Merck	–	–	4	4	–	–	–	–
Diatomaceous earth[#]	–	Biofa InsectoSec	–	–	4	4	–	–	–	–
Glycerol[@$]	–	Merck	–	–	–	–	6.3	6.3	–	–
Linseed oil[$]	Vitaal	Teutoburger Ölmühle	–	–	–	–	–	–	6.3	6.3
Tween80[$]	–	Merck	–	–	–	–	0.06	0.06	0.06	0.06

[a] Spores per gram or ml; # gram of powder per trap; @ >99.5% purity product in gram; $ ml product.

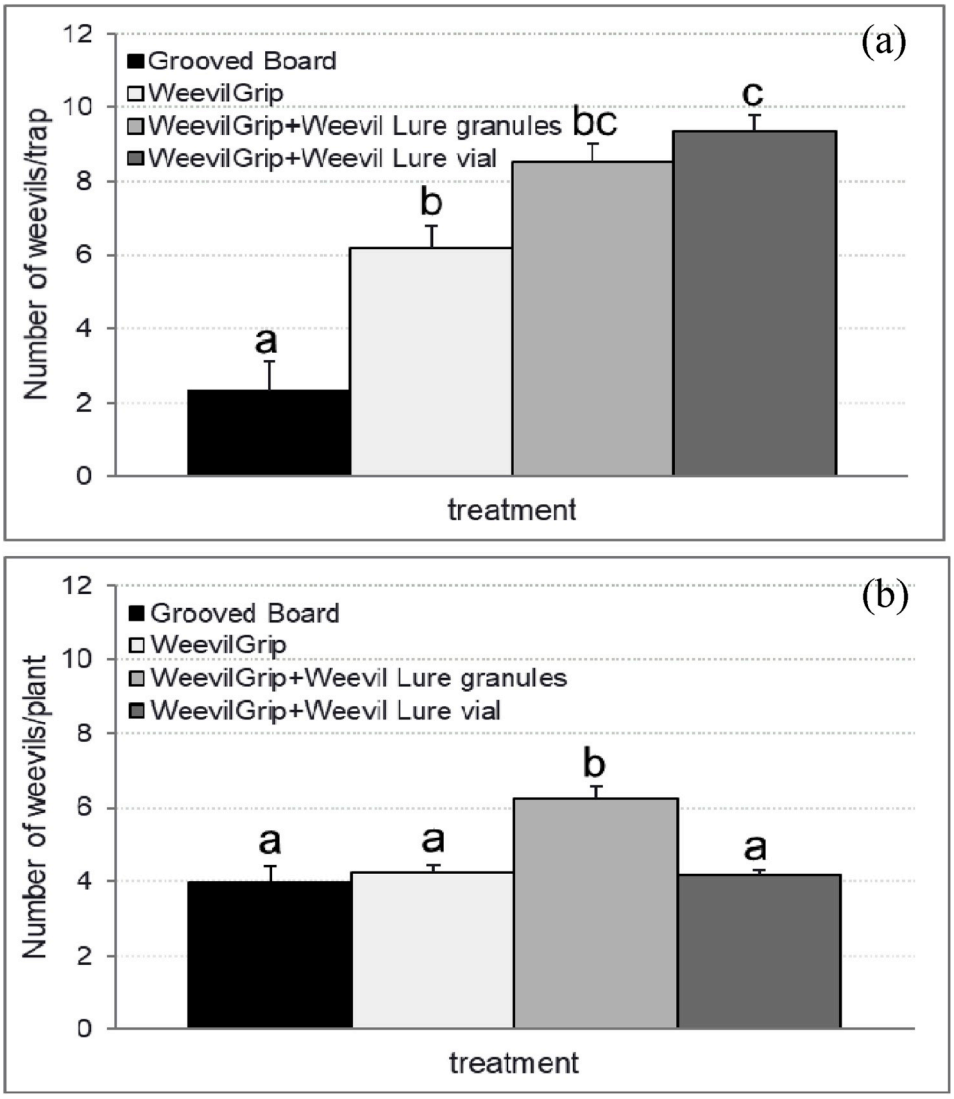

Fig. 4. Average number of *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae) found per date (seven dates in three weeks) in (a) four different trap types and (b) 30 cm plant row next to the four different trap types in a field with *Malus* 'M-9 (RN-29)' seedlings in 2012. Values with different letters (a, b, ….) on top of the bars are significantly different from each other at $P = 0.05$. Presented are means and standard error bars calculated on the original data.

