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UTILISATIONS  
(54) Title: ATTRACTANT COMPOSITIONS FOR WEEVILS OF THE GENUS OTIORHYNCHUS AND USES THEREOF

(57) Abrégé/Abstract:

The present invention relates to formulations of volatile organic compounds having effects on *Otiorhynchus* weevils e.g., *Otiorhynchus sulcatus*. In some embodiments, volatile organic compounds selected from (E)-2-hexenol, (Z)-2-pentenol, methyl eugenol and a combination thereof are effective for attracting *Otiorhynchus* weevils. The invention also relates to traps and particularly to a "weevil trap" combined with any one or more of the disclosed volatile organic compounds selected from (E)-2-hexenol, (Z)-2-pentenol, methyl eugenol, and light which are effective for monitoring and controlling *Otiorhynchus* weevils.

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(54) Title: ATTRACTANT COMPOSITIONS FOR WEEVILS OF THE GENUS OTIORHYNCHUS AND USES THEREOF

(57) Abstract: The present invention relates to formulations of volatile organic compounds having effects on *Otiorhynchus* weevils e.g., *Otiorhynchus sulcatus*. In some embodiments, volatile organic compounds selected from (E)-2-hexenol, (Z)-2-pentenol, methyl eugenol and a combination thereof are effective for attracting *Otiorhynchus* weevils. The invention also relates to traps and particularly to a "weevil trap" combined with any one or more of the disclosed volatile organic compounds selected from (E)-2-hexenol, (Z)-2-pentenol, methyl eugenol, and light which are effective for monitoring and controlling *Otiorhynchus* weevils.

## ATTRACTANT COMPOSITIONS FOR WEEVILS OF THE GENUS *OTIORHYNCHUS* AND USES THEREOF

### FIELD OF THE INVENTION

[0001] The invention relates to volatile organic compounds effective for attracting *Otiorhynchus* weevil species (*Otiorhynchus* sp.) and to methods for using the volatile organic compounds to control *Otiorhynchus* weevils.

### BACKGROUND OF THE INVENTION

[0002] Vine weevils of the genus *Otiorhynchus* are an important pest of hardy ornamentals, fruit tree and nursery stock worldwide. Though *Otiorhynchus* is of European origin, it has travelled from Europe to other regions of the world via plant material and thus, has become one of the most destructive pests in nursery and small fruit production areas throughout the United States and Canada as well as Europe.

[0003] Typically, adult vine weevils feed on plant leaves and deposit eggs in the soil. Once hatched, the larvae, born in the soil, feed on plant roots weakening and sometimes, killing the plants. Accordingly, *Otiorhynchus* weevils are responsible for a considerable amount of economically important damage. Indeed, based on USDA data, \$25-\$70 million is spent annually in the USA and Canada alone to combat this worldwide horticultural pest.

[0004] Unfortunately, combating *Otiorhynchus* weevil attack is problematic because adult weevils are active at night. The nocturnal behavior makes monitoring and timing of control measures difficult because growers frequently are not able to observe the emerging weevils in a timely manner. Thus, oviposition and thus, the next crop of destructive larvae, often starts before effective control measures are taken.

[0005] Typically, weevil presence is determined by monitoring feeding damage to plants. The most extensive feeding occurs during the four week preoviposition period and several weeks during oviposition. If adult weevils can be located and either captured or killed before they lay eggs, and initiate a new generation, much damage can be averted.

[0006] Unfortunately, localizing affected areas in nurseries is labor intensive, especially since the adults are active at night and hide during the day. Thus, to combat weevil infestation without encountering excessive labor costs, growers typically apply broad

spectrum pesticides over the entire area of affected or potentially affected plants for the entire growing season, which typically lasts from June to October. Amongst other issues, the use of broad spectrum pesticides over a large area for a prolonged period, limits the ability of growers to practice less toxic integrated pest management techniques.

**[0007]** Therefore, what is needed in the art are effective means for monitoring and controlling *Otiorhynchus* weevils before oviposition such that damage to nursery stock can be minimized or eliminated.

**[0008]** Fortunately, as will be clear from the following disclosure, the present invention provides for these and other needs.

## **SUMMARY OF THE INVENTION**

**[0009]** In an exemplary aspect, the invention provides a composition of one or more attractant volatile organic compounds effective for attracting *Otiorhynchus* weevils, the volatile organic compounds selected from the group consisting of (X)-Y-pentenol, (X)-Y-hexenol, methyl eugenol, methyl *iso*-eugenol and a combination of said members, wherein X is E or Z, and Y is 1 or 2.

**[0009a]** In another exemplary aspect, the invention provides a composition of one or more attractant volatile organic compounds effective for attracting *Otiorhynchus* weevils, wherein the volatile organic compounds are members selected from (i) to (iv):

- (i) (X)-Y-pentenol;
- (ii) (X)-Y-pentenol and (X)-Y-hexenol;
- (iii) (X)-Y-pentenol and methyl eugenol; and
- (iv) (X)-Y-pentenol, (X)-Y-hexenol, and methyl eugenol;

wherein X is E or Z, and Y is 1 or 2.

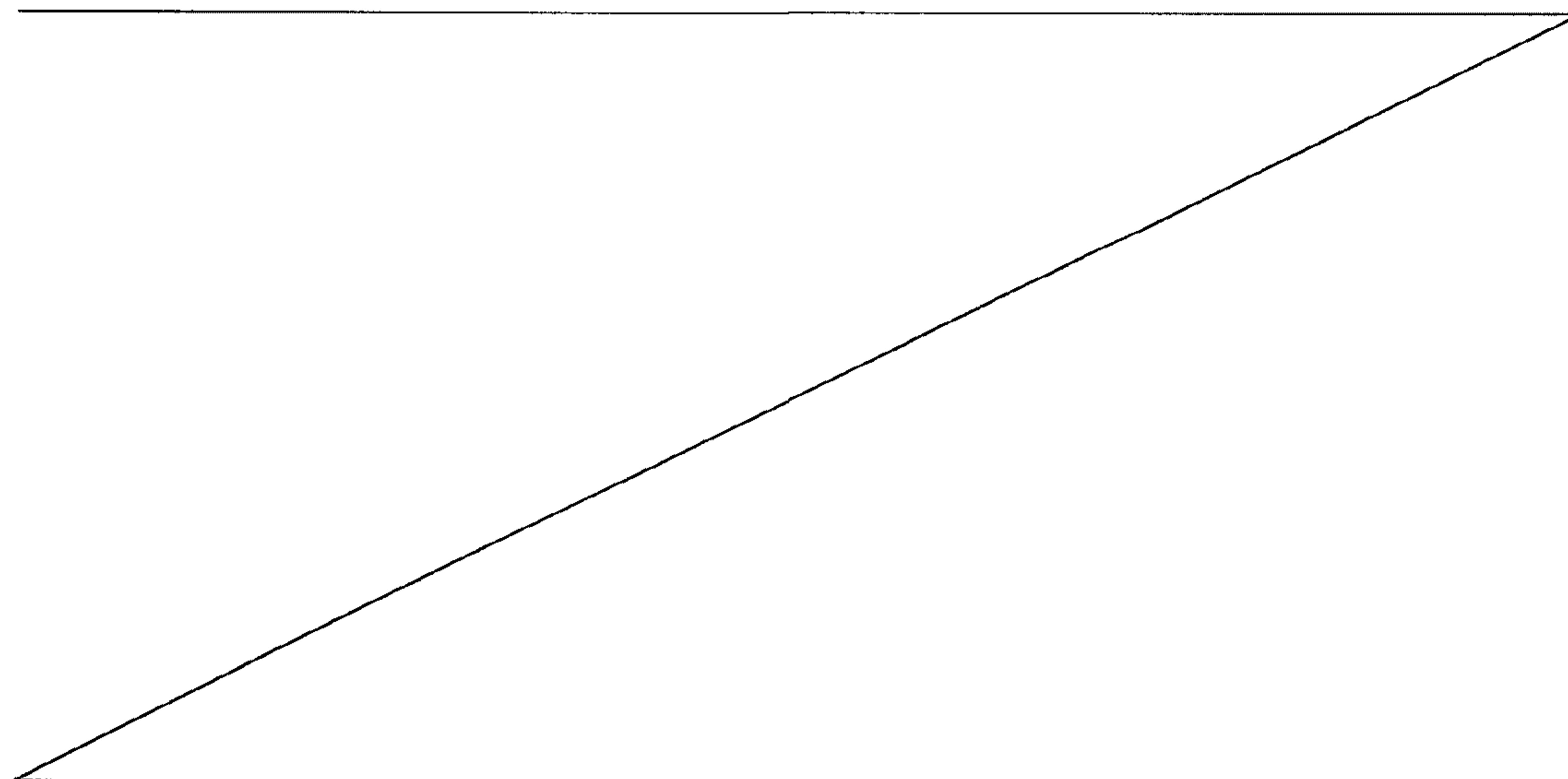
**[0009b]** In one exemplary embodiment, the *Otiorhynchus* weevils are *Otiorhynchus sulcatus*. In another exemplary embodiment, the one or more attractant volatile organic compound is (E)-2-hexenol. In another exemplary embodiment, the one or more attractant volatile organic compound are (E)-2-hexenol and (Z)-2-pentenol. In another exemplary embodiment, the one or more attractant volatile organic compound is (Z)-2-pentenol. In another exemplary embodiment, the one or more attractant volatile

organic compounds are (*Z*)-2-pentenol and methyl eugenol. In still another exemplary embodiment, the *Otiorhynchus* weevils are members selected from the group consisting of *Otiorhynchus ovatus* and *Otiorhynchus rugosostriatus*, and the attractant volatile organic compounds are members selected from the group consisting of (*Z*)-2-pentenol and (*E*)-2-hexenol.

