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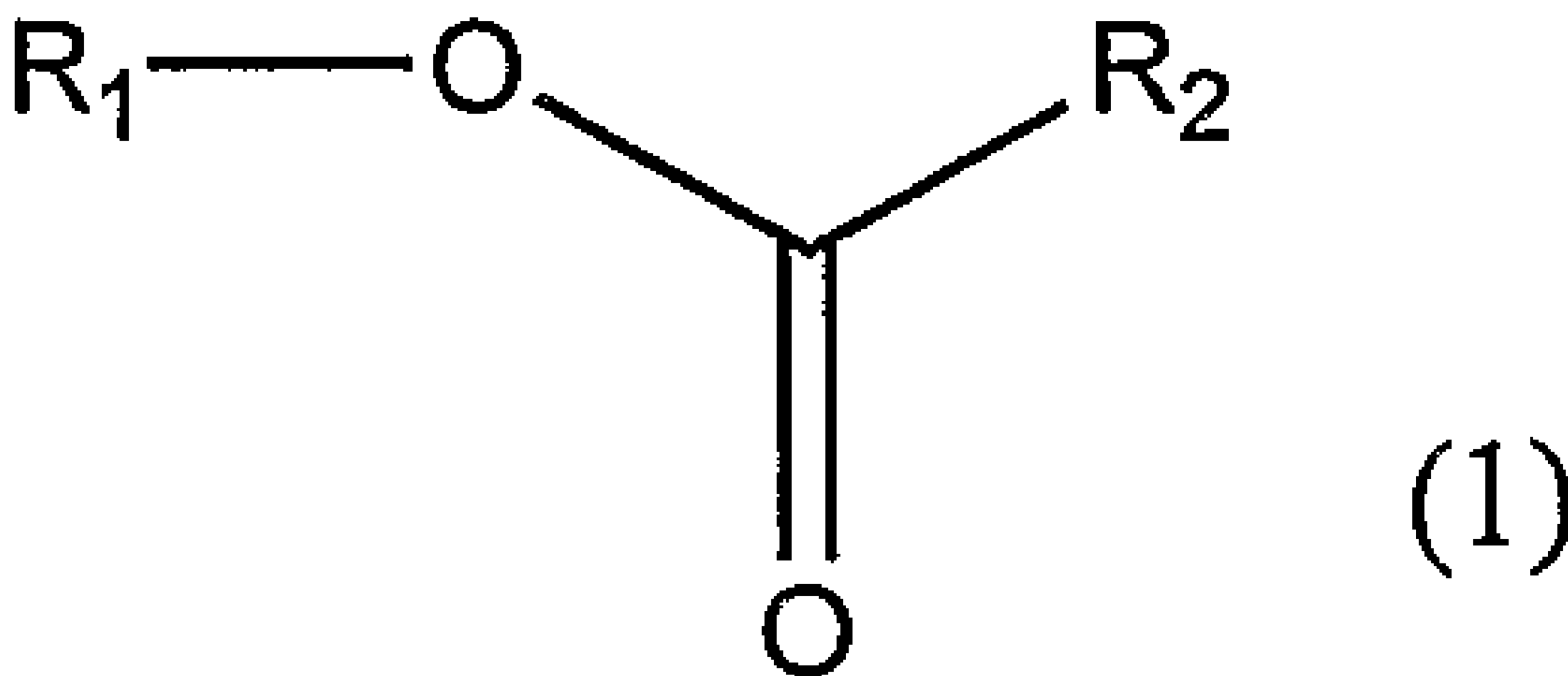
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A method of monitoring/controlling Thysanoptera (hereafter thrips) by the use of a behaviour modifying compound of Formula (1), wherein Formula (1) is : where R₁ is a C₈-C₁₂ group and R₂ is a C₂-C₈ group.

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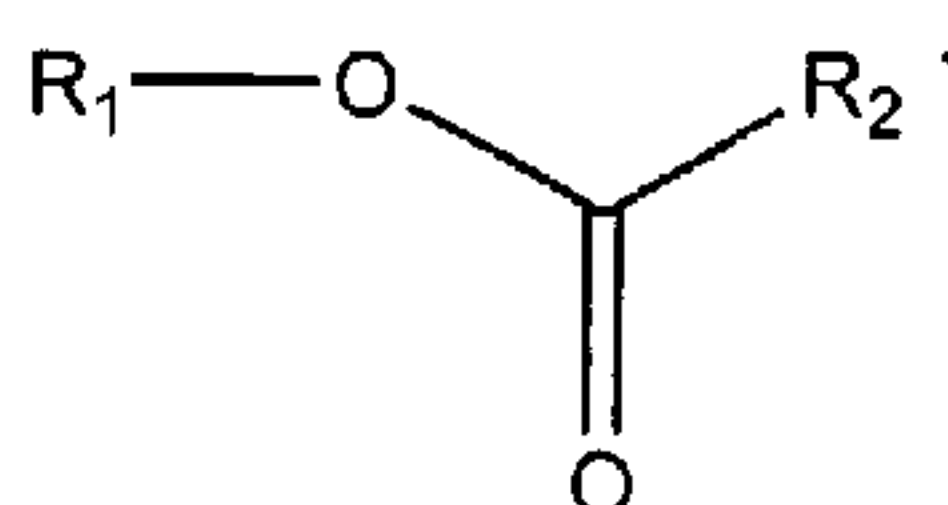
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(54) Title: METHOD OF MONITORING/CONTROLLING THYSANOPTERA



(1)

(57) Abstract: A method of monitoring/controlling Thysanoptera (hereafter thrips) by the use of a behaviour modifying compound of Formula (1), wherein Formula (1) is : where R₁ is a C₈-C₁₂ group and R₂ is a C₂-C₈ group.

WO 03/055309 A1

METHOD OF MONITORING/CONTROLLING THYSANOPTERA

The present invention relates to a method of monitoring/controlling Thysanoptera (thrips), particularly but not exclusively for monitoring/controlling Western Flower Thrips (*Frankliniella occidentalis* (Pergande)).

Spoilage of cultivated plants/crops by insect pests is a widespread problem. Insects are recognised to cause direct damage by eating the plants/crops and also by laying eggs therein. Additionally insects often carry transferable diseases that cause damage of plants/crops.

One method of controlling insects is the direct application of pest control agents/pesticides to the plant/crop. However, as the pest control agents/pesticides used are commonly toxic to other animals their use is becoming increasingly less acceptable on environmental grounds. Also there is the problem of the triggering of undesirable reactions, such as poisoning or allergies, for agricultural and horticultural workers. Many consumers are reluctant to purchase edible crops that have been treated with pesticides, because of fears of adverse effects on their health from pesticide residues.

