CATCH ME IF YOU CAN: THE INFLUENCE OF REFUGE / TRAP DESIGN, PREVIOUS FEEDING EXPERIENCE, AND SEMIOCHEMICAL LURES ON VINE WEEVIL (COLEOPTERA: CURCULIONIDAE) MONITORING SUCCESS

RUNNING TITLE: FACTORS INFLUENCING VINE WEEVIL MONITORING SUCCESS

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ABSTRACT

BACKGROUND:

Vine weevil, *Otiorhynchus sulcatus* F. (Coleoptera: Curculionidae), is one of the most economically important pest species of berry and ornamental crops globally. Monitoring this nocturnal pest can be difficult and time consuming and the efficacy of current tools is uncertain. Without effective monitoring tools, implementation of integrated pest management strategies is challenging. This study tests the relative efficacy of a range of vine weevil monitoring tools. Whether host-plant volatiles and weevil feeding experience influence vine weevil capture is also tested.

RESULTS:

Monitoring tool efficacy differed overall between the six monitoring tool designs tested and ranged from catches of 0.4 % to 26.7 % under semi-field conditions. Previous feeding experience influenced vine weevil behaviour. In yew conditioned populations, 39 % of the weevils responded to and were retained in the trap baited with yew foliage while 37 % of weevils from *Euonymus fortunei* conditioned populations responded to and were retained in the trap baited with *E. forunei* foliage. A simple synthetic lure consisting of (*Z*)-2-pentenol + methyl eugenol also increased vine weevil catches compared with an unbaited trap.

CONCLUSION:

Demonstrating differences in the efficacy of different monitoring tool designs is an important first step for developing improved methods for monitoring vine weevil populations within crops. This study presents the first direct comparison of vine weevil monitoring tool designs and indicates that trap efficacy can be improved by baiting with host-plant material or a synthetic lure based on host-plant volatiles.

KEY WORDS: vine weevil; pest management; monitoring tools; semiochemicals; feeding experience

HEADINGS

1 INTRODUCTION

1 INTRODUCTION

Vine weevil, or black vine weevil, *Otiorhynchus sulcatus* F. (Coleoptera: Curculionidae), is one of the most economically important pest species of berry and ornamental crops globally.[1,2] Only female vine weevils are known, and reproduction is via thelytokous parthenogenesis.[3] As a result, little genetic diversity exists within this species.[3] The flightless adults are nocturnal and lay their eggs at night into cracks in the soil or growing medium or occasionally on the leaves, stems and crowns of plants.[4] Upon hatching, larvae complete four to nine moults before pupating in earthen cells.[5] Typically, vine weevils are univoltine, but as a winter diapause is not required and their development rate is temperature dependent,[6] overlapping generations may occur in protected environments, such as glasshouse grown crops. Crop damage, and the subsequent economic losses, are largely the result of feeding on the roots, corms and rhizomes by larvae and on the leaves by adults.[7]

Broad-spectrum synthetic chemical insecticides, applied either through incorporation into plant growing media or as foliar sprays are used to control vine weevil populations by targeting both the larval and adult life-stages.[2] Use of these chemical control measures does, however, have a negative impact on beneficial arthropod populations,[8] often leading to an increased risk of secondary pest outbreaks within a crop.[2] Recently there has been a shift from using synthetic chemical insecticides for control of vine weevil larvae to the use of entomopathogenic nematodes and fungi.[9-14] Control of adults, however, still largely relies on broad-spectrum insecticides,[2,7] although the potential of entomopathogenic fungi [15] and the plant extract azadirachtin [16] has been demonstrated.

One of the underlying principles of an integrated pest management (IPM) programme is to base the use of any control measure on careful pest population monitoring in relation to action thresholds.[17] Effective monitoring of vine weevil populations is difficult due to their nocturnal feeding activity as adults and the subterranean lifestyle of the larvae, often resulting in growers not realising that they have an economically damaging pest population until crop losses have been inflicted.[2] In addition to night-time assessments of crops, the presence of vine weevil adults may be determined through the use of artificial refuges or traps. These approaches exploit the nocturnal behaviour of adult vine weevil, which means that weevils seek out shelters during daylight hours. A number of refuge designs have been used for monitoring vine weevil populations, including: grooved wooden boards placed on the ground,[18,19] pitfall traps,[20] corrugated cardboard wrapped around stems of larger bushes [21] or rolls of cardboard placed on the ground, traps used for other species of weevil and plastic crawling insect traps.[15] Despite the availability of a range of vine weevil refuge and trap designs, there is little information on their relative efficacy for monitoring populations of vine weevil adults. Studies that have been undertaken provide contradictory information, with Maier[22] and Li *et al.*[18] suggesting that grooved wooden boards are more effective than pitfall traps while Hanula[20] argues that pitfall traps are the more effective of these approaches.

