Repellence of the red bud borer *Resseliella oculiperda* from grafted apple trees by impregnation of rubber budding strips with essential oils

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Abstract: The red bud borer *Resseliella oculiperda* (Rübs.) is a pest insect of apple trees when rootstocks are grafted with scion buds by 'shield budding'. The female midges are attracted to the wounds of the grafted buds where they lay their eggs. The larvae feed on the cambium and destroy the buds completely or partially, leading to bad union of the buds with the rootstocks. Budding strips are used very often by growers to bind scion buds to rootstocks. These strips cannot prevent midges from reaching the damaged tissue. Chemical treatments applied to the grafts and other types of strip do not provide better protection against the pest and may cause other risks for growers. In orchard experiments in 2000 and 2001, the authors evaluated the repellent action provided by three essential oils and five compounds of plant origin against the midges by impregnating budding strips with them. The essential oils of lavender, *Lavandula angustifolia* (P. Mill.), and α-terpineol decreased the infestation of buds by more than 95 and 80% respectively. The other potential repellents tested [the essential oil of *Juniperus virginiana* (L.), citronellal, the essential oil of *Cinnamomum camphora* (L.) J. Presl, *R*-carvone, linalool and *R*-fenchone] decreased infestation by 67, 66, 51, 45, 37 and 25% respectively. The formulation and commercial development of budding strips impregnated with lavender oil is discussed.

Keywords: red bud borer; *Resseliella oculiperda*; *Thomasiniana oculiperda*; plant essential oils; repellents; *Malus*; budding strips

1 INTRODUCTION

The producers of apple rootstocks in the Netherlands and Belgium have increasing problems controlling infestations of the red bud borer *Resseliella oculiperda* (Rübs.) [syn. *Thomasiniana oculiperda* (Rübs.)] (Diptera: Cecidomyiidae).

The female midges are attracted to the odour of freshly grafted buds of apple trees where they lay their eggs. The larvae feed on the cambium, thereby destroying the grafted buds. After 2–3 weeks, the larvae fall to the ground where they pupate in the top layer of soil. Pupation usually takes 2–3 weeks (depending on temperature and day length) before the adult midges emerge from the soil. In the Netherlands, three or four main flights of the midge occur,[1] usually when apple trees are being grafted or pruned. The only other plant species considered a true host for this midge is *Rosa* sp., although the results of host-plant preference studies have indicated that *R. oculiperda* from apple trees strongly prefer apple for egg laying, while *R. oculiperda* from rose do not show any preference for egg laying.[2] It was concluded from these studies that at least two strains of *R. oculiperda* exist, with the apple strain probably being dominant in apple-growing areas.[2] Taxonomic differences between the two subspecies have not been demonstrated. Other plant species that are attacked occasionally by *R. oculiperda* are pear (*Pyrus communis* L.), plum (*Prunus domestica* L.), peach (*Prunus persica* Batsch), apricot (*Prunus armeniaca* L.) and some ornamental tree species of *Acer* and *Tilia*.[1,3,4] As damage to these budded ornamental trees occurs only in areas where amenity tree growers and apple orchards are in close proximity, the ornamental species are not considered to be true hosts for the red bud borer. However, in the Netherlands, apple orchards and amenity tree nurseries are often close and the midge frequently causes serious problems. The damage caused by *R. oculiperda* to budded rootstocks of apple in the Netherlands varies from year to year in different locations. It may result in the loss of 10–80% of unprotected trees (Dutch Plant Advisory Service, unpublished). Budding strips are very often used by growers to bind scion buds to rootstocks. Growers in different regions use different types of strip for budding; only one (Buddy-Tape), a paraffinic strip that can be stretched over the whole bud after grafting, provides a mechanical barrier preventing the midge

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from egg laying. The disadvantages associated with 'Buddy-Tape' are its high cost and, more importantly, the risk of buds rotting (the strips do not break down in autumn by weathering) or the strip breaking down early (through melting in high autumn temperatures) before the bud/rootstock unions heal. In both cases, growers face the costs of the extra labour needed to strip off the budding strips by hand and retape the trees, as well as the costs of replacing trees having damaged bud/rootstock unions.