The use of traps in the vicinity of the crop that contain an attractant sex pheromone and a pest control agent is becoming increasingly widespread. The traps may be selective in attracting one sex (usually the male) of the insect concerned so as to remove them from the population and hence control population growth. Alternatively the traps may be more general in their operation.

The general release of an attracting sex pheromone is also sometimes used as a control measure. The released sex pheromone "confuses" the sex that is attracted so that it cannot locate a mate. This disruption of the mating process slows down or stops the build up of the pest population.

The Order Thysanoptera (thrips) is a group of insects that is recognised as causing damage to a wide range of cultivated crops. Some thrips are a pest of a particular crop, e.g. avocado thrips are a pest to avocado crops, whereas some species are a pest to a wide range of crops. Thrips cause damage to crops by feeding upon the crops and laying their eggs therein. Their feeding method comprises penetrating parts of the plant and sucking out the liquid contents, thus causing aesthetically unappealing scarring and stunting the growth of the crop. The presence of insects alone or small feeding marks can make ornamental crops unsaleable. Some thrips spread plant viruses, which can cause considerable damage to many crops.

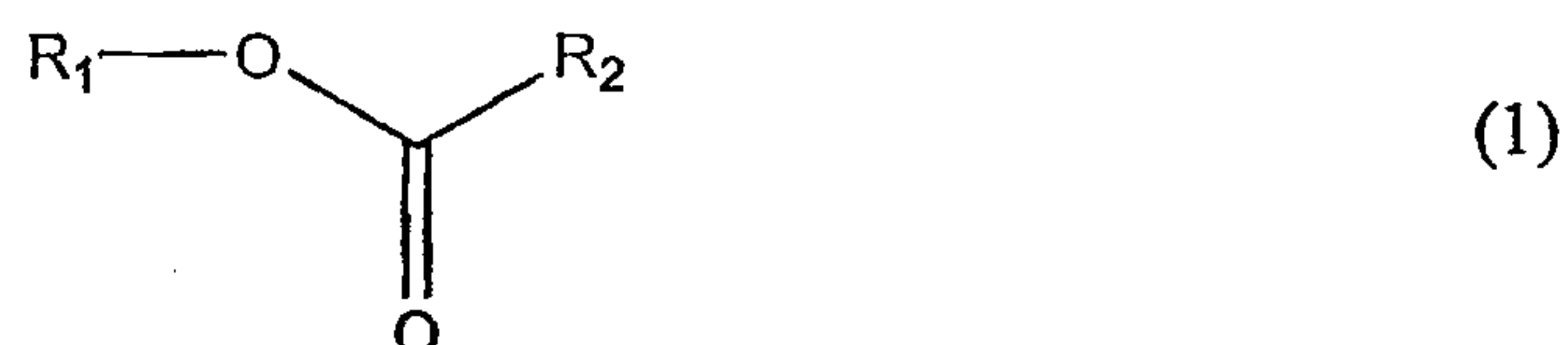
Thrips are commonly active within enclosed parts of the crop, such as flower buds and leaf buds. Thus commonly the damage caused by thrips, in the form of direct damage such as feeding and in the form of indirect damage such as transmission of a virus, has often occurred before the thrips themselves have been observed. For crops such as commercially harvested flowering plants the management of thrips is a particularly acute problem as the thrips damage is sometimes only observed in the late stages of the flower development when the bud finally opens. Thrips are also a problem because they breed rapidly and large pest populations can build up very quickly if unchecked.

Thrips are difficult to control with insecticides because they retreat into minute recesses on the plant where insecticides are less likely to reach them and because the main pest species have high levels of insecticide resistance. Additionally pesticides applied to crops are perceived to be a poor solution to the problem of thrips since the pesticides used are normally detrimental to the population of beneficial arthropods that prey upon the thrips and other insect and mite pests on the crop.

Biological control agents such as predatory mites or fungal pathogens, are sometimes used to control things, but they are not always reliable and they are not very effective on some crops.

It is an object of the present invention to obviate and mitigate the problems outlined above.

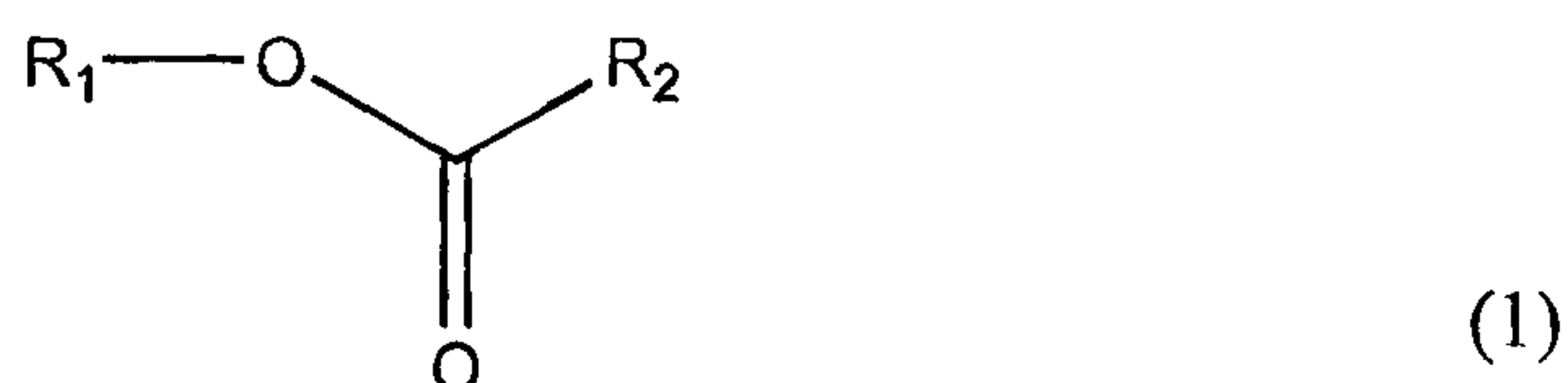
According to the present invention there is provided a method of monitoring/controlling Thysanoptera (hereafter thrips) by the use of a behaviour modifying compound of Formula 1, wherein Formula 1 is:-



where R_1 is a $\text{C}_8\text{-C}_{12}$ group and R_2 is a $\text{C}_2\text{-C}_8$ group.