It has previously been demonstrated for other beetle species that monitoring tool efficacy can be improved through the addition of a semiochemical lure.[23-27] To date there has been little progress in identifying vine weevil specific semiochemicals suitable for this purpose, with previous work on aggregation pheromones proving inconclusive.[28,29] Without identification of vine weevil pheromones, the focus has shifted toward other semiochemical sources, primarily in the form of plant-originating volatile organic compounds (VOCs). Several studies have shown that vine weevil adults detect and respond to plant-derived odours, which are used to locate suitable host-plants for feeding and oviposition. For example, odours of yew (*Taxus baccata* (L.)) and *Euonymus fortunei* (Turcz.) Hand.-Maz damaged by adult vine weevils are attractive to other adult vine weevils, but *Rhododendron* and strawberry (*Fragaria* x *ananassa*) are not.[30] It has similarly been reported that vine weevil adults also respond positively to synthetic versions of (*Z*)-2-pentenol and methyl eugenol, which are found in the odour of one of their host-plants *E. fortunei*, when provided in a 1:1 binary blend in a strawberry field.[2] The synthetic blend tested by van Tol *et al*[2] led to increased numbers of weevils near the traps with a lure placed inside the top part of the tested boll weevil trap. Bruck *et al.*[31] tested (*Z*)-2-pentenol as a single component lure, in combination with the 'WeevilGrip' ruffle trap, which also led to increased vine weevil catches, albeit less than the 1:1 binary blend of (*Z*)-2-pentenol and methyl eugenol reported by van Tol *et al*..[2] A synthetic lure based on (*Z*)-2-pentenol has recently been patented for vine weevil monitoring.[31]

Despite the availability of a range of artificial vine weevil refuges and traps the relative efficacy of these approaches for capturing and retaining vine weevil adults, and therefore their usefulness for monitoring this pest, remains largely unknown. Furthermore, without baiting these refuges and traps with an attractive semiochemical, there is a lack of sensitivity for early, reliable detection of infestations. This study reports on the relative efficacy of six different monitoring tool designs, whether host-plant material can be used to increase catches of adult vine weevils and whether previous feeding experience influences responses to host-plant odours with the aim of improving monitoring methods for this economically important pest.

2 MATERIALS AND METHODS

2.1 Insect cultures

Adult vine weevils (*Otiorhynchus sulcatus* F.) were collected during the summer of 2016 from commercial strawberry crops grown in Newport, Shropshire and Penkridge, Staffordshire in the UK for the trap efficacy experiment and from the same farms during the summer of 2018 for the feeding experience experiments. In both cases the recovered vine weevils were initially maintained on branches of yew (*T. baccata*) and moist paper towels, which were replaced weekly, inside insect cages (47.5 x 47.5 x 47.5 cm) (Bugdorm, MegaView, Taiwan) placed in a controlled environment room (20 °C; 60 %RH; L:D 16:8) (Fitotron, Weiss Technik, Ebbw Vale, Wales). Vine weevils were maintained under these conditions for at least one month before use in experiments during which time it was confirmed that the weevils were reproductively active.

2.2 Monitoring tool efficacy experiment

The efficacy of six different monitoring tool designs was tested in a 'semi-field' environment simulating a susceptible crop (Fig. 1). To create this 'semi-field' environment, five potted strawberry plants (*cv*. Elstanta) were placed in a 'tent' cage (145 x 145 x 152 cm) (Insectopia, UK) situated within a polytunnel (mean day-time temperature = 23.7°C and mean night-time temperature = 14.5°C). Monitoring tools were used as supplied by the manufacturer except for the pitfall trap, which was modified by painting the top of the catching box with PTFE paint (Fluon^{TM}) to prevent weevils escaping.