**[0010]** In another exemplary aspect, the invention provides a trap for capturing *Otiorhynchus* weevils, wherein the trap comprises a container that has an interior and an exterior, wherein the container comprises openings in the exterior that are large enough for the weevils to pass through and thereby enter the interior; and wherein the interior contains a “ruffle” which serves as a hiding place for weevils that enter the trap.

**[00010a]** In another exemplary aspect, the invention provides a trap for capturing *Otiorhynchus* weevils, wherein the trap comprises a container that has an interior and an exterior, wherein the container comprises openings in the exterior that are large enough for the weevils to pass through and thereby enter the interior; and wherein the interior contains a “ruffle” which serves as a hiding place for weevils that enter the trap; and wherein the trap is baited with the composition as defined herein.

**[00010b]** In one exemplary



embodiment, the trap is placed in a tree or other plant wherein it is desired that weevils are to be trapped/captured/monitored. In another exemplary embodiment, the trap is baited with or placed in the vicinity of attractant volatile organic compounds that are members selected from the group consisting of (X)-Y-pentenol, (X)-Y-hexenol, methyl eugenol, methyl *iso*-eugenol and a combination of such members, wherein X is E or Z, and Y is 1 or 2. In another exemplary embodiment the trap is baited with or placed in the vicinity of attractant (E)-2-hexenol. In still another exemplary embodiment, the trap is baited with or placed in the vicinity of attractant (Z)-2-pentenol and (E)-2-hexenol. In still another exemplary embodiment, the trap is baited with or placed in the vicinity of attractant (Z)-2-pentenol. In still another exemplary embodiment, the trap is baited with or placed in the vicinity of attractant (Z)-2-pentenol and methyl eugenol. In still another exemplary embodiment, the trap is baited with or placed in the vicinity of attractant volatile organic compounds that are members selected from the group consisting of (Z)-2-pentenol, methyl eugenol and a combination of such members. In still another exemplary embodiment, the trap further contains a bait laced with synthetic insecticide or pathogens. In still another exemplary embodiment, the pathogens are members selected from the group consisting of bacteria, fungi, nematodes and microspora. In still another exemplary embodiment, the trap is used in conjunction with light at an intensity of between about 0.1-1.1 lux for the duration of overnight hours.

[0011] Other features, objects and advantages of the invention will be apparent from the detailed description which follows.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0012] **FIG. 1** Shows different pattern in release of compounds from the headspace of mechanically damaged and weevil-damaged *E. fortunei* 'Dart's Blanket' plants.

[0013] **FIG. 2** Show the number of weevils per replicate for each treatment after statistical analysis of the total number of each weevil species caught during the whole season.

[0014] **FIG. 3** Illustrates an exemplary "weevil trap".

[0015] **FIG. 4** Is a graphical depiction of data obtained from field studies that demonstrate the capture efficacy of several *Otiorhynchus* sp. trap designs. See, Example 3.

## DETAILED DESCRIPTION OF THE INVENTION

### Definitions

**[0016]** Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

**[0017]** The term “*Otiorhynchus* weevil”, “*Otiorhynchus* weevils”, “*Otiorhynchus* sp.”, “vine weevil(s)”, and/or “weevil(s)” as used herein, refer to weevil species of the genus *Otiorhynchus*. “*Otiorhynchus* sp.” are a major insect pest of agriculture. Exemplary *Otiorhynchus* weevils include, but are not limited to *Otiorhynchus sulcatus* (Black vine weevil), *Otiorhynchus ovatus*, *Otiorhynchus salicicola*, *Otiorhynchus rugosostriatus*, etc.

**[0018]** As used herein, the term “control” or “controlling” as in e.g., the phrase: the “control” of *Otiorhynchus* sp., or “controlling” *Otiorhynchus* weevils, or as in the phrase: “controlling” agricultural pests, refers to any means for preventing infection or infestation, reducing the population of already infected areas or organisms, or elimination or reduction in numbers of the population of pests e.g., *Otiorhynchus* sp. or other species whose “control” is desired. Indeed, “controlling” as used herein refers to any indicia of success in prevention, elimination, reduction or amelioration of a pest population or pest problem. In an exemplary embodiment, *Otiorhynchus* weevils are “controlled” by attracting *Otiorhynchus* weevils using volatile organic compounds as disclosed herein and trapping and/or killing the attracted weevils.

**[0019]** The term “reduce” as used herein refers to any indicia of success in the diminishment in size, amount, extent, and/or severity of *Otiorhynchus* weevil infestation. The term “reduce” as used herein also refers to any indicia of success in the diminishment of reproductive capacity (e.g., through killing or trapping etc); diminishment of spread (e.g., rate or extent of spread) e.g., from an un-treated nursery stock to a treated nursery stock; diminished damage to nursery stock or other susceptible plant species caused by *Otiorhynchus* weevils (adults and/or larvae) etc.

**[0020]** As used herein, the term “attracting” refers to the action of causing an insect pest e.g., an *Otiorhynchus* weevil e.g., *Otiorhynchus sulcatus* either directly or indirectly, to move in a direction towards the source of a stimulus e.g. toward a selected attractant VOC

as disclosed herein. One of skill in the art will recognize that suitable stimuli may include a large variety of methods including, but not limited to chemical stimulus *e.g.*, volatile chemicals such as *e.g.*, those disclosed herein, *e.g.*, (Z)-2-pentenol; pheromones; kairomones; etc. A chemical stimulus can be an individual compound or a composition, including *e.g.*, more than one compound *e.g.*, a 1:1 ratio mixture of (Z)-2-pentenol and methyl eugenol, that either directly or indirectly, causes the insect to move toward the source of the stimulus.

**[0021]** Thus, the term "attractant" as used herein refers to a stimulus such as *e.g.*, an attractant VOC *e.g.*, (Z)-2-pentenol, which causes an *Otiorhynchus* weevil, either directly or indirectly, to move in a direction towards the stimulus. In some exemplary embodiments, an "attractant" takes the form of a "bait composition". Typically, a bait composition comprises at least one attractant VOC as disclosed herein either together with other attractant or non-attractant chemicals or alone in amounts effective to attract *Otiorhynchus* weevils to the bait composition. For *Otiorhynchus* attraction, attractant VOCs are effectively used in a concentration range of from about 1.0% to about 10% (wt:v) for granular baits. Indeed, attractant VOCs disclosed herein are typically 10-100 fold less well sensed by the weevils than pheromones and/or have to compete with natural high background levels of these compounds released by the plants. Thus when attracting *Otiorhynchus* weevils, exemplary effective amounts typically are about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10% or even more. Thus, a person having of ordinary skill in the art is readily able determine optimal concentration ranges for the attraction of any given attractant VOC.

**[0022]** The term "bait" as used herein, refers to an ingredient or combination of ingredients comprising an attractant.

**[0023]** The term "pheromone" as used herein, refers to a substance or mixture of substances which are secreted and released by an organism for detection and response by another organism of the same species. Pheromones mediate a variety of interactions between organisms. Thus, pheromones are typically classified by the interaction that they most strongly influence *e.g.*, alarm, aggregation or sex pheromone.

**[0024]** As is known in the art, "pheromones" belong to the larger class of chemical compounds referred to as semiochemicals. The term "semiochemical" as used herein refers to chemicals that mediate interactions between organisms. Semiochemicals include

allelochemicals and pheromones depending on whether the interactions are interspecific or intraspecific, respectively. As used herein the term “allelochemical” refers to chemical substances that induce a response in the receiver of the signal that is either adaptively favorable to the emitter but not the receiver (allomones), or that is favorable to the receiver but not the emitter (kairomones) or is favorable to both emitter and receiver (synomones). Allelochemicals and pheromones are useful *e.g.*, as arrestants, attractants, repellents, deterrents, and/or stimulants.