to the trap (30 cm row/refuge) and analyzed both numbers for the different treatments (Fig. 4). The number of weevils found in the WeevilGrip significantly differed between treatments and date (Date by treatment F probability = 0.032; Treatment F probability < 0.001). The highest numbers were found in the WeevilGrip with the kairomone. The treatment WeevilGrip with the kairomone in a vial caught significantly more weevils than the WeevilGrip without the kairomone at $P = 0.05$ but not different from the WeevilGrip with the kairomone in granulated form. All WeevilGrip treatments caught significantly more weevils than the grooved board. Overall the traps did not affect the number of weevils found in the plant (Fig. 4b). A small significant positive effect was however found for the treatment where granules with the kairomone were present under the WeevilGrip refuge (Date by treatment F probability = 0.791; Treatment F probability = 0.011). The results per date also differed and some dates the differences seem much higher than on other dates. In the week of 14 June for example, when the highest total

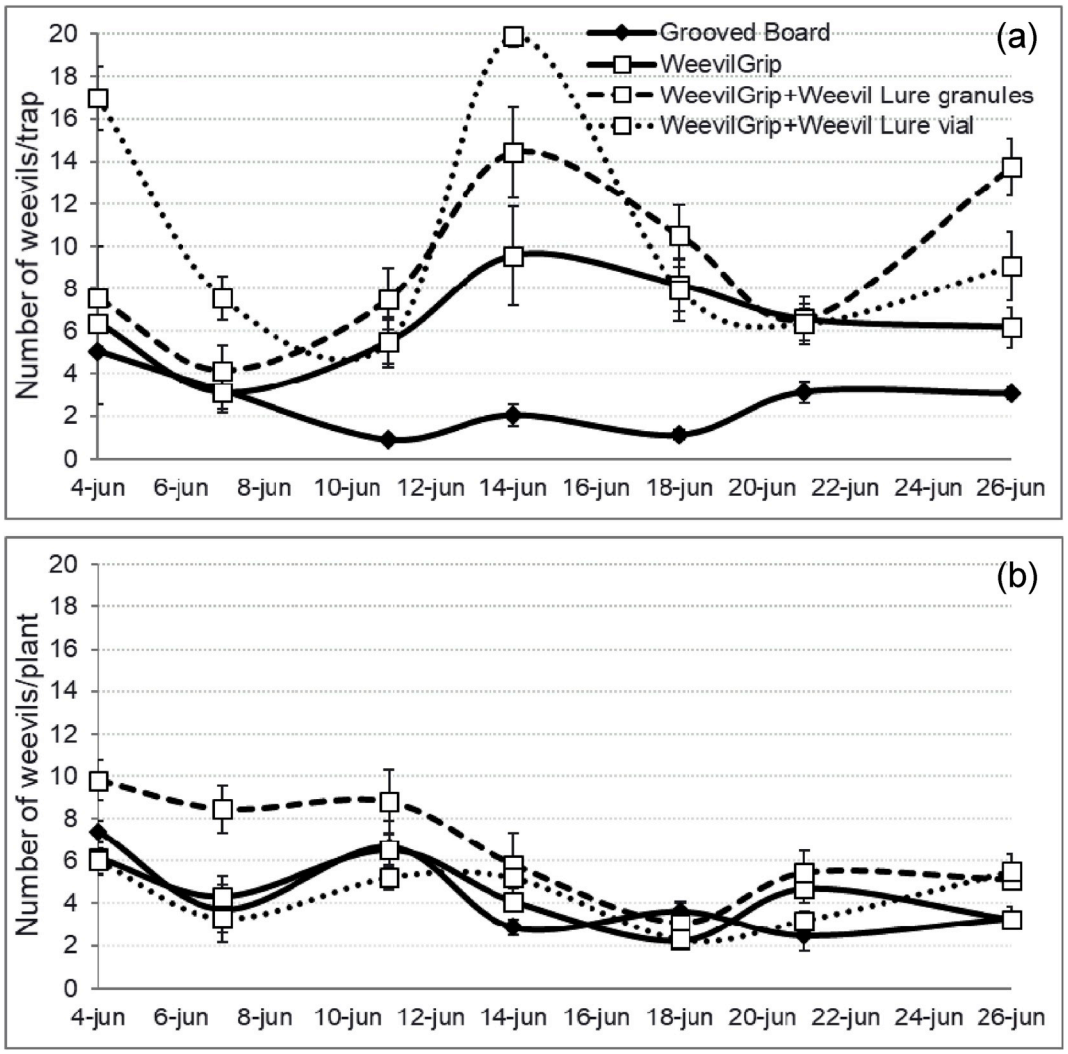