According to one aspect of the invention there is provided a method of monitoring or controlling Thysanoptera (thrips) comprising:

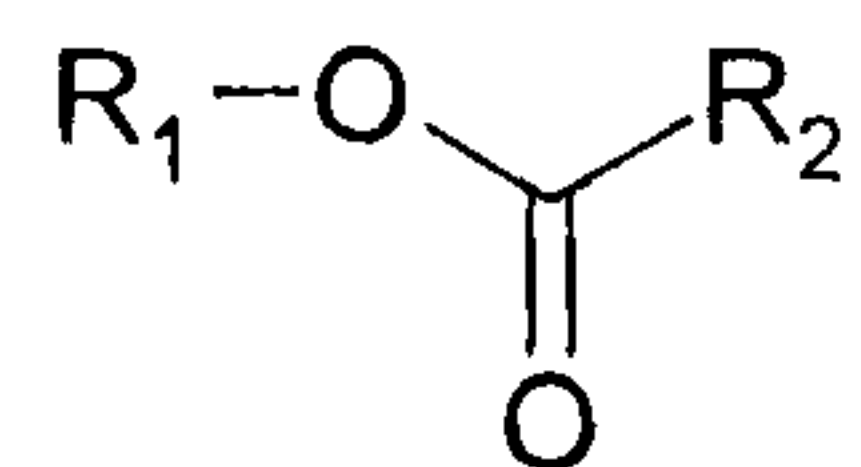
providing to an area infested by or at risk of infestation by thrips a behavior modifying compound of Formula (1), wherein Formula (1) is:



wherein R_1 is a monoterpenyl group and R_2 is a $\text{C}_3\text{-C}_5$ group.

According to a further aspect of the invention there is provided a device for providing a behavior modifying compound to an area infested by or at risk of infestation by Thysanoptera (thrips), said device comprising:

a compound of Formula (1) and being adapted to broadcast or release the compound of Formula (1) in said area, wherein Formula (1) is:



wherein R_1 is a monoterpenyl group and R_2 is a $\text{C}_3\text{-C}_5$ group.

3a

The method of the present invention has been found to be particularly effective in mimicking the effects of a natural thrips pheromone and thus the thrips may be effectively attracted for monitoring or control purposes. They may also be confused for control purposes. The invention, therefore, does not rely on any toxic qualities of the compound of formula (1) but rather on modification of thrips' behaviour for its effect.

When either sex of the thrips is attracted and monitored the method of the present invention may be used to gauge the population density of thrips in a particular area from analysis of the number and/or sex of the thrips caught. Based on the number of thrips attracted a decision may then be made as to what further action, if any, is required. At an extreme level the level of monitoring may be selected so that as high a portion of the number of thrips are removed from the population as possible.

When female thrips are attracted and monitored management of the overall thrips population is achieved as the number of female thrips is reduced. It has been observed that when the method of the invention is used to attract and monitor female thrips it is particularly efficacious as with removal of a portion of the number of egg-laying females from the thrips population not only is the damage caused by females

laying eggs in a crop to be protected quickly reduced but also population growth is quickly curbed with its associated advantages in terms of reduced damage.

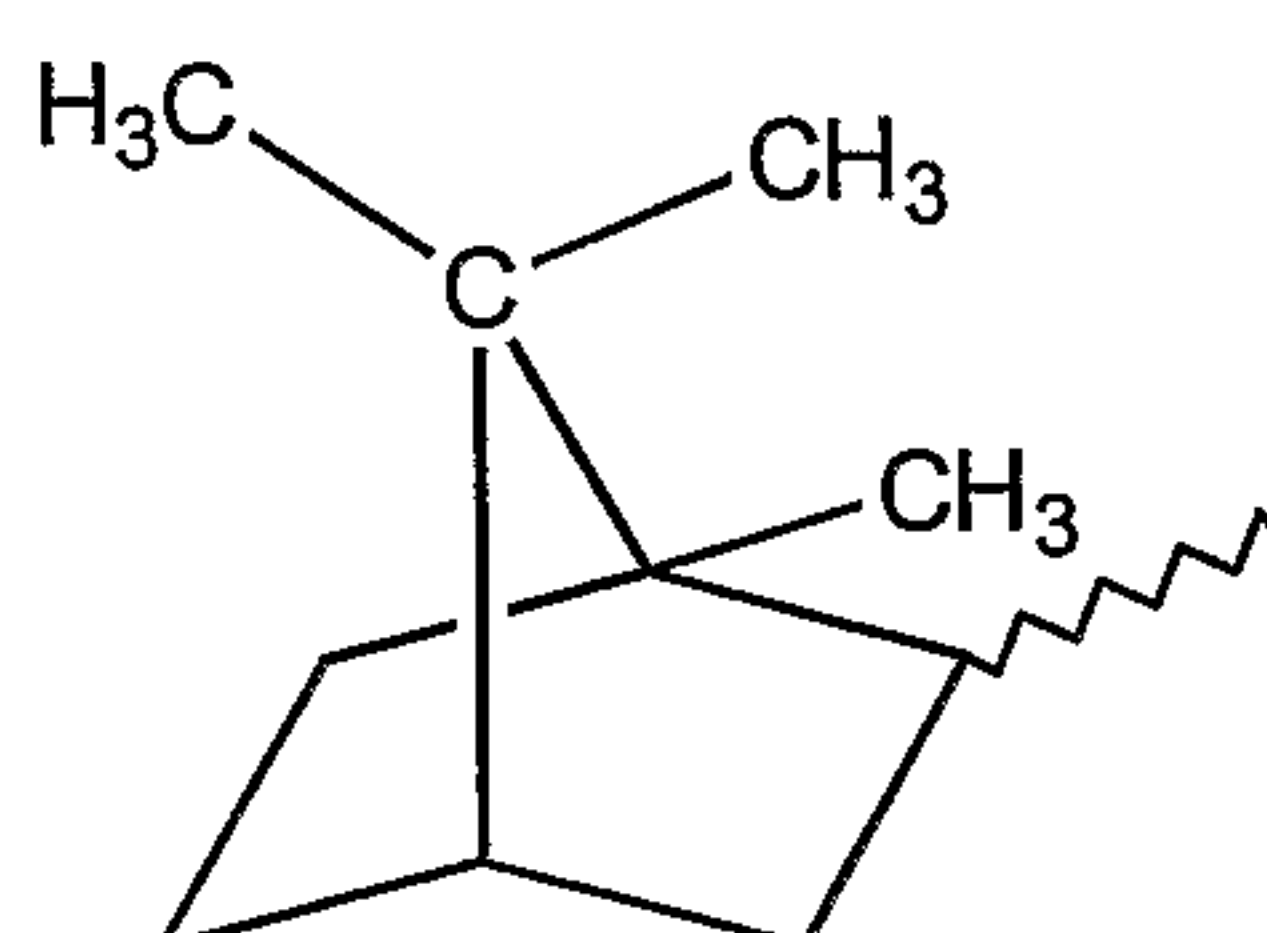
When male thrips are attracted and monitored management of the overall thrips population is achieved as the female thrips are not fertilised. Unfertilised females then produce only male thrips thus the number of females in the next generation is reduced and so population increase is curbed.