Two alternative options to protect budded rootstocks against the red bud borer were considered. One was to identify the pheromone of the midge and use it to monitor and/or decrease the numbers of the pest. The pheromones of several midges have been identified,[5,6] but the practical value of using them other than for the better timing of chemical treatments is questionable. Monitoring the red bud borer with pheromones would be of little value for growers as infestations occur immediately after budding. The current treatment in the Netherlands against the pest is a single application with deltamethrin immediately after grafting. The efficacy of this treatment appears to be highly variable, and the treatment is not considered by growers to provide very reliable protection. The other option is the use of a repellent odour, impregnated in the budding strips, to prevent midges from laying their eggs. The repellence of insects with essential oils is an interesting, relatively new tool for the control of insects that mostly does not share the problems traditional insecticides have in relation to environmental and human toxicity.[7–9] Owing to the unique site of action (disturbance of octopaminergic system), compounds in most essential oils affect only arthropods.[7] The fumigation of insect pests in stored products[10] is very successful, often giving nearly 100% control, but the treatment of plants in greenhouses and open fields is not as effective on account of the high volatility of most essential oils. The slow rates of release from current formulations, ineffective application and high costs are some of the main problems that presently limit practical possibilities of using essential oils in open crop situations. For the red bud borer, some of these problems would be limited, as the repellents can be impregnated in the budding strips to release the odours at high concentrations on small areas of trees that need protection over only a short period (often only 1–2 weeks).

The authors therefore evaluated the efficacy of several essential oils and single components of plant origin in orchards after impregnating budding strips. Cost considerations partially determined the selection of the products tested. A small survey among growers participating in the research revealed that the maximum acceptable cost for each impregnated strip (in 2001) should not exceed 4 eurocents. The cost of currently available unimpregnated budding strips varies between 1 and 1.3 eurocents per strip; this limits the total cost of impregnating repellents to approximately 2 eurocents per strip.

2 MATERIALS AND METHODS

2.1 Budding strips, essential oils and single compounds of plant origin

Rubber budding strips are used by many growers in the Netherlands to bind scion buds to rootstocks. The strips usually break down by weathering, or must be removed 2–3 weeks after the unions have healed. Budding strips, 180 mm long and 6 mm wide, (Flexiband; Fleischhauer, Germany) were impregnated with one of three essential oils or five pure compounds. The strips were impregnated by mixing with essential oil or compound dissolved in distilled dichloromethane in a rotating powder flask until dryness (~20 min at 30 rpm). Impregnated strips were then placed on filter paper in a fume cupboard for ~2 h until the solvent had evaporated, sealed in aluminium-coated envelopes and stored at −20 °C until used in the field. The essential oils tested (Table 1) were from camphor tree [*Cinnamomum camphora* (L.) J. Presl], red cedar (*Juniperus virginiana* L.) and lavender (*Lavandula angustifolia* P. Mill.). The single compounds tested (Table 1) were α-terpineol, (*R*)-(−)-carvone, linalool, (*R*)-(−)-fenchone and citronellal. As a positive control treatment for comparison in 2000 and 2001, unimpregnated Flexiband strips were used; as negative controls, 'Buddy-Tape' (paraffinic band, 2 cm wide) (Aglis, Japan) was used in both years, and in 2001 Flexiband with deltamethrin 25 g L^{-1} EC (Decis; Agrevo, The Netherlands) diluted at 20 mL in 100 L water and sprayed immediately after connection of buds with the strip to the rootstock. Deltamethrin is an insecticide used by growers as an alternative for Buddy-Tape. Buddy-Tape consists of a thin paraffinic strip stretched over the buds after grafting, forming a mechanical barrier to prevent female midges from laying their eggs.

2.2 Release of compounds from budding strips

To determine the release of the volatile compounds from the impregnated budding strips on different days after exposure to the open air at 20 °C, 1 cm long strips were cut from five randomly chosen rubber strips and placed in one vial with 5 mL distilled dichloromethane and 1 µg undecane mL^{-1} as an internal standard. The strips were shaken for 5 h, and the dissolved components released from the strips were analysed by gas chromatography/mass spectrometry (GC-MS). Each treatment was performed in duplicate.

The amounts of each of the compounds in the impregnated Flexibands were measured and expressed as the percentages released from the strips on days 1, 2, 3 and 7 relative to day 0 (= 0%); for the essential oils, only the major compounds are shown (Fig. 1): linalool and linalyl acetate for lavender oil; camphor, 1,8-cineole and linalool for camphor tree oil; and α-thujone, 1,8-cineole and camphor for red cedar oil).