Fig. 5. Average number of *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae) found per date in (a) four different trap types and (b) 30 cm plant row next to the four different trap types in a field with *Malus* 'M-9 (RN-29) seedlings in 2012. Presented are means and standard error bars calculated on the original data.

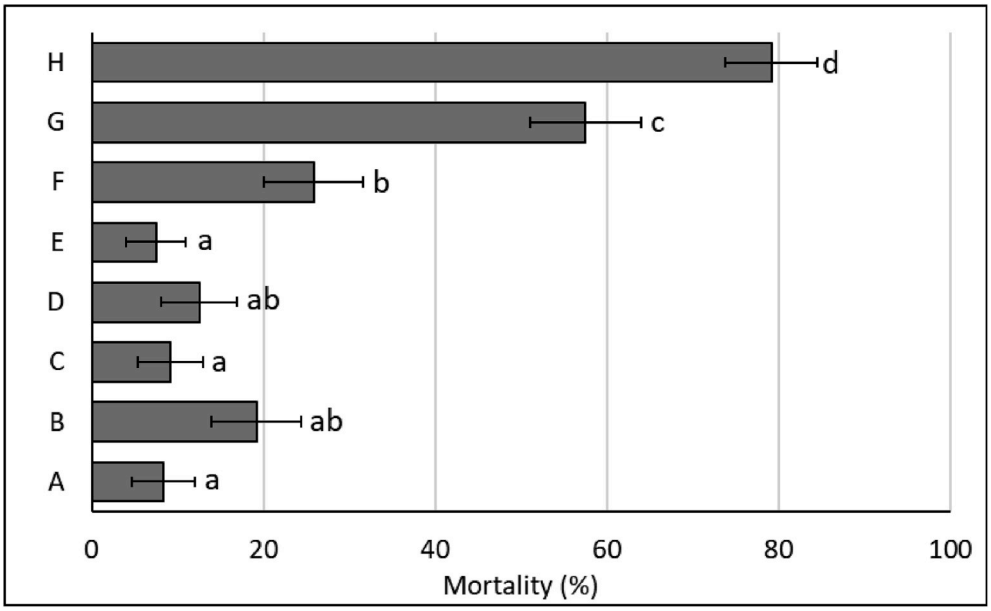

Fig. 6. Mortality (%) of weevils of *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae) after 35 days in cages with three *Euonymus fortunei* 'Dart's Blanket' (Turcz.) Hand.-Mazz. surrounded by one WeevilGrip trap, planted in a soil layer of 10 cm per cage (N = 4; 30 weevils/cage). Values with different letters (a, b, ….) on top of the bars are significantly different from each other at $P = 0.05$. Values are based on back transformed data. The shown approximate standard error bars are appropriate for interpretation of the predictions as summaries of the data rather than as forecasts of new observations. A = Control (water), B=Botanigard®, C=Kaolinite + Diatomaceous earth, D = Botanigard®+Kaolinite/ Diatomaceous earth, E = Glycerol, F=Botanigard®+Glycerol, G = Linseed oil, H=Botanigard®+Linseed oil.

number of weevils were caught in the WeevilGrip, 10 times more weevils were caught with the WeevilGrip and vial kairomone compared to the grooved board.