R_1 is preferably aromatic, aliphatic or cycloaliphatic. R_1 is preferably a cyclic and most preferably a multi-cyclic (e.g. a bi-cyclic) moiety.

It is preferred that R_1 is C_9 - C_{11} and most preferably C_{10} .

A preferred form of R_1 is a monoterpene.

Most preferably R_1 is isobornyl, as shown below.

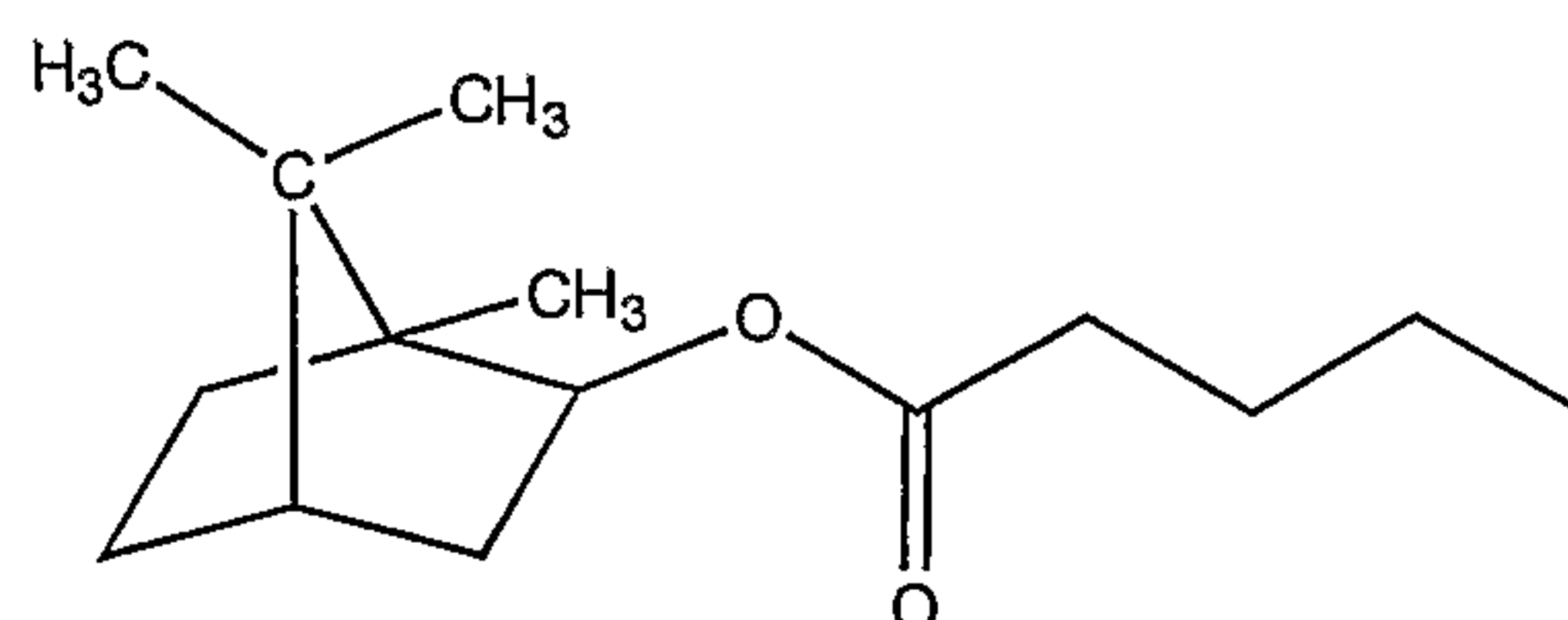


Alternatively R_1 may be a lavandulyl group.

R_2 is preferably straight or branched alkyl or alkylene, most preferably straight alkyl.

It is preferred that R_2 is C_3 - C_5 and most preferably C_4 (i.e. the $R_2C(O)O$ group in the compound of formula (1) has a total of 5 carbon atoms).

Suitable examples of the $R_2 C(O)O$ group in the compound of formula (1) are the valerate, isovalerate, 2-methyl butanoate and pivalate groups.



The compound of Formula 1 may be isobornyl valerate, i.e. :-

Alternatively the compound of formula 1 may be isobornyl isovalerate, isobornyl 2-methyl butanoate or isobornyl pivalate. Further possibilities include lavandulyl valerate, lavandulyl isovalerate, lavandulyl 2-methyl butanoate and lavandulyl pivalate.

The method of the invention, i.e. the way in which the compound of Formula 1 is used, may be carried out in several ways. For a method of controlling thrips these include use as a confusant.

In this method a compound of Formula 1 is broadcast within an area infested (or potentially infested) by thrips. As the compound of Formula 1 mimics the effect of a thrips pheromone the thrips become "confused", namely the application of a compound of Formula 1 overcomes the effect of any natural thrips pheromones present with the result that the thrips cannot find a mating partner.

For a method of monitoring thrips a compound of Formula 1 may be provided in a release device located in an area infested (or potentially infested) by thrips. The release device may include a means for immobilising and/or killing the thrips so that the thrips cannot leave the release device once attracted thereto. Namely the release device may be used as a pest control device by attracting and then removing thrips from an area.

Preferably the compound of Formula 1 is held in/on a support of the release device. Generally the support medium is an adhesive material so that the thrips stick thereto.

A preferred example of a release device comprises a sheet of plastic having a sticky adhesive coating which has been soaked/coated with a formulation including a compound of Formula 1. Such release devices are commonly referred to as "Sticky Traps". Preferably the plastic is porous to allow penetration of said formulation. Generally the solvent for said formulation is hexane or acetone. As thrips are attracted to certain colours the sticky paper is preferably coloured to act as a secondary attractant. Most preferably the sheet of sticky plastic is coloured blue, white or yellow. The use of coloured release devices is described in the paper *Zeitschrift für Angewandte Entomologie* 107, 136-140 (H. F. Brødsgaard). In this paper a sheet having a colour close to Pantone® 279 (a shade of blue) was found to be the most attractive for thrips.

Generally each sheet of plastic is rectangular having dimensions of around 7cm and between 10-15cm, although sheets of other sizes are not precluded. For example the sheet may be in the form of a strip and extend around/across a portion of the growing area.

Whilst it is to be appreciated that the method of the present invention may be applied to monitor thrips in a variety of different locations it is preferred that the method of the present invention is used in an enclosed structure such as a greenhouse. In this case the release device is preferably in the form of a strip extending along a side of the greenhouse.