Each unbaited monitoring tool was individually placed in a tent cage (145 x 145 x 152 cm) (Insectopia, UK) with five potted strawberry plants to provide both a food source and a range of alternative refuges e.g. under pots, around rims, within compost. A known population of 40 vine weevils (approx. 19 weevils/m^2) was collected from the culture and placed into 'mini' insect cages (12.5 x 11.4 cm) (BugDorm, MegaView, Taiwan) and then released into the centre of the experiment cage by gently upending the 'mini' insect cage. The efficacy of each monitoring tool was assessed on 12 occasions (between 9th and 14th August 2016) by recording numbers of weevils within the traps between 09:00 and 12:00 each day. The tent cage to which each monitoring tool was allocated was re-randomised each day to exclude the effect of tent cage position and/or simulated crop. Weevil populations were changed between each replicate.

2.3 Feeding experience experiments

2. 3. 1 Vine weevil preconditioning

Prior to their use in 'feeding experience' experiments, adult vine weevils were preconditioned on either yew or *E. fortunei* depending on the experimental design. Preconditioning was undertaken by transferring twenty-five vine weevils into 'mini' insect cages and providing them with material from one of the two plant species for ten days. Plant material was prepared by cutting branches from the main stem and wrapping the cut end in moist tissue paper, which was replaced every two days. A ball of dry tissue paper was also placed within the insect cage to provide a refuge area. As the insect culture was maintained on yew, individuals preconditioned on yew had more than thirty days feeding experience on this plant species while those preconditioned on *E. fortunei* were initially fed on yew before switching to *E. fortunei* for conditioning.

2. 3 .2 Preference bioassays

The behavioural responses of preconditioned adult vine weevils to a variety of chemical stimuli were tested during three experiments in a 'semi-field' environment simulating a strawberry crop (Table 1). To create this 'semi-field' environment, four potted strawberry plants (*cv.* Elsanta) were placed in a 'tent' cage (145 x 145 x 152 cm) (Insectopia, UK) situated within an unheated glasshouse (mean daytime temperature = 28.4°C and mean night-time temperature = 16.9°C). Two vine weevil traps were then positioned an equal distance from one another inside the 'tent' cage, with each trap containing one of the experimental treatments. For experiments one and two the treatments were 15 g of plant material from yew or *E. fortunei* plants or unbaited (i.e. empty) while in experiment three the treatments were 15 g of plant material from yew, 100 µl of a synthetic lure (100 mg/ml) or unbaited. Plant material consisted of small branches (~ 5 cm) containing foliage, which was secured in a perforated nylon bag (30 x 17 cm and with mesh aperture 160 µm) to prevent the vine weevils from accessing the plant material while allowing treatment VOCs to enter the surrounding environment. Lures used for this study were based on the design described by Fountain *et al.*[32] with some minor modifications. In brief, lures were constructed from opaque 1 ml polypropylene pipette tips with a 0.2 mm aperture (Fisher Scientific Loughborough, UK). The synthetic lure, a 1:1 blend of (*Z*)-2-pentenol and methyl eugenol,[2] was dissolved in analytical grade paraffin oil (Sigma-Aldrich, Gillingham, UK) at a concentration of 100 µl/ml before impregnating onto a cellulose acetate cigarette filter (14 x 6 mm) (Swan, High Wycombe, UK) placed in the pipette tip. Lures were sealed at one end with a 11 mm PTFE-lined crimp seal (Sigma-Aldrich, Gillingham, UK).

Four 'tent' cages were set up to enable one replicate of each of the four treatments to be undertaken at one time over 10 consecutive days. Treatment positions were randomised between each replicate to account for any bias arising from environmental conditions or trap position. Once the environments had been set up, a known population of 15 preconditioned vine weevils was collected and placed into 'mini' insect cages and then released into the centre of the experiment cage between 18:00 and 20:00 by gently inverting the 'mini' insect cage. The number of vine weevils in each of the traps was then recorded the following morning between 08:00 and 09:00. After each assessment the vine weevils were returned to the insect cages in the controlled environment room (20 °C; 60 %RH; L:D 16:8) (Fitotron, Weiss Technik, Ebbw Vale, Wales) to continue feeding on the preconditioning plant until the next bioassay. Weevil populations were changed between each replicate.

2.4 Statistical analyses

All statistical analyses were performed using R (Version 3.5-3).[33] Monitoring tool performance (i.e. the number of individuals within the monitoring tool) was evaluated with a general linear model (GLM) with a quasipoisson probability distribution and 'trap type' as a factor using the *glm* function from the *stat*s R package.[33] Multiple comparisons for the GLM were evaluated by Tukey's HSD tests implemented in the *HSD.test* function in the R package *agricolae*.[34,35]

Feeding experience experiment observations were individually analysed using binomial exact tests against the null hypothesis that the number of vine weevils in each trap had a 50:50 distribution using the *binom.test* function in the *stats* R package. The replicated results were pooled for each trial and un-trapped individuals were excluded from statistical analyses, where *n* = the number of trapped individuals for these analyses.