**[0025]** The term “ratio” as used herein, refers to the relative proportion of at least two compounds with respect to one another.

**[0026]** The term “nursery stock” as used herein, refers broadly to field-grown and/or container-grown hardy perennial and woody plants or biennial trees, shrubs, vines, and plants, evergreens, fruit pits, and other plants or plant parts capable of propagation *e.g.*, vegetative or propagative parts. Other plants considered nursery stock include plants grown for commercial purposes, such as *e.g.*, flowering annuals, strawberry, blueberry, caneberries, etc and vegetables for transplanting.

**[0027]** The terms “isolated,” “purified” or “biologically pure” as used herein, refer to a chemical or microorganism that is substantially or essentially free from components that normally accompany it as found in its native state.

**[0028]** In some exemplary embodiments, the term “isolated” is used to describe an isolated chemical compound, *e.g.*, isolated volatile organic compound, *e.g.*, (Z)-2-pentenol. Thus, in some exemplary embodiments, the terms “isolated” or “purified” refer to a chemical species that is the predominant species present in a preparation. Thus, in some exemplary embodiments, “purity” of an isolated species is determined using analytical chemistry techniques such as *e.g.*, high performance liquid chromatography. A chemical species that is the predominant species present in a preparation is substantially purified. Typically, a “purified” chemical species denotes that a chemical species that is at least about 85% pure, at least about 95% pure, or at least about 99% pure.

**[0029]** The expression “effective amount” or “amount effective for” or any grammatically equivalent expression as used herein, refers to that amount of attractant *e.g.*, a VOC as disclosed herein *e.g.*, (Z)-2-pentenol, which is sufficient to attract *Otiorhynchus* weevils, *c.g.*, *Otiorhynchus sulcatus*, relative to a control that does not contain the attractant

as disclosed herein. In an exemplary embodiment, an effective amount is that quantity of an attractant VOC or attractant VOC blend that provides a release rate of the attractant VOC(s) that attracts *Otiorhynchus* weevils to the location of a bait at a rate higher than the rate at which weevils are attracted to a nonbaited location. In an exemplary embodiment, an effective amount is provided at a release rate that is about 0.06 ml/day. In another exemplary embodiment, an effective amount is provided at a release rate that is about 0.0024 ml/hour. In another exemplary embodiment, an effective amount is provided at a release rate that is between about 0.02 ml/day to 0.1 ml/day. However, effective release rates are not limited to these rates and any suitable rate may be effective so long as it is sufficient to attract *Otiorhynchus* weevils to the vicinity or location of a bait comprising the attractant VOC(s) at a rate higher than the rate at which weevils are attracted to a nonbaited location.

**[0030]** As used herein, the term "trap" refers to any device into which the volatile organic compounds or blends of compounds *e.g.*, (Z)-2-pentenol; a 1:1 ratio mixture of (Z)-2-pentenol and methyl eugenol, etc as disclosed herein are placed such that *Otiorhynchus* weevils are attracted to the trap and can be monitored, collected and/or killed. In some exemplary embodiments a "trap" prevents the *Otiorhynchus* weevil from escaping once the weevil has come into contact with the trap. However, in other exemplary embodiments, a "trap" effective for controlling *Otiorhynchus* weevils does not prevent the weevil from escaping. Indeed, since *Otiorhynchus* weevils hide during daylight hours, in some exemplary embodiments effective "traps" for *Otiorhynchus* weevils simply provide a daytime hiding place for the weevils. Thus, in an exemplary embodiment, a piece of paper placed on a baited piece of ground is an effective "trap" for *Otiorhynchus* weevils. Weevils hiding in such a "trap" can be collected and/or killed by a person monitoring a nursery or nursery stock for *Otiorhynchus* weevils. Traps can be of various sizes, shapes, colors, and materials. In an exemplary embodiment, traps are designed and manufactured specifically for use as an insect trap. In other exemplary embodiments a trap is a container converted and adapted from other uses such as, for example, a glass Petri dish, a metal coffee can, a cardboard box or any ordinary plastic, paper, metal, wood, fiberglass, composite or ceramic container, etc. Exemplary materials for use in making the traps include, but are not limited to, paper, cardboard, metal, wood, metal alloys, glass, paper, plastic, acrylic, fiberglass, composite,

ceramic, etc. Typically, traps have a bottom, sidewalls, and a top. The bottom, sidewalls and top of the trap can be solid, or be perforated. An exemplary perforated sidewall is a screen. In an exemplary embodiment, traps are configured such that insect pests can enter the trap but are unable to escape once inside the trap. In other exemplary embodiments, traps are commercially available (e.g., from Suterra Inc.). In still other exemplary embodiments, traps have only a bottom or only a top e.g., a piece of paper placed on a baited piece of ground.

## I. Introduction:

[0031] Insects have plagued people throughout history. Intercontinental travel and trade have enabled the importation of *Otiorhynchus* weevils into countries to which they are not indigenous. As a result, numerous species of *Otiorhynchus* weevils now plague the horticultural industry.

[0032] Root weevils of the genus *Otiorhynchus*, are flightless parthenogenic and/or sexual weevil species (see e.g., Downes, 1922; Smith, 1932; Suomalainen et al., 1987) that have become a major worldwide pest of the horticultural industry.

[0033] Although a number of natural enemies, such as hedgehogs, frogs, predatory beetles, and insect pathogens such as entomopathogenic fungi and nematodes help to maintain *Otiorhynchus* weevils populations at a low level in natural environments, such natural predators and pathogens are less successful or costly and difficult to apply in intensive horticultural systems where persistent chemicals have been relied on to keep populations low and/or when the number of cultured plants is beyond the capacity of the ecosystem.

[0034] Adult weevils are nocturnal and oviposition, which produces the next generation of destructive larvae, typically occurs at night. Thus, monitoring and control of the weevils is difficult.

[0035] Weevil presence is typically determined by monitoring weevil damage to the plants. To prevent weevil oviposition, growers have available a number of more or less effective measures such as various cultural measures, and chemical and biological agents that can be deployed both above and below ground as necessary (see e.g., Kakouli-Duarte, et al. (1997) Annals of Applied Biology, 131: 11–27; Lola-Lutz, T. and Downes, M. (2007) Biological Control 40(3): 314-319; Moorehouse, E. et al. (1992) Annals of Applied Biology,

121: 431–454; Van Tol, R. W. H. M., & Raupp, M. J. (2005). Nursery and tree application. *Nematodes as biological control agents*. Wallingford: CABI Publishing, 167-190.; Dolmans, N.G.M. and R.W.H.M. Van Tol (1996). Prospects for chemical control of black vine weevil (*Otiorhynchus sulcatus*) in nursery stock. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft* 316, 108-112; Van Tol, R.W.H.M. (1996). A strategy for control of black vine weevil (*Otiorhynchus sulcatus*) in an Integrated Pest Management programme in nursery stock. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft* 316, 76-80.).

[0036] Unfortunately, as noted above, adults are nocturnal and oviposition typically occurs at night. Thus, a major problem in combating weevil attack is monitoring and timing of control measures. Indeed, due to the night-activity of the adults, growers and gardeners typically do not observe the first emerging weevils in a timely manner and oviposition often starts before effective control measures are taken.

[0037] Fortunately however, the instant invention provides, for the first time, compositions of volatile organic chemicals (VOC) effective for attracting *Otiorhynchus* weevils so that they can be effectively monitored and/or controlled despite their nocturnal habits. The compositions and methods utilize a unique combination of VOCs to not only attract *Otiorhynchus* weevils but also to induce them to enter a trap or get in contact with an active ingredient such as a chemical or biological agent so that they can be captured and killed or otherwise controlled.

[0038] Thus, as disclosed herein, the effective volatile organic compounds are deployed in the field *e.g.*, nurseries, gardens, etc, for the control of *Otiorhynchus* weevils.

## II. Compounds

### A. General Methods

[0039] Methods disclosed herein utilize routine techniques in the field of chemistry and chemical analysis. Basic texts disclosing the general methods of use in this invention include, *e.g.*, *GC-MS A Practical User's Guide* by Marvin McMaster, Wiley-VCH (1998); *Modern Analytical Chemistry*, by David T. Harvey, McGraw-Hill Science/Engineering/Math (1999).