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Table 1. Main compounds present in essential oils and single compound products (% of total product) impregnated in rubber budding strips for control of the red bud borer, *Resseliella oculiperda*

	Treatment[a]							
Compound	Camphor tree oil	Red cedar oil	Lavender oil	Linalool	α-Terpineol	(*R*)-(−)-Carvone	(*R*)-(−)-Fenchone	Citronellal
1,8-Cineole	30	14						
Camphor	35	11						
Linalool	11		39	96				
Linalyl acetate			44					
α-Terpineol	4	4			99			
α-Thujone		31						
(*R*)-(−)-Carvone						>99		
(*R*)-(−)-Fenchone							>99	
Citronellal								95
Mean amount per strip (g)[b]	0.14	0.06	0.08	0.08	0.12	0.12	0.08	0.06
Origin (company)	Roth	Roth	Roth	Fluka	Acros	Aldrich	Fluka	Acros

[a] Camphor tree, *Cinnamomum camphora*; red cedar, *Juniperus virginiana*; lavender, *Lavandula angustifolia*.
[b] Total amount of essential oil or compound product present per budding strip at the start of the field experiments.

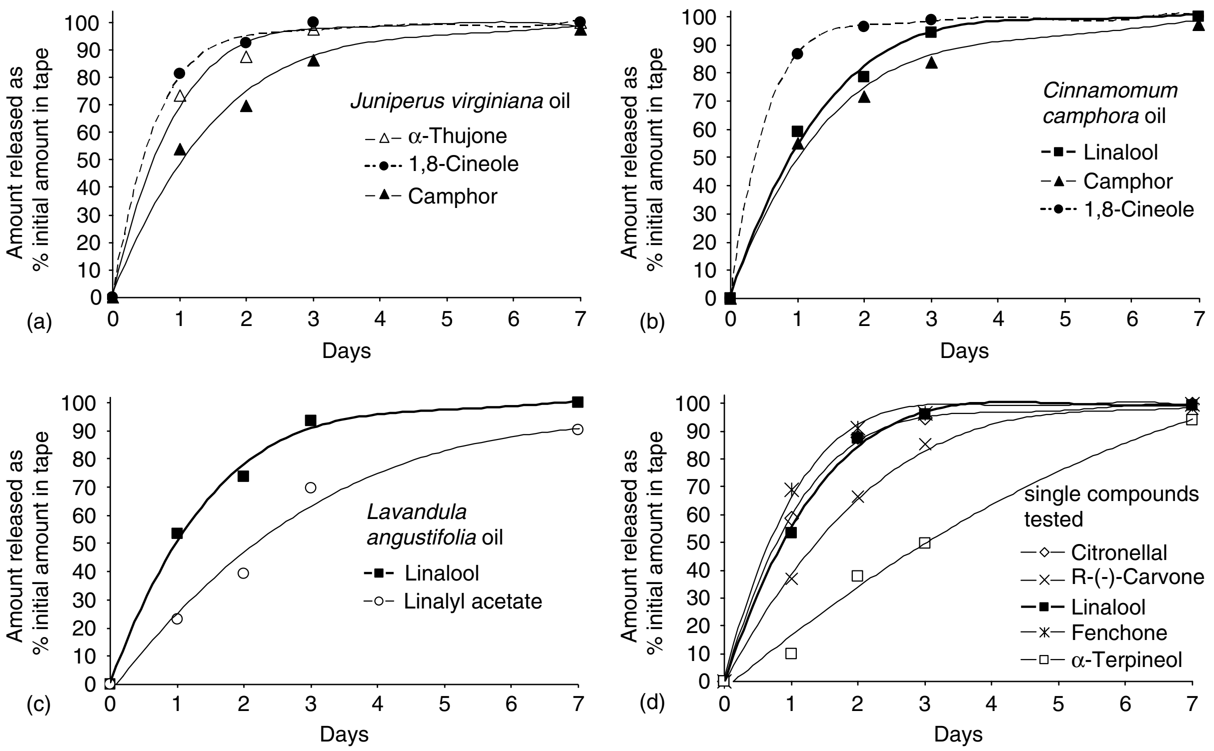

Figure 1. Release over time ($n = 2$) of the main compounds from Flexiband rubber budding strips after exposure to the open air at 20 °C. A: red cedar oil, *Juniperus virginiana*; B: camphor oil, *Cinnamomum camphora*; C: lavender oil, *Lavandula angustifolia*; D: single compounds, citronellal, (*R*)-(−)-carvone, linalool, (*R*)-(−)-fenchone and α-terpineol. Lines presented are the best fitting lines ($R^2 > 0.96$) for the release of the specific volatiles over 7 days.