3 RESULTS

3.1 Vine weevil monitoring tool performance

Monitoring tool efficacy differed overall between the designs tested (generalised linear model: $X_5^2 =$ 249.71, *df* = 66, *P < 0.001*) and ranged from catches of 0.4 % to 26.7 % of the vine weevil populations introduced into the tent cage arenas (Fig. 2). The vine weevil trap was most effective for retaining vine weevils (26.7 %) (Fig. 2), while the pitfall trap (6.6 %), cockroach bait station (5.8 %), and red palm weevil trap (5.2 %) showed similar performance to one another (Fig. 2). Grooved boards and cardboard rolls were the least effective monitoring tools tested in this experiment, catching 0.4 and 0.8 % respectively (Fig. 2).

3.2 Feeding experience experiment 1 – vine weevils preconditioned on yew

Vine weevils preconditioned on yew for ten days exhibited a preference for the traps baited with plant material from either of the plant species when offered against unbaited traps: unbaited vs *E. fortunei* (binomial exact test: *P* < 0.001, *n* = 54) and unbaited vs yew (binomial exact test: *P* < 0.001, *n* = 63) (Fig. 3). However, when vine weevils preconditioned on yew were offered a choice between traps baited with either yew or *E. fortunei* plant material, they exhibited a preference for traps baited with yew (binomial exact test: *P* < 0.001, *n* = 82) (Fig 3).

3.3 Feeding experience experiment 2 – vine weevils preconditioned on Euonymus fortunei

Vine weevils preconditioned on *E. fortunei* for ten days exhibited a preference for the traps baited with plant material from either of the plant species when offered against unbaited traps, unbaited vs *E. fortunei* (binomial exact test: *P*< 0.001, *n* = 82) and unbaited vs yew (binomial exact test: *P* < 0.001, *n* = 57) (Fig. 4). However, when vine weevils preconditioned on *E. fortunei* were offered a choice between traps baited with either yew or *E. fortunei* plant material, they exhibited a preference for traps baited with *E. fortunei* (binomial exact test: $P < 0.001$, $n = 77$) (Fig 4).

3.4 Feeding experience experiment 3 – synthetic lure

Vine weevils preconditioned on yew for ten days exhibited a preference for the traps baited with yew plant material when offered against an unbaited trap (binomial exact test: $P < 0.001$, $n = 65$) or a binary synthetic lure (binomial exact test: $P < 0.05$, $n = 65$) (Fig. 5). However, when vine weevils preconditioned on yew were offered a choice between an unbaited trap or one containing the binary synthetic lure, they exhibited a preference for traps containing the lure (binomial exact test: $P < 0.01$, $n = 59$) (Fig 5).

4 DISCUSSION

A range of refuges and traps have been developed to monitor for the presence of vine weevil adults within crops. Until now there has been little work to directly compare the efficacy of the tools available for vine weevil monitoring. Results from this comparison of different tools for vine weevil monitoring indicates that each tool can detect the presence of vine weevil adults, but there were large differences in terms of their efficacy to retain vine weevils (Fig. 2). The most effective monitoring tool design tested was the vine weevil trap commercially available for monitoring this pest species. Why this trap design proved to be more effective than the other monitoring tool designs tested is unclear, but with no semiochemical lure used it could be attributed to monitoring tool size, colour, shape or the number and design of the entrances. This is especially evident when comparing the vine weevil and red palm weevil traps, where the designs (colour and silhouette) are similar but displayed significant differences in efficacy. Perhaps the key difference between these two trap designs is the location of the entrance, which is at the bottom of the vine weevil trap and the top of the red palm weevil trap. Although the vine weevil trap retained the most weevils in this study, in work testing the efficacy of the same trap for monitoring the cranberry weevil, *Anthonomus musculus* Say (Coleoptera: Curculionidae), it was found to be the least effective of those tested.[36] This difference is likely, however, to be a consequence of the cranberry weevil being able to fly while vine weevil adults are restricted to walking.