## B. Volatile Organic Compounds Effective for Attracting *Otiorhynchus* weevils

[0040] In exemplary embodiments (E)-2-hexenol, (Z)-2-pentenol, methyl eugenol are used alone in combination to effectively attract *Otiorhynchus* weevils. Typically, volume ratios are 1:1 or 1:1:1 for single, double and triple mixtures of (E)-2-hexenol, (Z)-2-pentenol, methyl eugenol.

## III. Methods for Attracting *Otiorhynchus* weevils

[0041] Insect traps are typically used to monitor or directly reduce insect populations. Traps may reduce insect populations directly or may reduce future populations by negatively affecting the reproductive capacity of a present generation of insects e.g., by directly or indirectly preventing oviposition. Thus, one embodiment, the volatile organic compounds disclosed herein, are used to attract and trap *Otiorhynchus* weevils.

[0042] Fortunately, it has now been discovered that *Otiorhynchus* weevils are selectively attracted by a well defined release of attractive chemicals. The ability to attract *Otiorhynchus* weevils before they lay eggs adds greatly to the value of the trapping system because it permits the elimination or reduction of the very destructive larval state.

[0043] The addition of supplemental lighting at night alters the catch of *Otiorhynchus* sp. in trap devices. Indeed, light provided at a low intensity level (0.1-1.1 lux) for the duration of the overnight hours demonstrates a synergistic effect of supplemental light with experimental trap B in the overnight hours (see e.g. Example 2 and Table 1 hereinbelow).

## A. Determining an Effective Amount of VOC for Attracting *Otiorhynchus* weevils

[0044] In an exemplary embodiment, effective attractant ability is indicated when capture of *Otiorhynchus* weevils in locations baited with one or more volatile organic compounds as disclosed are higher than in an unbaited location.

[0045] Typically, because the attractant VOC(s) disclosed herein are about 10-100 fold less well sensed by the weevils than pheromones and/or have to compete with natural high background levels of these compounds released by the plants effective amounts of VOC(s) for attracting *Otiorhynchus* weevils is typically between about 1% to about 10%. Methods for determining release rates are known in the art (see e.g., R.W.H.M. van Tol et al. (2007)

Pest Manag Sci 63: 483-490; Pietrogrande and Basaglia (2007) Trends in Analytical Chemistry 26: 1086-1094; Yosha et al. (2008) J. Agric. Food Chem. 56: 8045-8049).

**[0046]** In an exemplary embodiment, effective release rates are achieved by placing 0.4 ml of pure compounds/mixtures in high volume plastic containers as follows “Plant volatile dispensers were made of 1.5 ml LDPE Pasteur pipettes (Labo Scientific, Ede, the Netherlands). The compounds to be tested 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. This type of ‘high release’ pheromone/kairomone dispenser have been developed and used by Pherobank® for several years for the attraction of *Phyllopertha horticola* L. (Coleoptera: Scarabaeidae) and has also been successfully tested for attraction of the rosy apple aphid, *Dysaphis plantaginea* (Passerini) (Hemiptera: Aphidae) (Van Tol et al. 2009, *supra*). Typically, closed dispensers do not release high enough amounts of the plant volatiles thus, keeping the are typically left partially opened. The dispenser is a simple and cheap existing design, known in the art, which enables a high release profile especially for larger quantities of plant volatiles. In exemplary embodiments, release rates of about 0.06 ml/day or 0.0024 ml/hour are achieved. In other exemplary embodiments release rates of between about 0.02 to 0.1 ml/day are achieved using this method. In still other exemplary embodiments a release rate range of attractant VOCs is between about 1 ul/hour to 240 ul/hour.

**[0047]** In another exemplary embodiment, a blend of attractant VOCs is contained in a release membrane made from, for example, polyethylene, polypropylene, polyvinylchloride, mylar, and acrylic as described in e.g., Leonhardt et al, *Insect Pheromone Technology: Chemistry and Applications*, ACS Symposium Series 190, 1982 and Kydonisus, *Controlled Release Pesticides*, ACS Symposium Series 53, 1977. In some exemplary embodiments, efficacy and composition of attractive VOC is affected by the nature of the crop or nursery stock and by the particular weevil species being attracted. Thus, in some exemplary embodiments different combinations of odors are attractive to the different weevil species.

#### IV. Traps for *Otiorhynchus* weevils

[0048] In an exemplary embodiment, the invention provides an apparatus that includes a movable housing or device that can be strategically placed within an area needing treatment. The apparatus may include a source of at least one volatile organic compound (VOC) as disclosed herein contained within or in the proximity of the housing and a dispenser that is adapted to initiate the release e.g., controlled release over a certain time period, of the composition over an area in need of treatment.

[0049] Typically, *Otiorhynchus* weevils leave plants they feed on at night to seek a hiding place during the day. Thus, in an exemplary embodiment, a minimal trap for *Otiorhynchus* weevils is a hiding place e.g., a piece of cardboard, from which the *Otiorhynchus* weevils can escape if they so choose. Although the *Otiorhynchus* weevils can escape such a trap, close monitoring of traps permits the horticulturalist to capture hiding weevils before they leave their hiding places at night.

[0050] In other exemplary embodiments, the attractant compositions of the present invention are used in conjunction with a trap by dissolving attractant in a non-volatile and non- solvent, such as, for example, mineral oil, ethylene glycol or any solid organic or non-organic material. This slow-release product is then placed in a trap vial or that is placed in the canopy of the plant. *Otiorhynchus* weevils attracted to the solution, will enter the trap. In another exemplary embodiment, the trap is the attractant VOCs dissolved in a non-volatile solvent, and placed in a trap system that incorporates a fast acting insecticide or biological agent(s) that will kill attracted *Otiorhynchus* weevils that visit the trap. The attractant can also be formulated into a controlled release matrix that attracts *Otiorhynchus* weevils.

[0051] In some exemplary embodiments, the attractant VOCs or blends of attractant VOCs is/are microencapsulated, by methods known in the art (see e.g., Bakan, J. A. *Microencapsulation Using Coacervation/Phase Separation Techniques*. In Controlled Release Technology: Methods, Theory, and Application; Kydonieus, A. F., Ed.; CRC Press: Boca Raton, FL, 1980; pp 83-105; and Herbig, S. M, *et al.* (1987) Am. Chem. Soc. Div. Polym. Chem. Prepr. 1987, 28, 92-9. However, any suitable method known in the art for dispersal/dispensation of volatile organic compounds disclosed herein for luring and/or trapping may be used e.g., CheckMate®

Puffer by Suterra.

**[0052]** In some exemplary embodiments, the attractant VOCs or blends of attractant VOCs are used with a trap designed for use in attracting and/or trapping *Otiorhynchus* weevils (a “weevil trap”). A typical weevil trap comprises a container which may be of variable form and size made of variable material. In an exemplary embodiment, a Wiffle® ball is used to construct the container part of the trap. Thus the trap comprises openings large enough for the weevils to enter. In the interior of the trap is a “ruffle” which is serves as a hiding place for weevils once they enter the trap. Typically, a “weevil trap” trap is placed in a tree or other plant wherein it is desired that weevils are to be trapped/captured/monitored etc. In some exemplary embodiments twigs and/or branches of the tree/plant are woven through holes in the trap. To trap weevils, the weevil trap is placed above and not on the ground. In some exemplary embodiments, the trap is placed in a plant or in a plant canopy. Thus, in an exemplary embodiment, the hiding place takes the form of ruffle in a container with holes placed in the tree. In other exemplary embodiments, the trap is simply raised off the ground, by any suitable means.

## V. Exemplary Uses of Invention

**[0053]** In exemplary embodiments the invention is used as for monitoring, control, and/or detection of *Otiorhynchus* weevils. In one exemplary embodiment, the trap is deployed to tabulate the catch to determine size and location of *Otiorhynchus* weevil infestation. Economic and effective use of appropriate pest management systems can then be determined. In other exemplary embodiments, trapping the *Otiorhynchus* weevils serves as a control method.

**[0054]** In some exemplary embodiments attractant VOCs as disclosed herein are used in combination with insecticide application or other control measures. Thus, in one exemplary embodiment, the invention is used to attract *Otiorhynchus* weevils and to induce them to enter a trap or location where they contact an effective amount of toxicant to achieve control. An effective amount of the toxicant is an amount that is lethal for an exposed *Otiorhynchus* weevil or at least sublethal but sufficient to incapacitate the *Otiorhynchus* weevil with regard to future oviposition activity. Exemplary of the wide variety of toxicants which may be used with the invention are, e.g., methomyl, malathion, dichlorvos, acephate,

indoxacarb and biological agents including but not limited to fungi, nematodes and bacteria or a combination of two or more of the above.