3.3. Lure-and-kill experiment

The tests revealed that the treatments BotaniGard® with or without kaolinite/diatomaceous earth and glycerol did not significantly differ in mortality from the water (control) treatment (Fig. 6). Botanigard®-water ($P = 0.12$), Botanigard®/kaolinite/diatomaceous earth-water ($P = 0.48$), Glycerol-water ($P = 0.87$), Kaolinite/diatomaceous earth-water ($P = 0.88$). Botanigard® with glycerol ($P = 0.03$), linseed oil ($P < 0.001$) and Botanigard® with linseed oil ($P < 0.001$) were all different from water (control) and each other whereby Botanigard® with glycerol was not significantly different from Botanigard® (LSD at $P = 0.05$ is 0.16) and Botanigard®+kaolinite/diatomaceous earth (LSD at $P = 0.05$ is 0.14). The viability test of the spores of Botanigard® revealed an approximate germination of 60%. The mortality over time (Fig. 7) indicated a 50% mortality after approximately 9 days for the treatment Linseed oil with Botanigard® and a 50% mortality after 14 days for Linseed alone. All other treatments never reached 50% mortality. As not all dead weevils could be found back in the cages measured mortality rates never reached 100%.

4. Discussion

Monitoring of the vine weevil *O. sulcatus* with available commercial traps has a low and variable efficacy and is mostly unsuitable to compete with the many hiding places in pots and debris that can be found outside. We developed a flexible refuge trap that can be used in groundcover plants or as a wrap around a tree. The WeevilGrip can be made to any length and provides multiple hiding places in the folds of the fabric. We caught on average in three weeks (seven monitoring measurements) 4–5 times more weevils, and in certain weeks 10 times more weevils, with the WeevilGrip than grooved-wooden boards which are the current standard for growers in the Netherlands. Addition of the vine weevil kairomone (*Z*)-2-pentenol (Weevil Lure) in a dispenser above the WeevilGrip refuge (Bruck et al., 2018; van Tol et al., 2012) increased the weevil catch with 50% compared to the WeevilGrip without the kairomone. The kairomone adds a strong plant odour attractant which encourages more weevils to aggregate and eventually seek refuge in the nearby WeevilGrip at daybreak when they leave the plant in search of a hiding place. We still need to determine an optimal release system through a dispenser (for example polyethylene bags) that mimic the release rate of the pipette so to avoid opening the tip and avoiding spill or other accidents while using in the field. We found some differences in efficacy between application as a granule under the WeevilGrip or a dispenser with the kairomone above the WeevilGrip. The granules release the odour in 3–4 days which hampers regular release over time and make it unlikely to become a commercial product. As the results from different dates indicate, there is also a variability in efficacy that may be correlated to the kairomone and application method. Other factors such as the weather, soil humidity and location of the kairomone (dispensers above WeevilGrip in the canopy versus granules on the soil near the WeevilGrip) may have influenced this variation as well but this needs further investigation. The variable weevil catch over the weeks is not connected to the day of renewing the granules (twice a week) and dispensers (weekly) and the dispensers are releasing the (*Z*)-2-pentenol linear over a prolonged time (>3–4 weeks).

The current refuge trap design, like most other available trap types for the vine weevil, have the disadvantage of non-permanent catch of the weevils. The weevils can freely enter and leave the trap again. As such, they are functioning well for timely monitoring the weevil presence and density in the field but they still don't function as a permanent trap which would allow testing mass-trapping for the weevil. Only the ChemTica trap in cages tested provided a 26.7% permanent catch rate compared to near zero with other traps or refuges tested (Roberts et al., 2019). As no catch of vine weevils in the open field in heavily infested rhododendron was accomplished with the ChemTica trap or grooved board refuges in our experience it remains unclear how effective these traps perform in the open field are (Bruck DJ, unpublished). As an alternative control strategy we therefore tested the lure and kill/infect option with the WeevilGrip. There are many examples of successful devices for other insects than the vine weevil described where entomopathogenic fungi (EPF) are used as the 'kill' component (Lyons et al., 2012; Mfuti et al., 2016; Niassy et al., 2012; Yasuda, 1999). In accordance with the set-up of Pope et al. (2018) we tested entomopathogenic fungi but also added natural oils to protect the fungi from getting inactivated. While Pope et al. (2018) managed to control 26–41% of the weevils in gauze cages we achieved 19% mortality (not significantly different from the control), Linseed oil alone killed 59% and the combination of linseed oil with Botanigard® 79% of the weevils. The fungal