2.3 Field experiment

Two field tests were done in a commercial orchard of apple (*Malus domestica* 'Jonagold') in Puiflijk, The Netherlands, in 2000 and 2001. The orchard, with trees at 2 m × 2 m spacing, was managed under MBT guidelines[11] (MBT is a system whereby fruit growers in the Netherlands can choose pesticides voluntarily limited to those with low damage to the environment and natural enemies) and was selected for its high infestation of the red bud borer.

A randomised complete block design, with four blocks, was used for both experiments. Each plot comprised a single tree on which four randomly chosen branches were used for budding with one treatment only; there were five trees for each treatment in each block. The blocks were separated by one row of untreated apple trees. The distances between the treated trees in each block were considered large enough because, in preliminary tests, the repellents tested influenced egg laying by the midge only at distances less than 1 cm from the impregnated budding strips on the branches. Artificial wounds on branches 1 cm from impregnated strips connecting grafted buds with the rootstock gave infestations similar to those on grafted buds with untreated strips (personal observation).

Ten different treatments were performed in 2000, and five in 2001 (Figs 2 and 3). In the 2000 experiment, the trees were grafted on 22 August 2000 and checked on 12 September for tissue damage caused by larvae, infestation by larvae, number of larvae per grafted bud and phytotoxicity. In the 2001 experiment, the trees were grafted on 14 August 2001 and, on 5 September, they were checked for the same damage parameters that were used in 2000. Tissue damage was expressed as the percentage of the surface of the grafted tissue damaged by the larvae, and infestations as the percentage of grafted buds infested by larvae. Phytotoxicity was expressed as the percentage of the surface of the grafted tissue showing symptoms of necrosis. In the 2001 experiment only, test trees were also checked (on 8 April 2002) for successful union of the buds with the rootstocks. For this observation, 40 additional graftings on five trees (= plots) per treatment per block were incorporated in the 2001 experiment because of the destructive sampling required for the observations on tissue damage and larval infestations. Successful union of the buds with the rootstock was determined by visual observation (successful connection and drying out or rotting of the buds). Only fully connected buds with no signs of rotting or drying out were considered as successfully united.

Data on temperature, rainfall and relative humidity during the experiments were not measured in the orchard but were collected from the weather station De Bilt (Royal Netherlands Meteorological Institute) situated at ~20 km from the test field.