When used in an enclosed structure it is preferred that each release device produces/releases an effective amount of the compound of Formula 1. Generally in order to produce/mimic the effect of a single thrips the release device is configured to produce/release a compound of Formula 1 in an amount which is at least and more preferably a multiple of the amount of pheromone which would be released by a single thrips. The preferred release rate of a release device is in the region of from 150 to 1,500,000 picograms (1.5µg) per hour with more preferably 1000 to 750,000

picograms ($0.75\mu\text{g}$) per hour and most preferably 2000 to 300,000 picograms ($0.3\mu\text{g}$) per hour.

Preferably the method of the present invention is used to monitor/control the population of thrips for cultivated flower crops, e.g. chrysanthemums and roses and additionally for other crops such as cucumbers and peppers.

It is proposed that the method of the present invention may be used to monitor/control walking and/or flying species of thrips. Preferred examples of such thrips include *Thrips palmi* (Karny), *Thrips tabaci* (Lindemann), *Frankliniella fusca* (Hinds), *Frankliniella schultzei* (Trybom), *Frankliniella tritici* (Fitch) and Western Flower Thrips (*Frankliniella occidentalis*). Most preferably the method of the present invention is used to control the population of Western Flower Thrips (*Frankliniella occidentalis*).

The invention will be now be further described with reference to the following non-limiting Examples.

Examples

Rearing Thrips

Thrips were originally obtained from a commercial glasshouse and then maintained on pot chrysanthemums (*Dendranthema x grandiflora* (Ramat.) Kitamura) at 25°C under a repeated cycle consisting of 18 hours of light and 6 hours of dark. The culture on chrysanthemums supplied mixed-age adult females, which were attracted to adult males in olfactometer bioassays, so these were used in the bioassays.

The Y-tube olfactometer

The Y-tube has two branches at an angle of 90° and a stem, which are all 60 mm long

with internal diameters of 5 mm. The Y-tube was held horizontal and illuminated from above by four fluorescent tubes (950 lux). It was screened by a 10 cm high matt-black card wall, forming a 280 mm x 280mm square, to minimise any external visual influences. A 50 ml round glass flask with a Drechsel head was inserted in the tube before each branch and a semi-circle of filter paper (Whatman No. 1, diameter 42.5 mm), moistened with water, was placed in each to humidify the air. Connections between tubes are either made with brass swagelock connectors or by inserting narrower tubes into slightly wider tubes. All the glassware and the glass wool were rinsed thoroughly in warm water with Teepol 'L' neutral detergent (BDH Laboratory Supplies, Poole), then in distilled water, then in acetone, and left to dry overnight in an oven at 200°C before each experiment. The connecting Teflon tubing and the swagelock connectors were rinsed thoroughly in warm water with Teepol 'L' neutral detergent, then in distilled water, then in hexane, and left to dry overnight in a fume cupboard before each run of an experiment.

Zero-grade clean air from a cylinder (supplied by British Oxygen Corporation, Manchester) was passed through an activated charcoal filter and then along Teflon tubes to two flow meters (Supelco, Sigma-Aldrich, Poole) that regulated the air flow to the two branches of a glass Y-tube. The rate in the two branches was 50 mm s⁻¹ (59 ml min⁻¹) and in the stem was 100 mm s⁻¹ (118 ml min⁻¹) and were checked with a bubble flow meter.

Y-tube olfactometer bioassays

Adults were placed one at a time in the stem of the Y-tube, by transferring them with a fine paintbrush, and allowed to walk up the Y-tube. A choice was recorded when they first crossed a line 20 mm down either branch from the junction of the "Y". If a choice was not made within 3 min, it was recorded as "no choice" and excluded from the analysis. Each individual was tested twice to provide two successive choices. The sample flasks, connecting tubing and Y-tube were reversed after every 5 individuals. This reduced possible bias from external influences. Each experiment consisted of 1 to 3 runs on separate days to reduce possible bias from external

influences. A total of 25 individuals were tested in each run of an experiment, which lasted about 2 h. Experiments were conducted at $25\pm 1^{\circ}\text{C}$

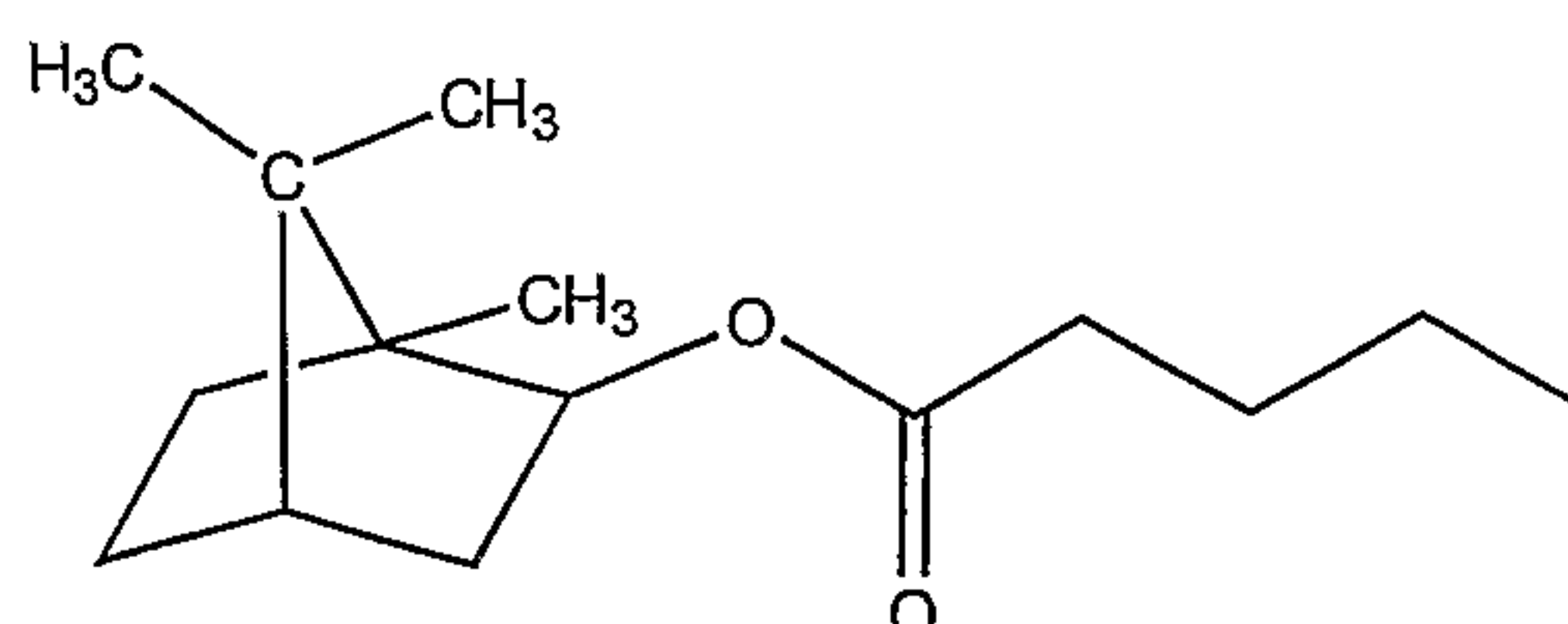
At the start of each run, 0.2 ml of distilled water was placed on the filter paper in each flask. 1 μl of the test substance (a known amount in hexane) was placed on the filter paper in one flask and 1 μl of hexane was placed on the filter paper in the other flask as a control. The filter papers were removed and replaced with fresh water and test and control substances every time the equipment was reversed.