**[0055]** In some exemplary embodiments, the attractant VOC(s) disclosed herein are used in conjunction with a “weevil trap” (see e.g., Example 3 hereinbelow). In some exemplary embodiments, the attractant VOC(s) disclosed herein are used in conjunction with a “weevil trap” and an effective amount of toxicant to achieve control.

**[0056]** In still other exemplary embodiments, control of *Otiorhynchus* weevils can be achieved by using the invention to detect the location and boundaries of localized *Otiorhynchus* weevil infestations and employ in the area chemosterilants, bioregulator agents, parasites, predators or other biological control agents..

**[0057]** The following examples are offered to illustrate, but not to limit the invention.

## EXAMPLES

### Example 1:

**[0058]** The following example illustrates formulation of volatile organic compounds suitable for attraction of *Otiorhynchus* weevils.

### METHODS AND MATERIALS FOR EXAMPLE 1

**[0059]** Volatiles were collected from a bio-active extract of *Euonymus fortunei* ‘Dart’s Blanket’ in paraffin oil and from cuttings of mechanically damaged and *O. sulcatus*-damaged *E. fortunei* ‘Dart’s Blanket’. The antennal response to these plant odours by *O. sulcatus* were measured by gas chromatography coupled with electroantennogram detection (GC-EAD). Compounds giving an electrophysiological response were tentatively identified by gas chromatography coupled with mass-spectrometry (GC-MS). When the tentatively identified compound showed similar Kováts indices on our chromatographic system as the purchased synthetic reference compound, it was considered to be a positive identification. A selection of plant volatiles that gave an electrophysiological reaction on the antenna were tested as single compounds and in several mixtures of two or more compounds on bioactivity using the olfactometer developed specifically to study vine weevil behaviour (see e.g., Van Tol et al., (2002) *Physiological Entomology* 27, 213-222). A small selection

of compounds bioactive in the lab was tested under field conditions in strawberry.

#### ***Headspace collection***

[0060] A filtered 10-ml extract of *E. fortunei* 'Dart's Blanket' in paraffin oil (Merck, Uvasol) and four *O. sulcatus*-damaged and four mechanically damaged 20-cm long cuttings of field-grown *E. fortunei* 'Dart's Blanket' were used for headspace collection. The extract was prepared by crushing 30 g of fresh leaves from the top 15cm part of stems with 50 ml of paraffin oil on ice. The extract was crushed for approximately 20 min followed by immediately filtering through Whatman<sup>TM</sup> No. 90 (diameter 15cm) paper filter. The filtered *Euonymus* extract was stored cool until use. Cuttings of *Euonymus* were mechanically damaged with a scissor by four incisions per leaf three hours prior to headspace collection.

[0061] The *O. sulcatus*-damaged *Euonymus* cuttings were prepared by offering 10 vine weevils 16 hrs prior to headspace collecting the cuttings. Weevils were removed and cuttings washed with deionized water prior to placing in a fresh 100 ml flask with water for headspace collection. For three days the *Euonymus* cuttings or a daily refreshed 10-ml paraffin oil extract of *Euonymus* in an open Petri-dish were placed under a glass bell jar (5 l) in a growth chamber at 20°C and under long day (L:D= 16:8 hr) light conditions. Air was purified by passage through a charcoal filter and drawn at 0.2 l min<sup>-1</sup> through the jar. Volatiles were entrained for a total of 69 hrs. For the collecting of volatiles, Gerstel<sup>TM</sup> thermodesorption tubes, filled with 80 mg Tenax<sup>TM</sup> TA 20/35 mesh (Grace-Alltech<sup>TM</sup>), were used. Before use, these tubes were cleaned by rinsing them with 10 ml hexane and, subsequently, flushing them for one hour at 280°C with 20 ml min purified nitrogen. Each Tenax<sup>TM</sup> tube was refreshed after approximately 8 hrs. The volatiles trapped on the Tenax<sup>TM</sup> were washed off with 15 ml hexane. The Tenax<sup>TM</sup> washings for each treatment were pooled and concentrated under argon to one extract (~200 µl) prior to GC-EAD and GC-MS testing (see e.g., Marco D'Alessandro and Ted C. J. Turlings (2006) Analyst 131:24–32; R.W.H.M. van Tol et al. (2009) Bulletin of Entomological Research 99: 593–602).

#### ***Coupled gas chromatography electroantennographic detection (GC-EAD)***

[0062] GC-EAD measurements were carried out using an Interscience<sup>TM</sup> Trace GC-2000 (Interscience<sup>TM</sup>, Breda, The Netherlands) equipped with a cold on-column injector. The

gas chromatograph was equipped with a Grace-Alltech<sup>TM</sup> 30 m EC-5 fused silica column, 0.25-mm ID and 0.25-mm film thickness. Conditions were: carrier gas, helium (constant flow 1.7 ml min<sup>-1</sup>); temperature programming, 80°C (0.8 min hold) to 260°C (10 min hold) at 25°C min<sup>-1</sup>; detector temperature, 250°C; the transfer line between the GC and the EAD (Syntech Laboratories, Hilversum, The Netherlands) followed the oven temperature. Over the antenna, a flow of purified, humidified air was maintained at a flow rate of 80 cm sec<sup>-1</sup>. The sample was equally split between a flame ionization detector (FID) and the EAG detector. Antennae were separated from the weevil heads and mounted between two glass electrodes filled with a ringer solution (6.4mM KCl, 12mM MgCl<sub>2</sub>.6H<sub>2</sub>O, 9.6mM KOH, 12mM NaCl, 20mM KH<sub>2</sub>PO<sub>4</sub>, 1mM CaCl<sub>2</sub> and 354mM glucose in deionized water). Antennal preparation and EAG recording were performed according to the procedure described by (Van Tol et al., (2002) *Physiological Entomology* **27**, 213-222). The EAG recorder plus peripheral equipment were manufactured by Syntech Laboratories. Approximately five antennal preparations with limited background noise for each treatment showing responses to several compounds in the extract were used for comparison. Only EAG responses that were present in all preparations at the same retention time (R<sub>t</sub>) were identified as an EAG positive response to a compound in the extract.

#### ***Gas Chromatography-Mass Spectrometry (GC-MS)***

[0063] GC-EAD active compounds were identified by mass spectrometry by injecting the same extracts used for GC-EAD on a GC-MS system under comparable conditions. GC-MS analyses were carried out on a Hewlett Packard<sup>TM</sup> 5973 mass selective detector (70 eV) coupled to a Hewlett Packard 6890 gas chromatograph equipped with a split/splitless injector. The gas chromatograph was equipped with an Alltech<sup>TM</sup> 30 m AT-5 fused silica column, 0.25 mm ID and 0.25 µm film thickness run in constant flow mode (1.3 ml/min Helium). Temperature programming: 50°C (2 min hold) to 300°C (8 min hold) at 5°C/min (AT-5 column); transfer line temperature, 300°C; injector temperature, 230°C. One µl of concentrated headspace volatiles were injected manually into the GC-MS system for analysis. Injections were done in splitless mode only (1 µl).

#### ***Compounds and treatments***

[0064] A selection of single plant compounds (A = (Z)-2-pentenol and B = (E)-2-

hexenol) and combinations of plant compounds (C = A+B in ratio 1:1, D = A+methyl eugenol in ratio 1:1, and E = A+B+methyl eugenol in ratio 1:1:1) were tested in a strawberry field (*Fragaria × ananassa* 'Tillamook') for attraction of *O. sulcatus*. Choice of compounds and mixtures were based on identification of EAD-active compounds from headspace of weevil-damaged *E. fortunei* 'Dart's Blanket' plants and paraffin oil extract of the same *Euonymus* plant species combined with bioassay results with the EAD-active compounds alone and in several mixtures. The compound (Z)-2-pentenol (purity 95%) was obtained from Bedoukian (Danbury, CT, USA), (E)-2-hexenol (purity >96%) from Acros (Geel, Belgium) and methyl eugenol (purity >98%) from Sigma-Aldrich (St. Louis, Missouri, USA). All chemicals were used without further purification. Single compounds and mixtures were introduced in dispensers prior to use and sealed. In each dispenser 0.4 ml of each compound was present.