Understanding the efficacy of the different monitoring tool designs available to detect the presence of vine weevil adults within crops, is an important step in developing more effective IPM strategies for this economically important pest. With growers often considering use of direct monitoring of vine weevil adults,[18,19,21,22] it is vital that the information obtained from monitoring tools is reliable and timely if control measures are to be applied before economic losses are incurred. It is interesting to note then that two of the most frequently used approaches, grooved wooden boards[18,19] and corrugated cardboard[21] retained the fewest vine weevils of the tested tools. As such, improvements in monitoring for vine weevil adults can be made by simply switching from the use grooved boards or corrugated cardboard to another monitoring tool design.

Research on attractants for vine weevil adults has primarily focused on potential aggregation pheromones produced by live weevils, volatiles emitted from their frass, and volatiles produced by host-plants. This is the first study, however, to report increased trap catches using semiochemicals, in this case the odour of cut foliage from one of their host plants, either yew or *E. fortunei*. Previous work had shown only that use of host plant volatiles could increase numbers of vine weevil adults in the area around the trap but importantly did not increase trap catches.[2]

In the first two experiments in this study, vine weevil adults showed a preference towards the traps baited with host-plant foliage compared to unbaited traps (Figs. 3 and 4). When given a choice between traps baited with different host-plant foliage, significantly more adult weevils were found in traps baited with the host-plant foliage on which they were conditioned for ten days before the start of the experiment. This behavioural plasticity in herbivorous insects has been thoroughly reviewed by Papaj and Prokopy[37] and Bernays[38] and is reported in several insect orders, including: Orthoptera,[39] Hemiptera,[40] and Lepidoptera.[41] With respect to phytophagous Coleoptera, there are several examples in which previous feeding experience has been found to influence feeding preference.[37] The Hopkins' host-selection principle (HHSP) suggests that many adult phytophagous insects exhibit a strong preference for their developmental plant species that cannot be 'reprogrammed'.[42] However, it appears that innate host plant preferences can be modified in adult insects in a relatively short period of time,[43] and some species of insect are able to switch to a new crop plants relatively quickly. Behavioural plasticity in vine weevil may have implications for designing effective monitoring strategies used as part of future IPM programmes. In this study, the background crop used differed from either host plant used as a bait. As such it may be that a semiochemical lure based on plant volatiles would need to incorporate VOCs from the crop it is being used in to be effective due to vine weevils becoming preconditioned to this host plant. Conversely, lures that simply mimic the odour of the crop in which they are placed may not always be effective. For example, in a study evaluating semiochemical baited traps for monitoring the pea leaf weevil, *Sitona lineatus* L. (Coleoptera: Curculionidae), traps containing only host plant volatiles were not effective.[27]

As vine weevil adults are nocturnal they feed at night and seek shelter during daylight hours.[4] Consequently, the trap tested in the preconditioning section of this study is primarily designed to act as daytime refuge and not to be used by the weevils while feeding at night. While it may appear counterintuitive to place host plant material within the traps, as vine weevils would be seeking refuge rather than feeding sites when they are entered, in the field vine weevils can be found to have aggregated on and around host plants, such as around the base of leaf petioles, during daylight hours.[7] The mechanism underlying this aggregation behaviour is largely unknown, but odours from damaged host plants may play a role.[30] Further research is required to investigate the effect of placing a lure inside or next to a trap on use of the trap as a refuge by weevils.

The behavioural response of adult vine weevil to synthetic chemical compounds identified in the headspaces of their host-plants has been studied by van Tol *et al*.[2] Using a binary blend of (*Z*)-2-pentenol and methyl eugenol together with the vine weevil trap design more weevils were recorded in the trap containing the synthetic lure than in the empty trap (Fig. 5). Previously van Tol *et al.*[2] reported that this binary blend only increased numbers of weevils within the boll trap vicinity and not in the trap itself. This is an important distinction as it highlights that with the correct lure and trap design it is possible to increase vine weevil catches. Nonetheless, it is possible that the lure is acting a similar way to that reported by van Tol *et al*.[2] by increasing weevil numbers close to the trap but that the improved design of the vine weevil trap led to increased numbers of weevils seeking refuge in this trap at sunrise. When the synthetic lure was, however, released from one trap and the host-plant lure on which the vine weevil adults had been preconditioned from the other trap, more weevils were caught in the trap releasing the host-plant lure (Fig. 5). Although van Tol *et al.*[2] did not report increased trap catches with their two-component synthetic lure, a single-component lure consisting of (*Z*)-2-pentenol in combination with the 'WeevilGrip' ruffle traps is reported to increase trap catches.[31] Synthetic lure efficacy could potentially be increased by adding further chemical compounds. It is generally accepted that herbivorous insects locate host-plants by sensing the entire odour profile of a plant rather than by a few key chemicals within the profile[44,45] and so a more effective synthetic vine weevil lure may contain more than two components. However, it is important to note that odour profiles of the host-plant foliage found to be effective in this study had been cut and so the odour profiles will differ to that of undamaged foliage.[46] A future line of investigation may then be to determine if the most effective lure is based on the odour profile of damaged or undamaged foliage.