It was shown that there was no preference in thrips response by testing mixed-age females (in the absence of a test composition) in the Y-tube olfactometer on 3 separate days. The apparatus was set up as before, but with only water and hexane in each flask. Since the arms of the Y-tube were reversed by rotating the stem by 180° after every five individuals, the difference in the number of choices between left and right and between arm A and arm B could be tested separately. There was no preference to the left or right or for arm A or arm B thus establishing that the apparatus was unbiased.

Results

Example 1

Using the method outlined above the compound isobornyl valerate (shown below), which was synthesised in the laboratory from isobornyl alcohol and valeric acid,



applied in a Y-tube olfactometer was found to be an attractant for female Western Flower Thrips. In each case 25 adult females were tested in 2 or 3 separate runs. The compound was applied in 1 μl hexane. The results were combined from the separate

runs and are shown in Table 1. Also for each result in Table 1 a figure showing the probability of this result happening merely by chance is shown.

Table 1

Amount of Compound (ng)	Solvent	Preference (%)	Probability
0.002	Hexane	64.8	0.007
0.02	Hexane	73.3	<0.0001
0.1	Hexane	76.9	<0.0001
0.2	Hexane	79.8	<0.0001
2	Hexane	60.3	0.026

In each case it can be seen that the probability of the result merely occurring by chance is extremely unlikely. In each case the thrips tested can be seen to exhibit a strong preference for the compound isobornyl valerate (shown schematically above). Thus it can be concluded that isobornyl valerate is a strong attractant for thrips.

Example 2

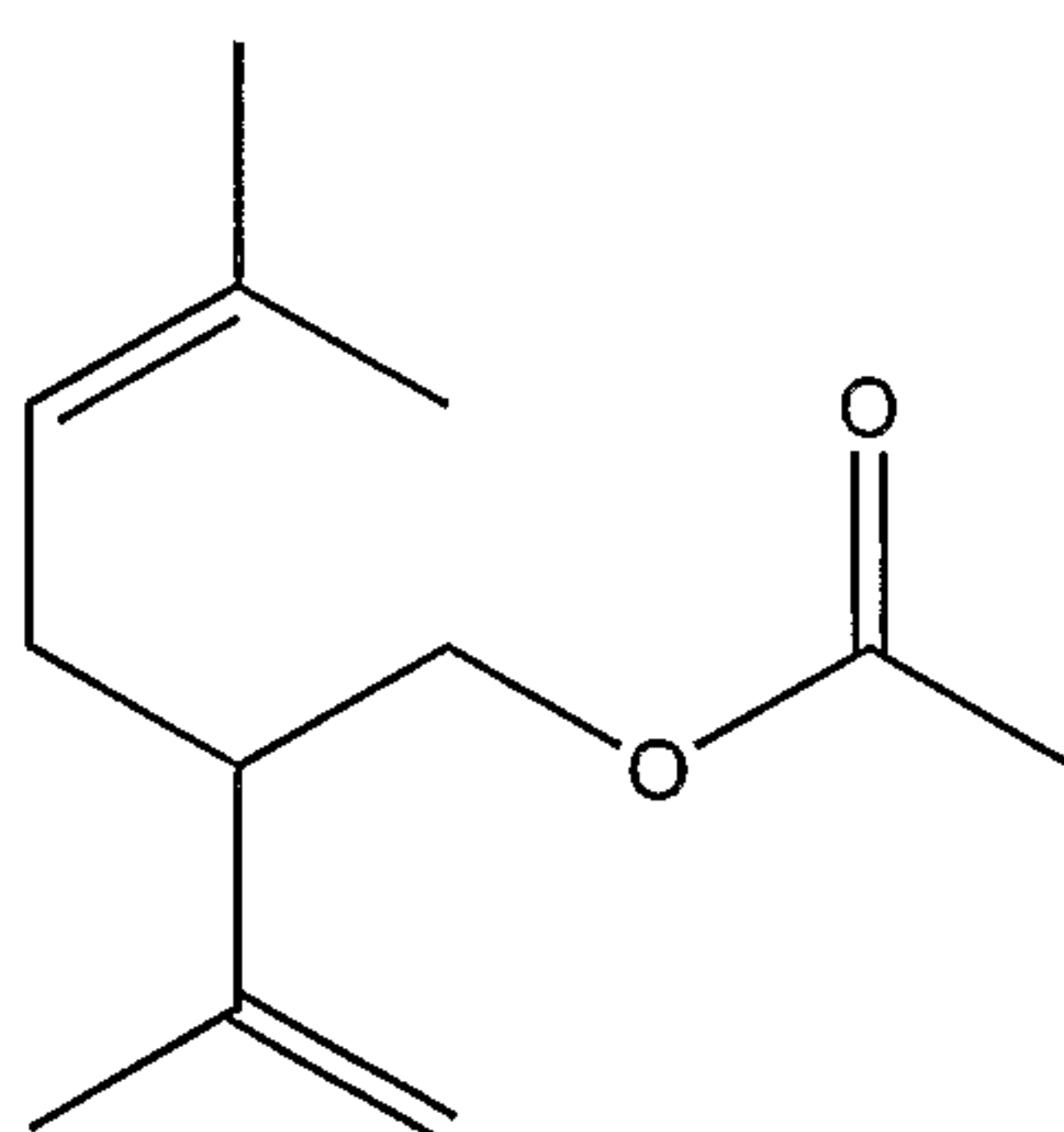
Example 1 was repeated using each of isobornyl 2-methyl butanoate, isobornyl pivalate and lavandulyl valerate in amounts of 0.1ng. The method was modified slightly so that the filter paper was placed in a length of Teflon tube instead of in a glass flask. The results obtained were as follows:

Compound	Preference (%)	P
Isobornyl 2-methyl butanoate	72.9%	0.018*
isobornyl pivalate	61.3%	0.010*
lavandulyl valerate	79.2%	<0.001

It can be seen from the above data that each of the above three compounds is a strong attractant for the thrips.