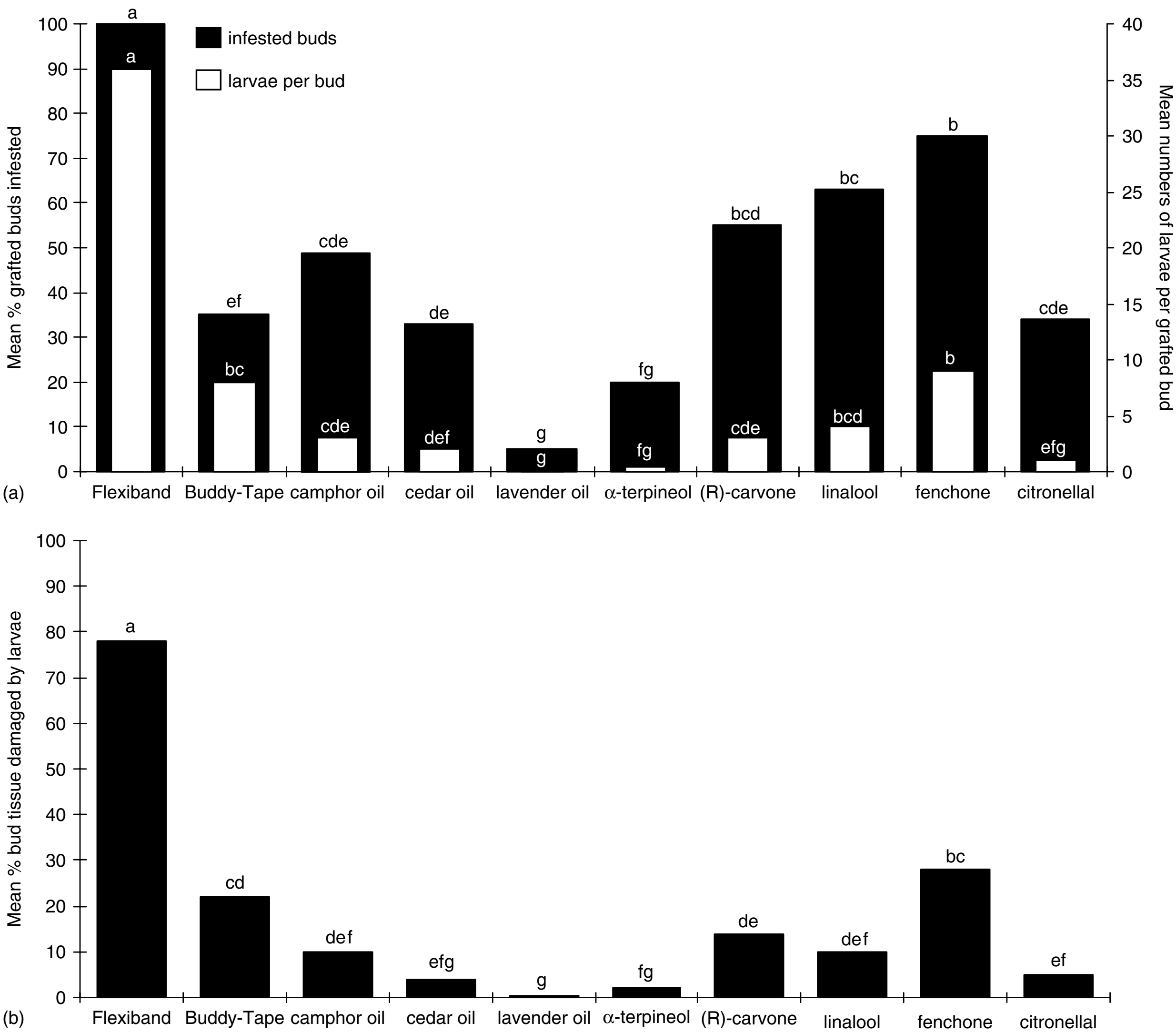

Figure 2. Infestation of grafted apple buds (*Malus domestica* 'Jonagold') by larvae of the red bud borer (*Resseliella oculiperda*) after impregnation of Flexiband budding strips with different essential oils and compounds in a field experiment in 2000. A: mean % grafted buds infested (black bars, $n = 80$) and mean numbers of larvae per bud (white bars, $n = 80$); B: mean % bud tissue damaged by larvae ($n = 80$). Control treatments for comparison are unimpregnated Flexiband and Buddy-Tape (a mechanical barrier to egg laying). A two-tailed regression analysis at $P = 0.05$ was performed on the data, and t-probabilities of pairwise differences were used to compare the different treatments. Means with different letters for the individual parameters are significantly different at $P \leq 0.05$.

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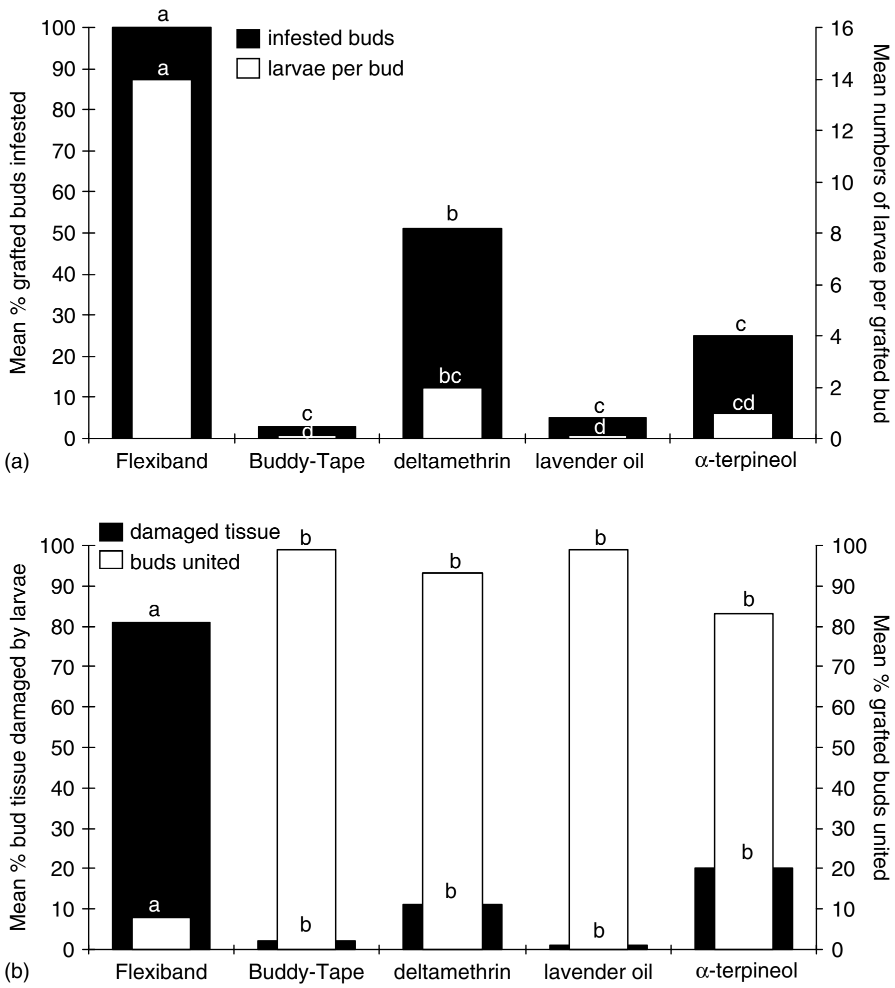