5 CONCLUSION

Demonstrating differences in the efficacy of different monitoring tool designs is an important first step for developing improved methods for monitoring vine weevil populations within crops. Even with this improved understanding there remains little known about what makes a good vine weevil monitoring tool in terms of shape and colour. Indeed, while vine weevil adults are known to exhibit thigmotactic behaviours it is noticeable that the two worst performing monitoring tool designs tested here exploit this aspect of vine weevil biology. Further work is required to understand the visual ecology and refuge requirements of vine weevil to optimise monitoring tool design and further increase their efficacy in the field. Silva *et al.*[36] highlight that for monitoring the cranberry weevil trap colour influences efficacy and argue that without semiochemicals traps have limited applicability. Without identification of a vine weevil pheromone for use as an attractant, host-plant volatiles are the most promising source to develop an attractant to improve vine weevil trapping. Combining a simple synthetic lure based on host-plant volatiles with a well-designed trap would provide an effective tool for monitoring vine weevil populations. This study provides evidence that host-plant volatiles can be exploited to improve monitoring tool efficacy by increasing the number of individuals responding to and being retained by vine weevil traps, but further work is required to develop more effective monitoring tools and establish whether a synthetic lure based on plant material can be usefully deployed in a range of crops.

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TABLES

Table 1: Feeding experience experiments.

Experiment	Trial	Preconditioning plant	Treatment 1 [a]	Treatment 2 [a]	No. of replicates
1	1	Yew	Unbaited	*E. fortunei*	10
	2	Yew	Unbaited	Yew	10
	3	Yew	*E. fortunei*	Yew	10
	4	Yew	Unbaited	Unbaited	10
2	1	*E. fortunei*	Unbaited	*E. fortunei*	10
	2	*E. fortunei*	Unbaited	Yew	10
	3	*E. fortunei*	*E. fortunei*	Yew	10
	4	*E. fortunei*	Unbaited	Unbaited	10
3	1	Yew	Unbaited	Yew	10
	2	Yew	Unbaited	Synthetic lure [b]	10
	3	Yew	Yew	Synthetic lure [b]	10
	4	Yew	Unbaited	Unbaited	10

[a] 15 g of 5 cm branches were used for yew and *E. fortunei* treatments

[b] 100 µl (*Z*)-2-pentenol + methyl eugenol (100 mg/ml) [2]

FIGURE LEGENDS

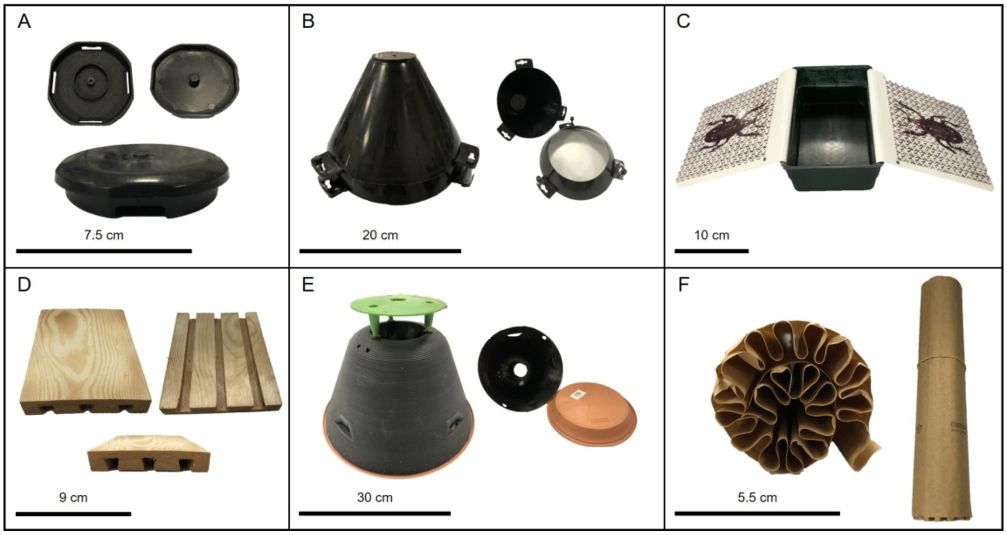
Figure 1: Monitoring tool designs tested in this study for vine weevil (*Otiorhychus sulcatus*): (A) Cockroach bait station (BASF, Cheadle Hulme, UK); (B) Vine weevil trap (ChemTica, Heredia, Costa Rica); (C) Pitfall trap modified by painting liquid PTFE around rim (Csalomon, Budapest, Hungary); (D) Grooved wooden board; (E) Red palm weevil trap (Sentomol, Monmouth, UK); (F) Corrugated cardboard roll (W 5.5 cm x L 30 cm). Scale bars indicate size in the largest image for A, B, and E.