### ***Dispensers***

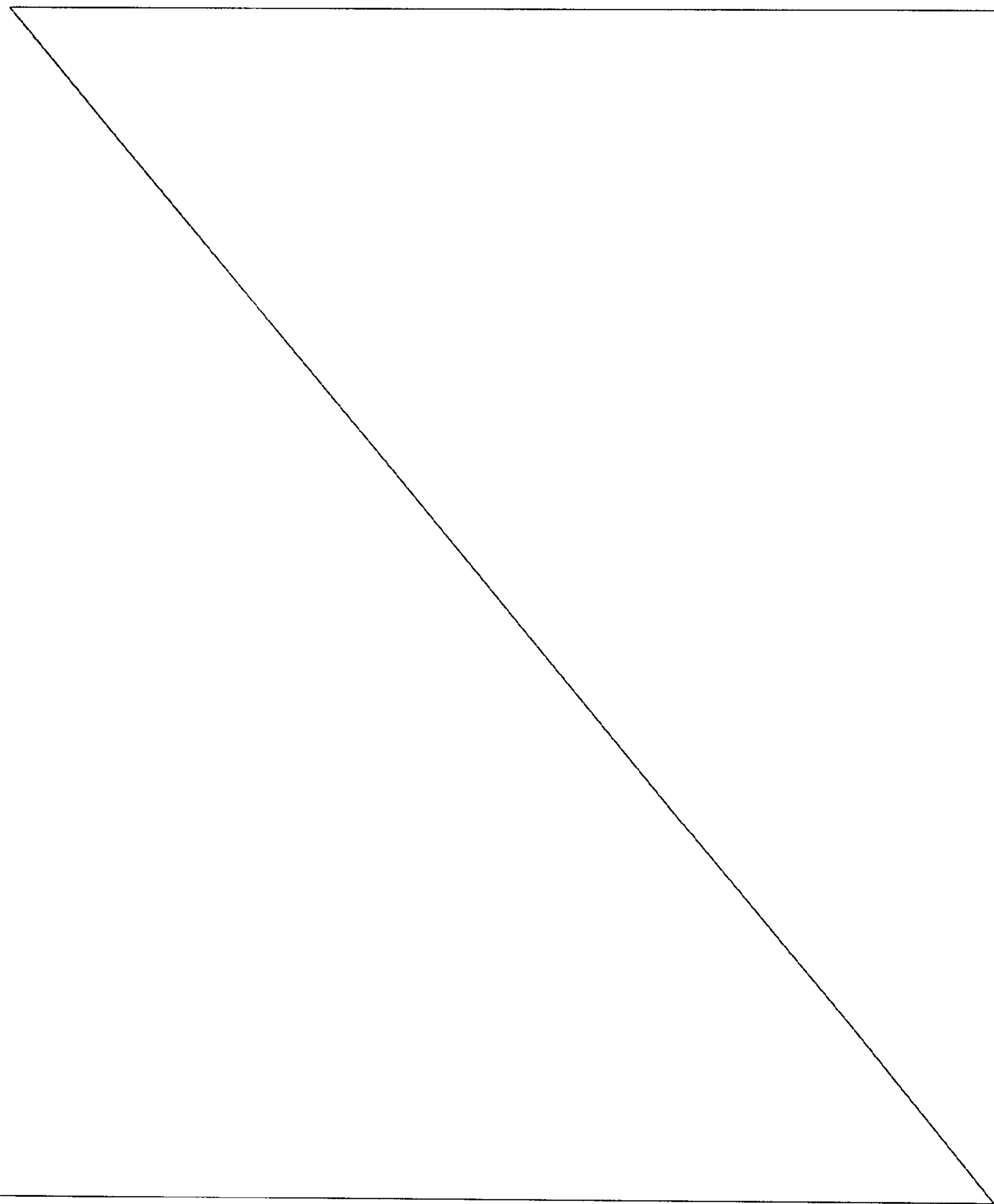
**[0065]** Plant volatile dispensers were made of 1.5 ml LDPE Pasteur pipettes (Labo Scientific, Ede, the Netherlands). The compounds to be tested were introduced into the pipette, the tip of which was then sealed by heat. Prior to use, the tip of the pipette was cut off at 1 cm above the reservoir portion. The open tip of the dispenser had an internal diameter of 3.5 mm. This type of 'high release' pheromone/kairomone dispenser are developed and used by Pherobank for several years for the attraction of *Phyllopertha horticola* L. (Coleoptera: Scarabaeidae) and has also been successfully tested for attraction of the rosy apple aphid, *Dysaphis plantaginea* (Passerini) (Hemiptera: Aphidae) (R.W.H.M. van Tol et al. (2009) Bulletin of Entomological Research 99: 593–602). Closed dispensers do not release high enough amounts of the plant volatiles through the polyethylene to attract these beetles and aphids compared to partially opened vials. The dispenser is a simple and cheap existing design which enables a high release profile especially for larger quantities of plant volatiles. The plant volatiles were present as pure commercial compounds single or mixed in one dispenser according to the compound composition.

### ***Field experiment***

**[0066]** Experiments were performed on a commercial strawberry field in Oregon (USA). Five different treatments (A, B, C, D and E) and one control were tested in the

field. The control consisted of an empty dispenser. Dispensers with odors were placed in the top part of a boll weevil trap (Great Lakes IPM, Inc., Vestaburg, MI). Each trap contained one odor-filled or control (empty) dispenser located in the top capture assembly. Boll weevil traps were placed in the rows between the strawberry plants.

Distance between each



17a

treatment was 10 meters. Each treatment of trap-odor was coupled with a trap-control treatment at a distance of 10 meters from each other and replicated four times for each field trial. Coupled odor-control set-up was performed to minimize effects of possible uneven weevil distribution throughout the fields. Dispensers were refreshed once a week. Traps and dispensers were placed in the fields and monitored for weevils' presence between May and August 2009. First weevils emerging from soil were found in the first week of June. Traps and plants surrounding each trap-dispenser combination were monitored for weevils weekly. Plants within the treatment row up to 60 cm distance from the dispenser in either direction were checked for weevil presence. Weevils found were removed from the field.

### **Statistics**

**[0067]** The field tests were set-up as block designs where the blocks consisted of four plots. Each plot was divided into two subplots where one subplot was the trap-odor treatment and the other subplot the trap-control treatment. The four subplots within a block were treated with the same odor. Each block contained therefore four identical odor traps coupled with four control traps. This set-up allows comparison of each odor with the control but not comparison between the different odors tested.

**[0068]** The total number of weevils for each weevil species per trap were analyzed using GLM (Generalized Linear Model) with logarithmic link, Poisson distribution and not fixed dispersion using the 12<sup>th</sup> version of the statistical package GenStat (Payne et al., 2009). The fixed part of the model consists of the additive effects of block/plot and odor (of which the control was an extra level). After the analysis, paired comparisons were performed on the transformed scale data with approximate t-tests between the odors and the control. Thereafter, estimates of the means of the weevils per trap are back transformed to the original scale with approximate standard errors.

### **RESULTS FOR EXAMPLE 1**

**[0069]** The headspace of mechanically damaged and weevil-damaged *E. fortunei* 'Dart's Blanket' plants and the leaf extract in paraffin oil showed a different pattern in release of compounds (FIG. 1). EAD-active compounds were present in all three headspace extracts and only differences in strength of antennal responses were found. Without being bound by theory it is believed that the differences in strength are related to the amount of the specific

compounds present in the headspace of the different treatments. The amounts of compounds and strength of the antennal responses were not quantified in this research. The results show that of the 14 EAD-active volatiles (*Z*)-2-pentenol, (*Z*)-3-hexenol and (*E*)-2-hexenol were present in larger quantities in the headspace of the *Euonymus* extract in paraffin oil than in the headspace of mechanically and weevil-damaged *Euonymus* leading to clearly stronger antennal responses for these compounds in the extract compared to the plant headspaces. High release of EAD-positive DMNT in weevil-damaged *Euonymus* compared to the mechanically damaged plant and paraffin oil extract indicate that this compound is produced and released by the plants in increased amounts as a response to weevil damage. There was, however, no stronger antennal response to this compound in the weevil-damaged plants compared to the other treatments.

**[0070]** Results presented in FIG. 2 show the number of weevils per replicate for each treatment after statistical analysis of the total number of each weevil species caught during the whole season. Except for an occasional weevil caught in the boll weevil trap all weevils were found in the plants surrounding the odor source.

**[0071]** Treatment A (11.3, s.e. = 3.3,  $p = 0.01$ ) and D (18.2, s.e. = 10.9,  $p = 0.03$ ) caught significant more *O. sulcatus* than the control (4.3, s.e. = 0.7). The treatments B (4.3, s.e. = 2.6,  $p = 1.0$ ), C (7.9, s.e. = 3.6,  $p = 0.22$ ) and E (3.3, s.e. = 1.3,  $p = 0.56$ ) were not significantly different from the control.