Figure 3. Infestation of grafted apple buds (*Malus domestica* 'Jonagold') by larvae of the red bud borer (*Resseliella oculiperda*) after impregnation of Flexiband budding strips with lavender oil or α-terpineol in a field experiment in 2001. A: mean % grafted buds infested (black bars, $n = 80$) and mean numbers of larvae per bud (white bars, $n = 80$); B: mean % bud tissue damaged by larvae (black bars, $n = 80$) and mean % grafted buds united successfully (white bars, $n = 40$). Control treatments for comparison were unimpregnated Flexiband, Flexiband sprayed with deltamethrin directly after grafting and Buddy-Tape (a mechanical barrier to egg laying). A two-tailed regression analysis at $P = 0.05$ was performed on the data, and t-probabilities of pairwise differences were used to compare the different treatments. Means with different letters for the individual parameters are significantly different at $P \leq 0.05$.

2.4 Statistical analyses

A two-tailed regression analysis (ANOVA) at $P = 0.05$ was performed using the GenStat 5.0 computer program,[12] after angular transformation of the original data on the numbers of infested buds, larval damage to the grafted tissues and the numbers of buds united successfully with the rootstocks, and after log transformation $[x' = \log_{10}(x + 0.5)]$ of the numbers of larvae found in the grafted buds. After the analyses, t-probabilities of pairwise differences were used to compare results given by different treatments.

3 RESULTS

3.1 Release of compounds from budding strips

The evaporation of the volatiles from the impregnated strip indicates a different release pattern in time for only a few of the main components (Fig. 1). The 'in duplo' measurement of each treatment does not allow statistical analysis of the differences, and only indications of differences in release of compounds from the strips are given. Of the single compounds tested (Fig. 1D), *R*-fenchone, citronellal and linalool showed a similar pattern of fast release in time, whereby after 2 days 85–90% of the initial amount was released, and after 3 days 95%. For *R*-carvone, 66% was released after 2 days, 85% after 3 days and 98% after 7 days. The compound with a relatively slow, near-linear release pattern in time was α-terpineol. Release of the initial amount present in the strip after 2, 3 and 7 days was respectively 38, 50 and 94%. Linalool, as one of the main compounds in the essential oil of *L. angustifolia* and *C. camphora*, showed a similar release pattern as part of the oils when compared with the single compound tested (Figs 1B, C and D). Camphor, as part of the essential oil of *J. virginiana* and *C. camphora* (Figs 1A and B), also showed a similar release pattern in both oils (Fig. 1D). The volatile 1,8-cineole, as part of the essential oil of *J. virginiana* and *C. camphora* (Figs 1A and B), showed an identical fast release in time, whereby 1 day after exposure to the open air more than 80% of the compound had evaporated. Unique compounds present in only one of the tested essential oils were α-thujone in *J. virginiana* and linalyl acetate in *L. angustifolia*. The volatile α-thujone showed a fast release pattern similar to that of most other components tested, while linalyl acetate resembled the slower release pattern of α-terpineol. After 2, 3 and 7 days exposure to the open air, 39, 69 and 90% respectively of the initial amount of linalyl acetate present in the strip had evaporated.