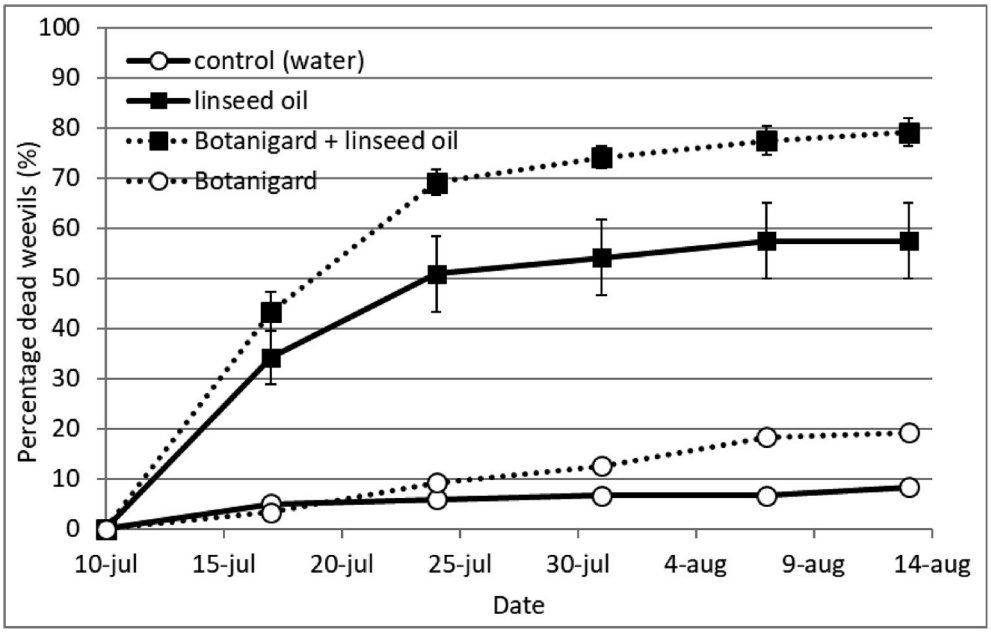

Fig. 7. Mortality (%) of dead weevils (N = 4; 30 weevils/cage) of *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae) over time in cages with three *Euonymus fortunei* 'Dart's Blanket' (Turcz.) Hand.-Mazz. surrounded by one WeevilGrip trap planted in a soil layer of 10 cm per cage. Values are based on back transformed data. The shown approximate standard error bars are appropriate for interpretation of the predictions as summaries of the data rather than as forecasts of new observations.

spore germination rate of 60% in the laboratory may be at least partially responsible for its poor performance. The oil is killing most of the weevils likely via the properties of the oil that block the spiracles, but this hypothesis requires more careful investigation. The protection of the fungus by the oil seems unlikely as the increased mortality of 20% equals the effect of the fungus alone. Per Ranger et al. (2009) quasisynergism (increased toxicity to insecticidal compounds attributed to oils improving cuticular penetration) can play a role but this is not the case in our set up where no insecticide has been applied together with the oil. More likely, similar compounds found in linseed oil and sesame oil inhibit the cytochrome P450 activity which may play a role in the increased toxicity of linseed oil for the weevils. The strategy of lure-and-kill with oils needs to be expanded to a full field trial outside of a cage to determine its efficacy. The kill by entomopathogenic fungi may be improved further with better fungal strains such as those tested by Pope et al. (2018) assuming compatibility with the oil they are combined with. Currently we are working on improving the WeevilGrip refuge trap by making it a permanent trap for the weevils through adaptations. Since vine weevils are not flying it is possible to perform mass-trapping under the conditions that there is no nearby infested field as a source of migrating weevils and no new introduction of infested plants. Currently a combination of monitoring, mass-trapping and application of entomopathogenic fungi and entomopathogenic nematodes in autumn and spring are the best method to eradicate a weevil infestation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Robert W.H.M. van Tol: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Ivonne A.M. Elberse:** Methodology, Validation, Formal analysis, Investigation, Resources, Supervision, Project administration, Funding acquisition. **Denny J. Bruck:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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References

Bruck, D.J., van Tol, R.W.H.M., Griepink, F.C., 2018. Attractant Compositions for Weevils of the Genus *Otiorhynchus* and Uses Thereof. European patent EP2540318.