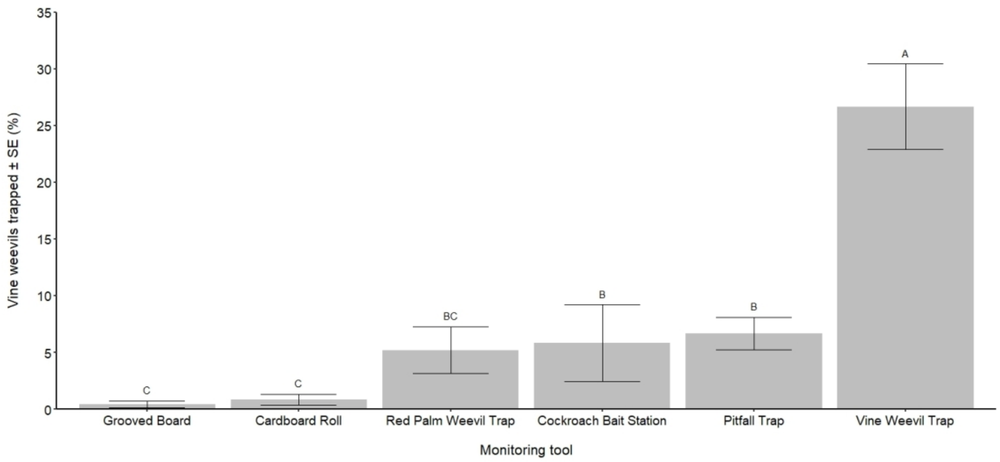
Figure 2: Mean (± SE) trap catch of populations of 40 adult vine weevils. Means capped with different letters are significantly different (generalised linear model: $X_5^2 = 249.71$, *df* = 66, *P* < 0.001; Tukey's HSD test: *P*< 0.05*)*.

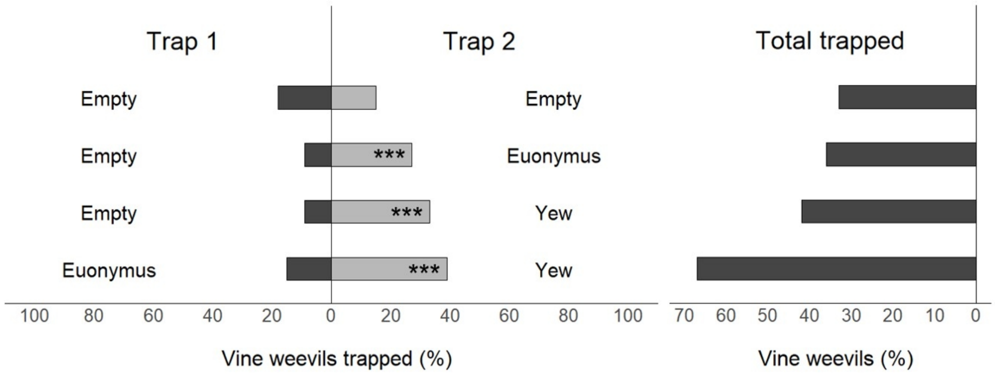
Figure 3: Behavioural responses of adult vine weevils preconditioned on yew under four 'semi-field' experimental scenarios. Asterisks indicate significance levels calculated using binomial exact tests: * *P* < 0.05; ** *P*< 0.01; *** *P* < 0.001.