3.2 Field experiments

The 2000 experiment was exposed to a heavy infestion of the red bud borer. All of the grafted buds taped with Flexiband without repellent were infested (Fig. 2A), with 36 larvae per bud (Fig. 2A) damaging 78% of the bud tissues (Fig. 2B) and no acceptable union of any of the buds with the rootstocks. All the potential repellents evaluated, and Buddy-Tape, reduced infestations, the numbers of larvae per bud and the amount of tissues damaged compared with the Flexiband control treatment. Buddy-Tape gave only limited protection against the red bud borer, with 35% of the grafted buds infested, eight larvae per bud and 22% of the grafted tissues damaged by the larvae. The volatiles decreased bud infestation by 25–95%, the most effective being lavender oil (95% reduction) and α-terpineol (80% reduction) (Fig. 2A). (*R*)-(−)-Fenchone decreased the numbers of larvae by 75%, to nine per bud; all the other repellents decreased the numbers of larvae by >85%, with cedar oil (1–2 larvae per bud), lavender oil (0.2), α-terpineol (0.5) and citronellal (1–2 per bud) being the most effective (Fig. 2A). The least damaged bud tissue was given by cedar oil (5%), lavender oil (0.3%) and α-terpineol (2.0%) (Fig. 2B). No phytotoxic symptoms were found with any of the treatments.

The mean daily air temperature (1 m above the soil) at the meteorological station during the experiment (21 days) varied between 13.8 and 19.5 °C. The maximum day temperature was between 20 and 23 °C on 13 days, 26 °C on 1 day, and <20 °C on the other 7 days of the experiment. Rainfall varied between 2.8 and 7.8 mm per day for 7 days, with no rainfall during the other days of the test period. Average relative humidity during the whole experiment varied between 70 and 96%.

The results of the 2001 field experiment (Fig. 3) were very similar to those obtained from the previous experiment, although the Buddy-Tape was more effective in 2001, giving only 3% infested buds, while the red bud borer larvae infested 100% of the buds with unimpregnated Flexiband strips (Fig. 3A). Flexiband gave 8% acceptable union of the grafts, while all other treatments gave >85% acceptable union of the grafts (Fig. 3B). No phytotoxic symptoms were found for any of the tested treatments.

The mean daily average air temperature in the test period (22 days) varied between 13.5 and 24.4 °C. The maximum air temperatures were between 25 and 32 °C on 9 days, between 20 and 23 °C on 8 days and <20 °C on 5 days in the test period. Rainfall varied between 1.3 and 13.9 mm per day for 7 days, with no rainfall during the other days of the test period. Average relative humidity during the test period was between 75 and 95%.

4 DISCUSSION

4.1 Field efficacy repellents

Protection of freshly grafted apple trees from the red bud borer is only needed during a short period of time, since the females are attracted only to the odour of fresh wounds of the trees, or at least they are only able to lay eggs and successfully infest the trees via these fresh wounds. Usually the unions have healed and become less attractive for the midges within 1 or 2 weeks after grafting. This short period of protection needed allows the use of highly volatile repellents impregnated in the budding strips. The authors were able to protect grafted buds of apple trees equally well by incorporation of α-terpineol (20% infested buds) and lavender oil (5% infested buds) in the budding strip. Lavender oil, an environmentally friendly alternative considered GRAS (Generally Recognized As Safe),[13] is protecting the grafted buds equally as well as the product Buddy-Tape and even better than the insecticide deltamethrin. The advantage of the product Flexiband with a repellent incorporated, compared with Buddy-Tape, is found in a reduced risk for the grower. Buddy-Tape, if damaged, can still give unacceptable infestation levels, as the field trial of 2000 showed, where 35% infested buds was found, as opposed to only 5% infested buds in 2001. Some growers are evidently not too careful with Buddy-Tape. They pull too strongly, which may result in small cracks in the strip. A more important reason for growers to replace Buddy-Tape with odour-impregnated Flexiband is, however, the risk that, owing to extreme weather conditions in autumn, an early breakdown (extreme warm) or no breakdown (extreme cold and wet) of the Buddy-Tape will occur. The extra labour growers faced in removing or replacing the strips in these years caused unacceptable economic damage. The insecticide deltamethrin, although killing the hatched larvae, did not seem to prevent oviposition by the midges. This caused some tissue damage (11%) by larvae feeding on a relatively high percentage of the trees initially infested (51%), which, although not leading to a lower percentage of graft union (93%), produced the risk of weak unions leading to breaking or slow growth of the fruit-producing trees. Growers generally consider more than 5% loss of grafted trees as economical damage (personal communication).