## Discussion

**[0072]** This is the first report of a successful attraction of weevils in the genus *Otiorhynchus* to a synthetic kairomone comprising plant volatiles. Three compounds from the spindle tree *Euonymus fortunei* 'Dart's Blanket', attractive for *O. sulcatus* (Van Tol et al., (2002) *Physiological Entomology* **27**, 213-222), and sensed by their antenna play a role in attraction. Of these compounds (*Z*)-2-pentenol alone and in combination with methyl eugenol is attractive for *O. sulcatus* in strawberry. For two other weevil species in the same genus (*O. ovatus* and *O. rugosostriatus*), present in the strawberry test field, there was attraction to a mixture of (*Z*)-2-pentenol and (*E*)-2-hexenol but not to the *O. sulcatus* attractive treatments.

**[0073]** Many weevils in the genus *Otiorhynchus* are polyphagous and reproduce parthenogenetically. Thus, without being bound by theory it is believed that unless these

weevil species also produce an aggregation pheromone it is likely that plant odors play a role in host-plant finding and/or aggregation (e.g. feeding-induced release of plant compounds attracting conspecifics). Indeed, EAD profile of *O. sulcatus* (Van Tol et al., (2002) *Physiological Entomology* 27, 213-222) showed strong antennal responses to typical Green Leaf Volatiles (GLVs) with strongest responses to the C6 alcohols but not the C6 aldehydes, acetates or ketones.

**[0074]** The headspace of a preferred *O. sulcatus*-damaged host-plant (*E. fortunei*) and an attractive host-plant extract tested on weevils' antennal response via GC-EAD coupled with GC-MS analysis revealed responses to two C6 alcohols present in the headspace of the plants, namely (*Z*)-3-hexenol and (*E*)-2-hexenol and also to a C5 alcohol, (*Z*)-2-pentenol. In laboratory and field tests only (*Z*)-2-pentenol was attractive to *O. sulcatus* next to methyl eugenol. Pentenols are compounds found in several plant species released in substantial amounts when exposed to pathogen attack and after freeze-thaw wounding of plants.

**[0075]** To our knowledge no behavioral responses of any insect species to pentenols has been described prior to this disclosure. The other compound, attractive in conjunction with (*Z*)-2-pentenol for *O. sulcatus* - methyl eugenol - occurs naturally in plants from over 200 species in 32 families.

**[0076]** Thus, in exemplary embodiments, odor composition is optimized for use in trap devices for monitoring in the field. *Otiorhynchus* weevils are night active feeders and are attracted to the host plants near the odor source at night while the traps tested are designed to act as daytime hiding locations and are not attractive for the weevils to enter during feeding. In an exemplary embodiment, new trap devices are constructed for capture efficacy.

### **Example 2:**

**[0077]** The following example illustrates formulation a synergistic effect of light on attraction of *Otiorhynchus* weevils exposed attractant volatile organic compounds.

**[0078]** A solar powered LED light (X watts) was positioned 30 cm above the ground in close proximity of the plant containing an *Otiorhynchus* sp. traps (described hereinbelow in Example 3). The light contained a sensor that powered the LED during the overnight hours. The proportion of *Otiorhynchus sulcatus* adults in the traps near a light source the following morning were compared to those traps absent of light. Results are shown in Table 1 below.

Table 1. Proportion of *Otiorhynchus* weevils recaptured from two example traps tested with supplemental overnight (0.1-1.1 lux) in comparison to the same trap absent of light in 2010.

Experimental date	Trap A	Trap B
7/8 June	*	.81
9/10 June	*	.65
14/15 June	.18	0.0
17/18 June	.42	.60
24/25 June	.56	1.0

\*No supplemental light treatment performed

[0079] As can be seen in the Table, a larger proportion of trapped weevils were trapped with supplemental overnight light, than were trapped without supplemental overnight light. For example, 7/8 June data Trap B. Here the data shows that all else being the same, 81% of the trapped weevils were trapped with the application of overnight light.

**Example 3:**

[0080] The following example illustrates a trap designed for use in attracting and/or trapping *Otiorhynchus* weevils.

[0081] In an exemplary embodiment a trap designed specifically for trapping *Otiorhynchus* weevils is used. An Exemplary *Otiorhynchus* weevil trap (or “weevil trap”) is shown in FIG. 3.

[0082] In general, a trap designed for use in attracting and/or trapping *Otiorhynchus* weevils comprises a container which may be of variable form and size made of variable material. In an exemplary embodiment, a Wiffle® ball is used to construct the container part of the trap. Thus the trap comprises openings where weevils can enter. The number and size of openings are not important except in-so-far as the openings must be large enough for the weevils to enter.

**[0083]** In the interior of the trap is a “ruffle” which is serves as a hiding place for weevils once they enter the trap. The “ruffle” is, as generally understood a gathered or goffered fluted frill of lace or cloth or paper or any convenient material. Exemplary “ruffle” material includes, but is not limited to cotton ruffles from the border of a garment.

**[0084]** Typically, the trap is placed in a tree or other plant wherein it is desired that weevils are to be trapped/captured/monitored etc. In some exemplary embodiments twigs and/or branches of the tree/plant are woven through holes in the trap.

**[0085]** To trap weevils, the weevil trap is placed above and not on the ground. In some exemplary embodiments, the trap is placed in a plant or in a plant canopy. In other exemplary embodiments, the trap is simply raised off the ground, by any suitable means available to a person having ordinary skill in the art.

**[0086]** In some exemplary embodiments, the trap is used in combination with light as described in Example 2. In other exemplary embodiments, the trap is used with various sources of light and attractants VOC(s). In still other exemplary embodiments the trap is used with attractant VOC(s) and in still other exemplary embodiments, the trap is used alone without attractant VOC(s) or light. In some exemplary embodiments, the trap is used in combination with any attractive and/or killing bait and thus in some exemplary embodiments is a “lure and kill” device.

**[0087]** Field studies were performed in 2010 to test the capture efficacy of several *Otiorhynchus* sp. trap designs. Traps tested included a ruffle of cotton secured around the base of a plant stem, a weevil trap as disclosed hereinabove (see e.g., FIG. 3) secured in the canopy of the plant, a PVC tube with four openings on the soil surface, and a standard grooved board. The experiment was replicated eight times over a period of four days. For each replicate, a single *Rhododendron* sp. plant was enclosed in 1 m<sup>3</sup> cage and 20 weevils (starved for 24 hrs) were released in each cage in late afternoon. The following morning, the cages were carefully inspected and the number of weevils captured in each trap design tabulated. The data is shown in FIG. 4.

**[0088]** The data shown in FIG. 4 indicate that the “weevil trap” recaptured more than twice as many of the released weevils as any other trapping device tested.

**[0089]** It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

**WHAT IS CLAIMED IS:**

1. A composition of one or more attractant volatile organic compounds effective for attracting *Otiorhynchus* weevils, wherein the volatile organic compounds are members selected from (i) to (iv):
  - (i) (*X*)-Y-pentenol;
  - (ii) (*X*)-Y-pentenol and (*X*)-Y-hexenol;
  - (iii) (*X*)-Y-pentenol and methyl eugenol; and
  - (iv) (*X*)-Y-pentenol, (*X*)-Y-hexenol, and methyl eugenol;wherein *X* is E or Z, and Y is 1 or 2.
2. The composition of claim 1, wherein the *Otiorhynchus* weevils are *Otiorhynchus sulcatus*.
3. The composition of claim 1 or 2, wherein the one or more attractant volatile organic compounds are (*E*)-2-hexenol and (*Z*)-2-pentenol.
4. The composition of claim 1 or 2, wherein the one or more attractant volatile organic compound is (*Z*)-2-pentenol.
5. The composition of claim 1 or 2, wherein the one or more attractant volatile organic compounds are (*Z*)-2-pentenol and methyl eugenol.
6. The composition of claim 1, wherein the *Otiorhynchus* weevils are members selected from the group consisting of *Otiorhynchus ovatus* and *Otiorhynchus rugosostriatus*, and the attractant volatile organic compounds are (*Z*)-2-pentenol and (*E*)-2-hexenol.
7. A trap for capturing *Otiorhynchus* weevils, wherein the trap comprises a container that has an interior and an exterior,  
wherein  
the container comprises openings in the exterior that are large enough for

the weevils to pass through and thereby enter the interior; and  
wherein  
the interior contains a “ruffle” which serves as a hiding place for weevils that  
enter the trap; and  
wherein the trap is baited with the composition as defined in any one of  
claims 1 to 6.