Comparative Example 1

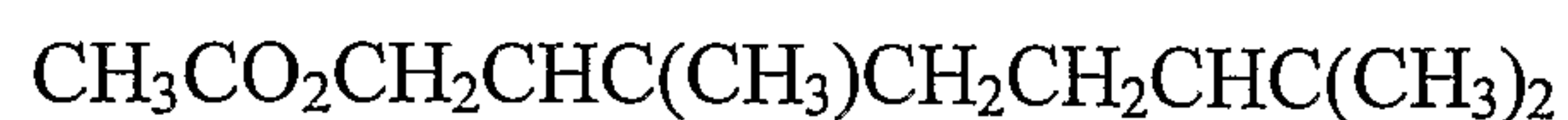
Using the method outlined above the compound (lavandulyl acetate) shown below



applied at doses of 0.1ng, 1ng, 10ng, 100ng and 1000ng (where ng is nanogram) under the conditions outlined above showed no attractant effect upon female Western Flower Thrips.

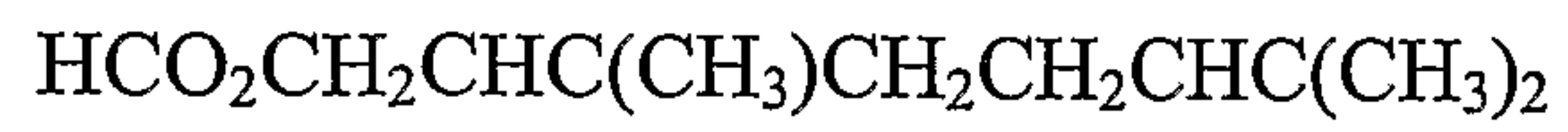
Comparative Example 2

Using the method outlined above the compounds shown below



Neryl Acetate

and



Neryl Formate

applied at doses of 1ng, 10ng, and 100ng under the conditions outlined above showed no effect upon female Western Flower Thrips.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method of monitoring or controlling Thysanoptera (thrips) comprising: providing to an area infested by or at risk of infestation by thrips a behavior modifying compound of Formula (1), wherein Formula (1) is:



wherein,

R₁ is a monoterpenyl group; and

R₂ is a C₃-C₅ group.

2. A method according to claim 1, wherein R₁ is an isobornyl group.
3. A method according to claim 1, wherein R₁ is a lavandulyl group.
4. A method according to claim 1, wherein R₁ is a neryl group.
5. A method according to any one of claims 1 to 4, wherein R₂ is a straight or branched alkyl or alkylene.
6. A method according to any one of claims 1 to 4, wherein R₂ is C₄.
7. A method according to any one of claims 1 to 4, wherein the R₂-C(O)O- group in the compound of Formula (1) is valerate, isovalerate, pivalate or 2-methylbutanoate.
8. A method according to any one of claims 1 to 4, wherein the R₂-C(O)O- group in the compound of Formula (1) is a 2-methylbutanoate group.
9. A method according to claim 1, wherein the compound of Formula 1 is isobornyl valerate, isobornyl isovalerate, isobornyl pivalate, isobornyl 2-methyl,

butanoate, lavandulyl valerate, lavandulyl isovalerate, lavandulyl pivalate or lavandulyl 2-methylbutanoate.

10. A method according to any one of claims 1 to 9, wherein the compound of Formula (1) is provided in a release device located in an area infested by or at risk of infestation by thrips.

11. A method according to claim 10, wherein the release device includes a means for immobilising or killing the thrips.

12. A method according to claim 11, wherein the compound of Formula (1) is held in or on a support medium of the release device.

13. A method according to claim 12, wherein the support medium is an adhesive material.

14. A method according to claim 13, wherein the release device comprises a sheet of plastic having a sticky adhesive coating which has been soaked or coated with a formulation including a compound of Formula (1).

15. A method according to claim 14, wherein the plastic is porous.

16. A method according to claim 14, wherein the formulation comprises a solvent which is hexane or acetone.

17. A method according to any one of claims 14 to 16, wherein the sheet of plastic is colored blue, white or yellow.

18. A method according to any one of claims 10 to 17, wherein the release device provides from 150 picograms per hour to 1,500,000 picograms (1.5 μ g) per hour of a compound of Formula (1).

19. A method according to claim 18, wherein the release device provides from 1000 picograms per hour to 750,000 picograms (0.7 μ g) per hour of a compound of Formula (1).

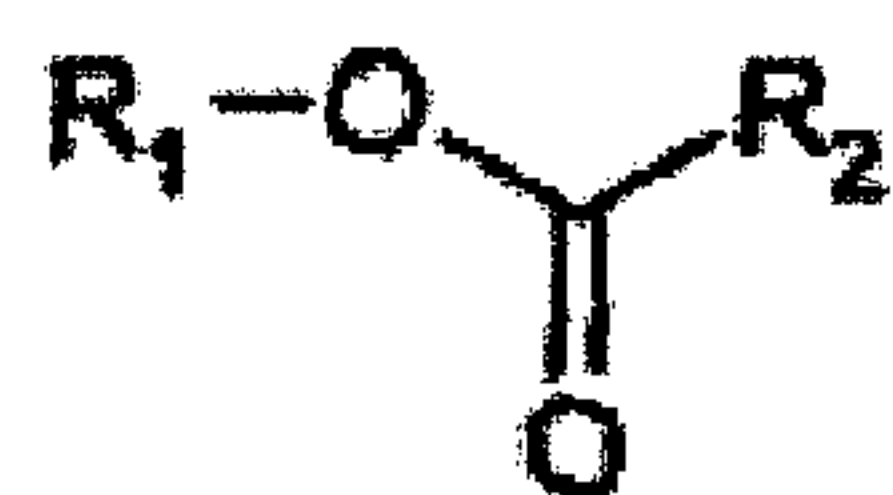
20. A method according to claim 19, wherein the release device provides from 2000 picograms per hour to 300,000 picograms (0.3 μ g) per hour of a compound of Formula (1).

21. A method according to any one of claims 1 to 20, wherein the method is used to control the population of thrips for edible or ornamental crops.

22. A method according to any one of claims 1 to 21, wherein the compound of Formula (1) is provided by broadcasting said compound in an area infested by or at risk of infestation by thrips.