Buxton, J., 2003. Vine Weevil Control in Hardy Nursery Stock, HDC Factsheet 02/03, Final Report for HDC Project SF103. Horticultural Development Company, p. 40.

Casteels, H., de Clercq, R., Miduturi, J.S., 1995. Phenological observations on the black vine weevil *Otiorhynchus sulcatus* F.. In: Belgium during the Decade 1985-1994. Med. Fac, vol. 60. Landbouww. Univ. Gent, pp. 657–661.

Cram, W.T., 1970. Acceptability of cultivars of highbush blueberry at varying temperatures by adult black vine weevils (Col.: Curculionidae). J. Entomol. Soc. B. C. 67, 6–7.

Cram, W.T., 1980. Fecundity of the black vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae), fed foliage from some current cultivars and advanced selections of strawberry in British Colombia. J. Entomol. Soc. B. C. 77, 25–26.

Cram, W.T., Daubeny, H.A., 1982. Responses of black vine weevil adults fed foliage from genotypes of strawberry, red raspberry, and red raspberry-blackberry hybrids. Hortscience 17, 771–773.

de Cássia Avellaneda Guimarães, R., Rodrigues Macedo, M.L., Leite Munhoz, C., Filiu, W., Viana, L.H., Taís Nozaki, V., Hiane, P.A., 2013. Sesame and flaxseed oil: nutritional quality and effects on serum lipids and glucose in rats. Food Sci. Technol. 33, 1. https://doi.org/10.1590/S0101-20612013005000029.

Faria, M., Hotchkiss, J.H., Hajek, A.E., Wraight, S.P., 2010. Debilitation in conidia of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* and implication with respect to viability determinations and mycopesticide quality assessments. J. Invertebr. Pathol. 105, 74–83.

Fisher, J.R., 2006. Fecundity, longevity and establishment of *Otiorhynchus sulcatus* (Fabricius) and *Otiorhynchus ovatus* (Linnaeus) (Coleoptera: Curculionidae) from the Pacific North-west of the United States of America on selected host plants. Agric. For. Entomol. 8, 281–287.

Georgis, R., Koppenhöfer, A.M., Lacey, L.A., Bélair, G., Duncan, L.W., Grewal, P.S., Samish, M., Tan, L., Torr, P., van Tol, R.W.H.M., 2006. Successes and failures in the use of parasitic nematodes for pest control. Biol. Control 38, 103–123.

Gordon, S.C., Woodford, J.A.T., Grassi, A., Zini, M., Tuovinen, T., Lindqvist, I., McNicol, J.W., 1995. Monitoring and importance of wingless weevils (*Otiorhynchus* spp.) in European red raspberry production. IOBC-WPRS Bull. 26, 55–60.

Li, S.Y., Fitzpatrick, S.M., Henderson, D.E., 1995. Grooved board traps for monitoring the black vine weevil (Coleoptera: Curculionidae) in raspberry fields. J. Entomol. Soc. B. C. 92, 97–100.

Lundmark, M., 2010. *Otiorhynchus sulcatus*, an autopolyploid general-purpose genotype species? Hereditas 147, 278–282.

Lyons, D., Lavallee, R., George, K.-P., van Frankenhuyzen, K., Johny, S., Guertin, C., Francese, J.A., Jones, G.C., Blais, M., 2012. Towards the development of an autocontamination trap system to manage populations of emerald ash borer (Coleoptera: buprestidae) with the native entomopathogenic fungus, *Beauveria bassiana*. J. Econ. Entomol. 105, 1929–1939.

Maier, C.T., 1981. Reproductive success of the black vine weevil, *Otiorhynchus sulcatus* (F.), fed different foliar diets and evaluation of techniques for predicting fecundity. Environ. Entomol. 10, 928–932.

Mfuti, D.K., Subramanian, S., van Tol, R.W.H.M., Wiegers, G.L., de Kogel, W.J., Niassy, S., du Plessis, H., Ekesi, S., Maniania, N.K., 2016. Spatial separation of semiochemical Lurem-TR and entomopathogenic fungi to enhance their compatibility and infectivity in an autoinoculation system for thrips management. Pest Manag. Sci. 72, 131–139.