Figure 4: Behavioural responses of adult vine weevils preconditioned on *Euonymus* under four 'semi-field' experimental scenarios. Asterisks indicate significance levels calculated using binomial exact tests: * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

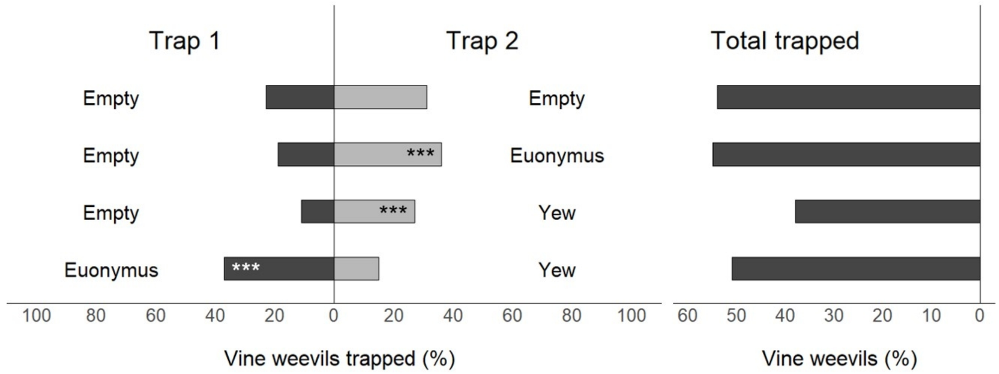
Figure 5: Behavioural responses of adult vine weevils preconditioned on yew under four 'semi-field' experimental scenarios. Asterisks indicate significance levels calculated using binomial exact tests: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

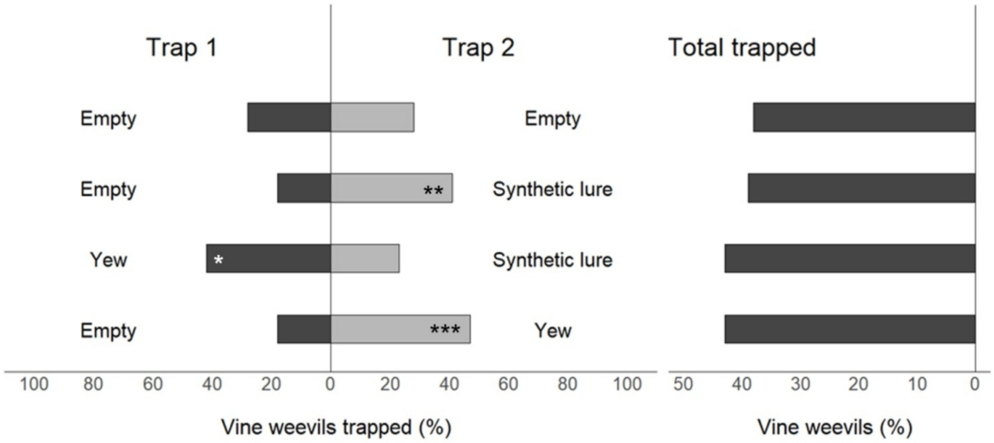

A
7.5 cm
B
20 cm
C
10 cm
D
9 cm
E
30 cm
F
5.5 cm


35
30
25
20
15
10
5
0
Vine weevils trapped ± SE (%)
A
BC
B
B
C
C
Grooved Board
Cardboard Roll
Red Palm Weevil Trap
Cockroach Bait Station
Pitfall Trap
Vine Weevil Trap
Monitoring tool



Trap 1
Trap 2
Total trapped
Empty
Empty
Empty
Euonymus
Empty
Euonymus
Yew
Yew
***
***
***
100 80 60 40 20 0 20 40 60 80 100
70 60 50 40 30 20 10 0
Vine weevils trapped (%)
Vine weevils (%)


Trap 1
Trap 2
Total trapped
Empty
Empty
Empty
Euonymus
Empty
Euonymus
Yew
Yew
***
***
***
100 80 60 40 20 0 20 40 60 80 100
Vine weevils trapped (%)
60 50 40 30 20 10 0
Vine weevils (%)


Trap 1
Trap 2
Total trapped
Empty
Empty
Empty
Synthetic lure
Yew
Synthetic lure
Empty
Yew
**
*
***
100 80 60 40 20 0 20 40 60 80 100
50 40 30 20 10 0
Vine weevils trapped (%)
Vine weevils (%)