23. A device for providing a behavior modifying compound to an area infested by or at risk of infestation by Thysanoptera (thrips), said device comprising:

a compound of Formula (1) and being adapted to broadcast or release the compound of Formula (1) in said area, wherein Formula (1) is:



wherein,

R₁ is a monoterpenyl group; and

R₂ is a C₃-C₅ group.

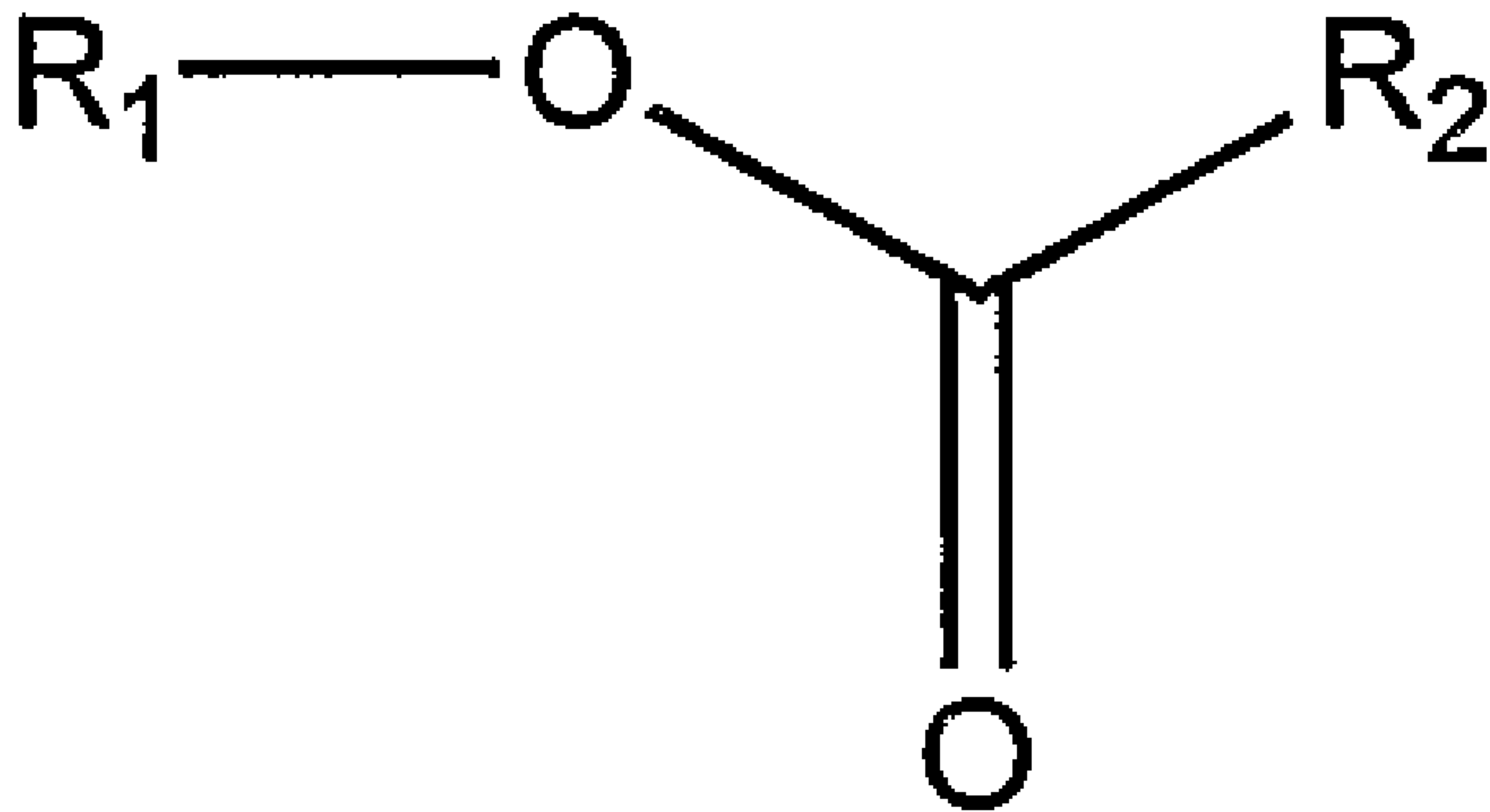
24. A device according to claim 23, wherein said device is effective for broadcasting the compound of Formula (1) in said area.

25. A device according to claim 23 or 24, wherein R₁ is an isobornyl group.

26. A device according to claim 23 or 24, wherein R₁ is a lavandulyl group.

27. A device according to claim 23 or 24, wherein R_1 is a neryl group.
28. A device according to any one of claims 23 to 27, wherein R_2 is a straight or branched alkyl or alkylene.
29. A device according to any one of claims 23 to 28, wherein R_2 is C_4 .
30. A device according to any one of claims 23 to 27, wherein the $R_2-C(O)O-$ group in the compound of Formula (1) is valerate, isovalerate, pivalate or 2-methylbutanoate.
31. A device according to any one of claims 23 to 27, wherein the $R_2-C(O)O$ group in the compound of Formula (1) is 2-methylbutanoate.
32. A device according to claim 30, wherein the compound of Formula (1) is isobornyl valerate, isobornyl isovalerate, isobornyl pivalate, isobornyl 2-methyl, butanoate, lavandulyl valerate, lavandulyl isovalerate, lavandulyl pivalate, or lavandulyl 2-methylbutanoate.
33. A device according to claim 23, wherein the compound of Formula (1) is neryl(S)-2-methylbutanoate.
34. A device according to any one of claims 23 to 33, wherein said device is a release device.
35. A release device according to claim 34, wherein the release device includes a means for immobilising or killing the thrips.
36. A device according to claim 34 or 35, wherein the compound of Formula (1) is held in or on a support medium of the release device.
37. A device according to claim 36, wherein the support medium is an adhesive material.

38. A device according to any one of claims 34 to 37, wherein the release device comprises a sheet of plastic having a sticky adhesive coating which has been soaked or coated with a formulation including a compound of Formula (1).
39. A device according to claim 38, wherein the plastic is porous.
40. A device according to claim 38 or 39, wherein the sheet of plastic is colored blue, white or yellow.
41. A device according to any one of claims 34 to 40, wherein the release device is a "sticky trap".
42. A device according to any one of claims 23 to 41, wherein the device provides from 150 picograms per hour to 1,500,000 picograms (1.5 μ g) per hour of a compound of Formula (1).
43. A device according to claim 42, wherein the device provides from 1000 picograms per hour to 750,000 picograms (0.75 μ g) per hour of a compound of Formula (1).
44. A device according to claim 43, wherein the device provides from 2000 picograms per hour to 300,000 picograms (0.3 μ g) per hour of a compound of Formula (1).



(1)