4.2 Odour composition and efficacy

As the release profile of the essential oils from the Flexiband show (Fig. 1), all major compounds in these oils have almost completely evaporated from the budding strip within 7 days after exposure to the

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open air. The best performing repellent, considering reduction in infestation and number of larvae per bud, is lavender oil, of which the major component, linalyl acetate, is also one of the slowest releasing from the strip compared with the other compounds. The next best performing compound, α-terpineol, also shows a relatively slow release pattern from the budding strip. Although it still needs experimental proof, a relation between effective midge repellence and release pattern of these specific components is possible. The high efficacy (5% infested buds) of the essential oil of lavender, *L. angustifolia*, is likely to be related to the presence of linalyl acetate (44% of total oil) because the only other main compound in this oil (linalool, 39% of total oil) shows poor performance in controlling the red bud borer as a pure compound (63% infested buds) as well as part of *C. camphora* oil (49% infested buds). The relationship discussed between repellence and the major compounds present in essential oils considers the minor compounds in the oils not to be important. This can, however, only be confirmed by testing all minor compounds singly for efficacy. Further, unknown synergistic effects between major compounds and minor compounds may contribute to the overall efficacy of plant essential oils. The importance of single non-active compounds present in natural oils was shown in the control of the spider mite *Tetranychus urticae* Koch by rosemary oil.[14] An artificial mixture of all single mite-toxic compounds found in the natural oil could not equal the toxicity of the natural oil, but addition of the non-toxic compounds to that mixture did equal the toxicity of the natural oil, indicating a synergistic effect of the active and inactive compounds. Although in that study toxicity and not repellence was studied, it is possible that single non-repellent compounds could also synergistically contribute to the repellence of the midges. However, this needs experimental proof.

As a last remark, the influence of weather conditions in the field should be considered. While the authors measured release from the tapes over time at fixed conditions of temperature (~20 °C) and no wind or other typical outside weather conditions (e.g. precipitation), release may be faster or slower in the field than in the laboratory, resulting in differences in efficacy of control. Comparing the weather conditions in both test years, the air temperature was on average higher in 2001 than in 2000. In 2001 there were 9 days with a maximum day temperature between 25 and 32 °C, while in 2000 only 1 day exceeded 25 °C. Other weather conditions were more or less the same in both years. The control results with the equivalent and more effective treatments, lavender oil and α-terpineol, in both test years do not indicate any influence of temperature on efficacy.

4.3 Product development

The authors are currently developing Flexiband with lavender oil and not with single compounds to a commercial product because of the following:

1. *Registration problems.* The natural oil is expected more easily to obtain legal registration for pest control than a single compound
2. *Organic growers.* For organic growers a natural oil is acceptable for use, while this is doubtful for a single compound that has no clear natural origin of extraction.
3. *Bud union.* A small increase in tissue damage (e.g. higher for budding strips treated with α-terpineol than for strips treated with lavender oil) may lead to partial and thus substantial weaker connection of the buds with the rootstock and hence more damage to the older trees when bearing fruit (breaking or bad growing through heavy weight of branches, fruit and or wind and storm damage).
4. *Cost aspects of the product.*
   A main concern for product development is to ensure constant high repellence, in spite of unavoidable variance in oil composition by the use of oil batches of different origin. For lavender oil the amount of linalyl acetate present seems critical for effective repellence of the red bud borer, but the total composition of all compounds in the natural oil may be critical for efficacy as well. Batches of oil should, therefore, always be checked for composition of the main compounds before use. The authors' tested lavender oil contained near-equal amounts of linalyl acetate (44%) and linalool (39%) and is currently used as a standard for use in their product. Further testing of different batches of lavender oil with different contents of these and other compounds (e.g. testing single compounds) is essential for determining how critical the composition of the oil is for repellence. Currently, field tests with several test products are being performed to optimise the product for control. The company Fleischhauer K.G., which produces Flexiband, is involved in developing a suitable product for growers.

Successful examples of repelling insect pests with essential oils in the open field that make it to marketable products are very limited. Most results are restricted to laboratory tests and very few to greenhouse trials where the costs of the amounts of oils relative to control efficacy are economically not feasible.[9] Only fumigation of stored products, giving near-100% control, may compete with the traditional control methods at this moment.[10] Success in the open field with the highly volatile repellents is due to the very specific local control of this pest insect over a short time period (3–7 days).

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