Moorhouse, E.R., Charnley, A.K., Gillespie, A.T., 1992. A review of the biology and control of the vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae). Ann. Appl. Biol. 121, 431–454.

Niassy, S., Maniania, N.K., Subramanian, S., Gitonga, L.M., Ekesi, S., 2012. Performance of a semiochemical-baited autoinoculation device treated with *Metarhizium anisopliae* for control of *Frankliniella occidentalis* on French bean in field cages. Entomol. Exp. Appl. 142, 97–103.

Nielsen, D.G., Dunlap, M.J., 1981. Black vine weevil: reproductive potential on selected plants. Ann. Entomol. Soc. Am. 74, 60–65.

Payne, R.W., Harding, S.A., Murray, D.A., Soutar, D.M., Baird, D.B., Glaser, A.I., Channing, I.C., Welham, S.J., Gilmour, A.R., Thompson, R., Webster, R., 2009. The Guide to GenStat Release 12, Part 2: Statistics, VSN International, Hemel Hempstead.

Phillips, P.A., 1989. Simple monitoring of black vine weevil in vineyards. Calif. Agric. 43, 12–13.

Pope, T.W., Hough, G., Arbona, C., Roberts, H., Bennison, J., Buxton, J., Prince, G., Chandler, D., 2018. Investigating the potential of an autodissemination system for managing populations of vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae) with entomopathogenic fungi. J. Invertebr. Pathol. 154, 79–84.

Ranger, C.M., Reding, M.E., Oliver, J.B., Moyseenko, J.J., Youssef, N.N., 2009. Toxicity of botanical formulations to nursery-infesting white grubs (Coleoptera: scarabaeidae). Hort. Entomol. 102, 304–308.

Reineke, A., Hirsch, J., Kubach, G., 2011. Aggregation, abundance and dispersal capabilities of *Otiorhynchus rugosostriatus* Goeze and *Otiorhynchus raucus* Fabricius (Coleoptera: Curculionidae) in plantations of ornamental plants. J. Pestic. Sci. 84, 297–302.

Roberts, J.M., Jahir, A., Graham, J., Pope, T.W., 2019. Catch me if you can: the influence of refuge/trap design, previous feeding experience, and semiochemical lures on vine weevil (Coleoptera: Curculionidae) monitoring success. Pest Manag. Sci. https://doi.org/10.1002/ps.5545.

Shanks, C.H., Doss, R.P., 1986. Black vine weevil (Coleoptera: Curculionidae) feeding and oviposition on leaves of weevil-resistant and –susceptible strawberry clones presented in various quantities. Environ. Entomol. 15, 074-1077.

van der Horst, M.J., van Tol, R.W.H.M., 1995. Integrated pest management in nursery stock in The Netherlands. Meded. Fac. Landbouwwet. Univ. Gent 60, 759–762.

van Tol, R.W.H.M., 1996. Prospects for biological control of black vine weevil in nursery stock. Mitt. Biol. Bundesanstalt 316, 69–75.

van Tol, R.W.H.M., Raupp, M.J., 2005. Nursery and tree application (Chapter 9). In: Grewal, P.S., Ehlers, R.-U., Shapiro-Ilan, D.I. (Eds.), Nematodes as Biological Control Agents. CABI Publishing, Wallingford, Oxfordshire, UK, pp. 167–190.

van Tol, R.W.H.M., van Dijk, N., Sabelis, M.W., 2004. Host plant preference and performance of the vine weevil *Otiorhynchus sulc*atus. Agric. For. Entomol. 6, 267–278.

van Tol, R.W.H.M., Bruck, D.J., Griepink, F.C., de Kogel, W.J., 2012. Field attraction of the vine weevil *Otiorhynchus sulcatus* to kairomones. J. Econ. Entomol. 105 (1), 169–175.

Yasuda, K., 1999. Auto-infection system for the sweet potato weevil, *Cylas formicarius* (Fabricius) (Coleoptera: Curculionidae) with entomopathogenic fungi, *Beauveria bassiana* using a modified sex pheromone trap in the field. Appl. Entomol. Zool. 34, 501–505.