8. The trap of claim 7, wherein the trap is for placing on a tree or other plant wherein it is desired that weevils are to be trapped, captured or monitored.
9. The trap of claim 7 or 8, wherein the trap is baited with attractant (Z)-2-pentenol.
10. The trap of claim 7 or 8, wherein the trap is baited with attractant (Z)-2-pentenol and methyl eugenol.
11. The trap of any one of claims 7 to 10, wherein the trap further comprises a bait laced with a synthetic insecticide or pathogens.
12. The trap of claim 11, wherein the pathogens are members selected from the group consisting of bacteria, fungi, nematodes and microspora.
13. The trap of any one of claims 7 to 12, wherein the trap is used in conjunction with a light at an intensity of between 0.1-1.1 lux for the duration of overnight hours.

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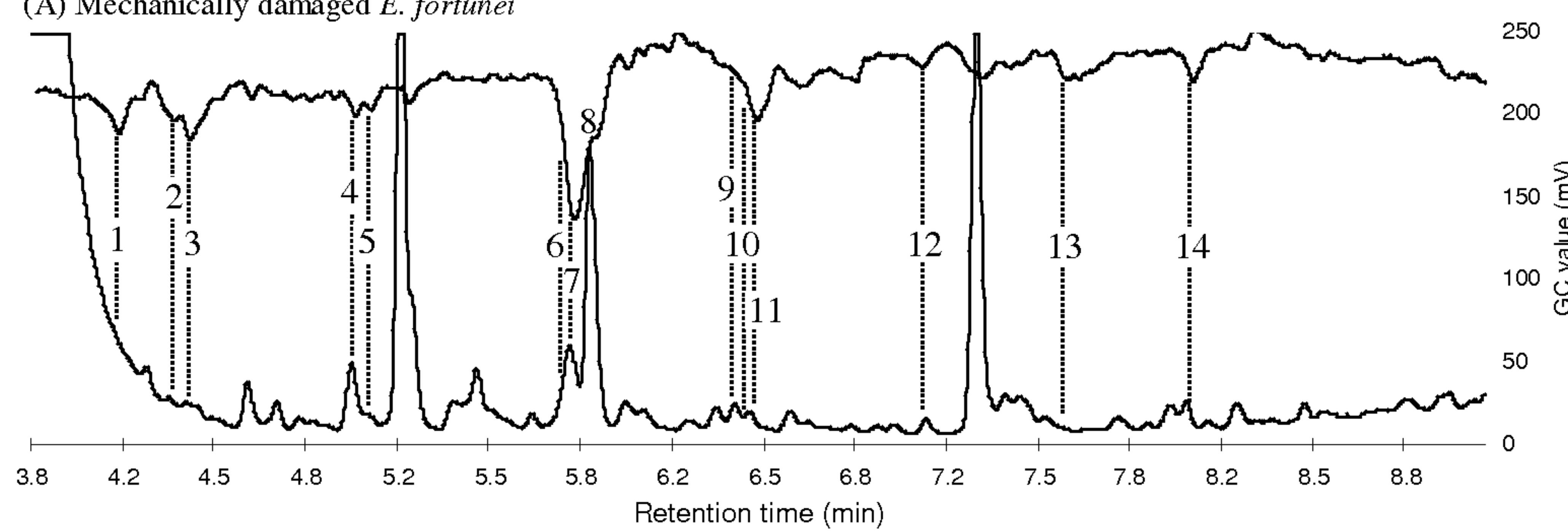
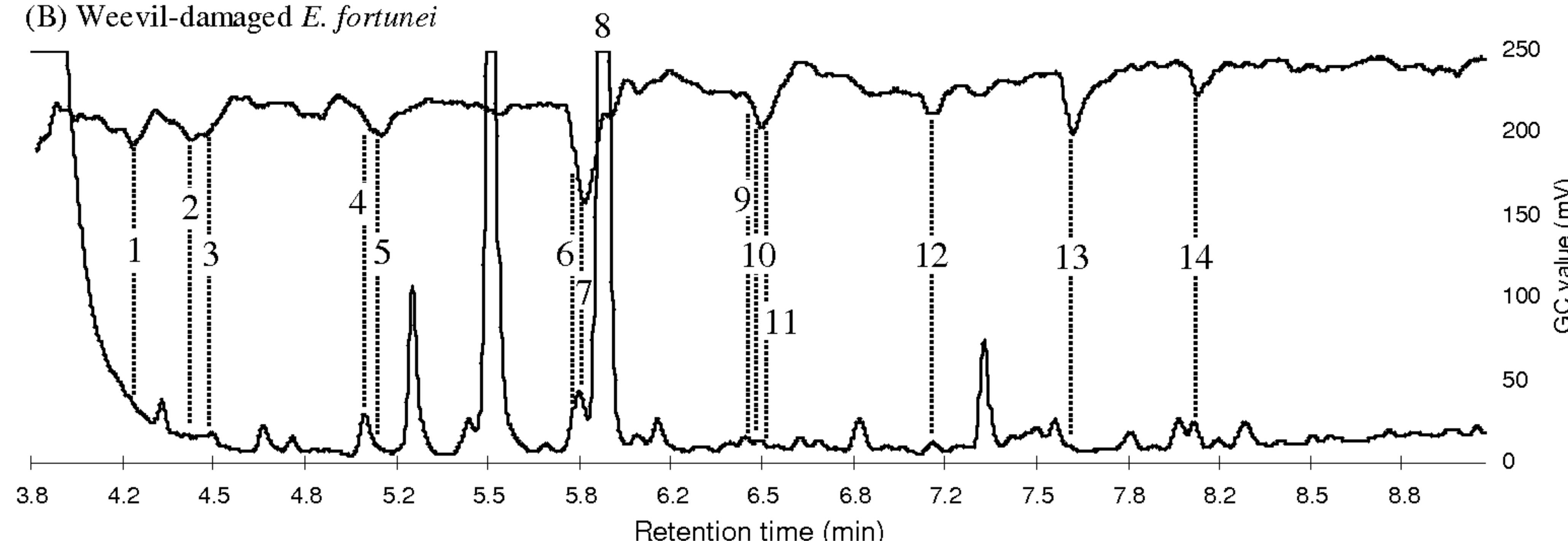
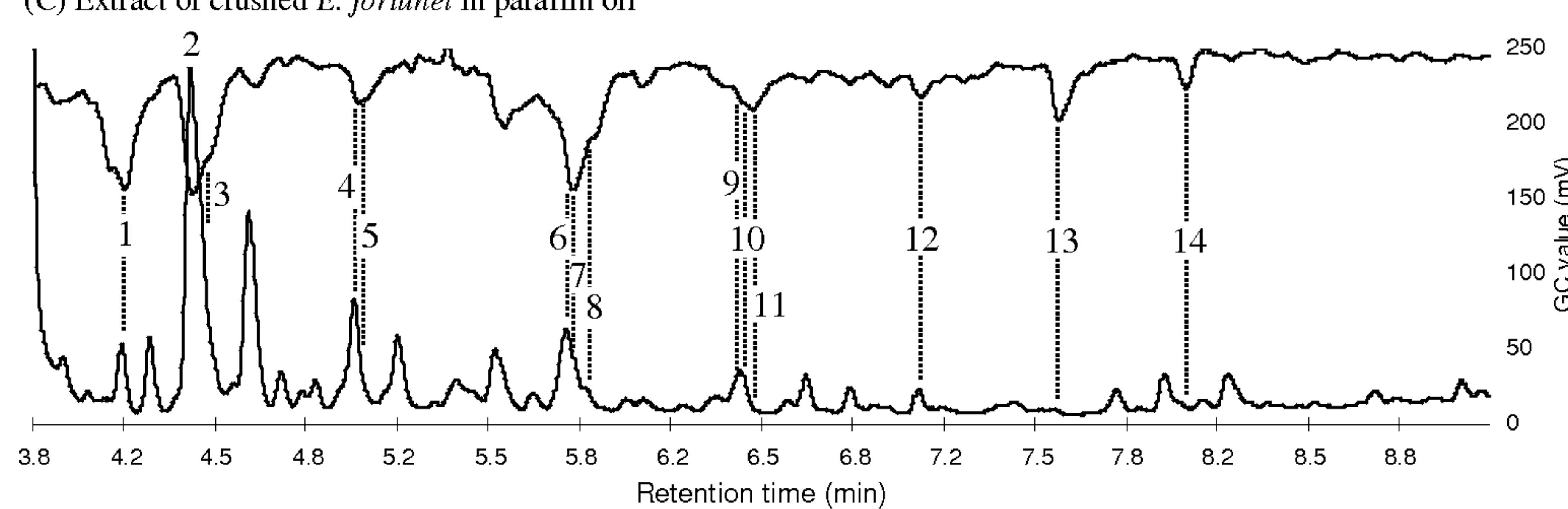
(A) Mechanically damaged *E. fortunei*(B) Weevil-damaged *E. fortunei*(C) Extract of crushed *E. fortunei* in paraffin oil

FIG. 1

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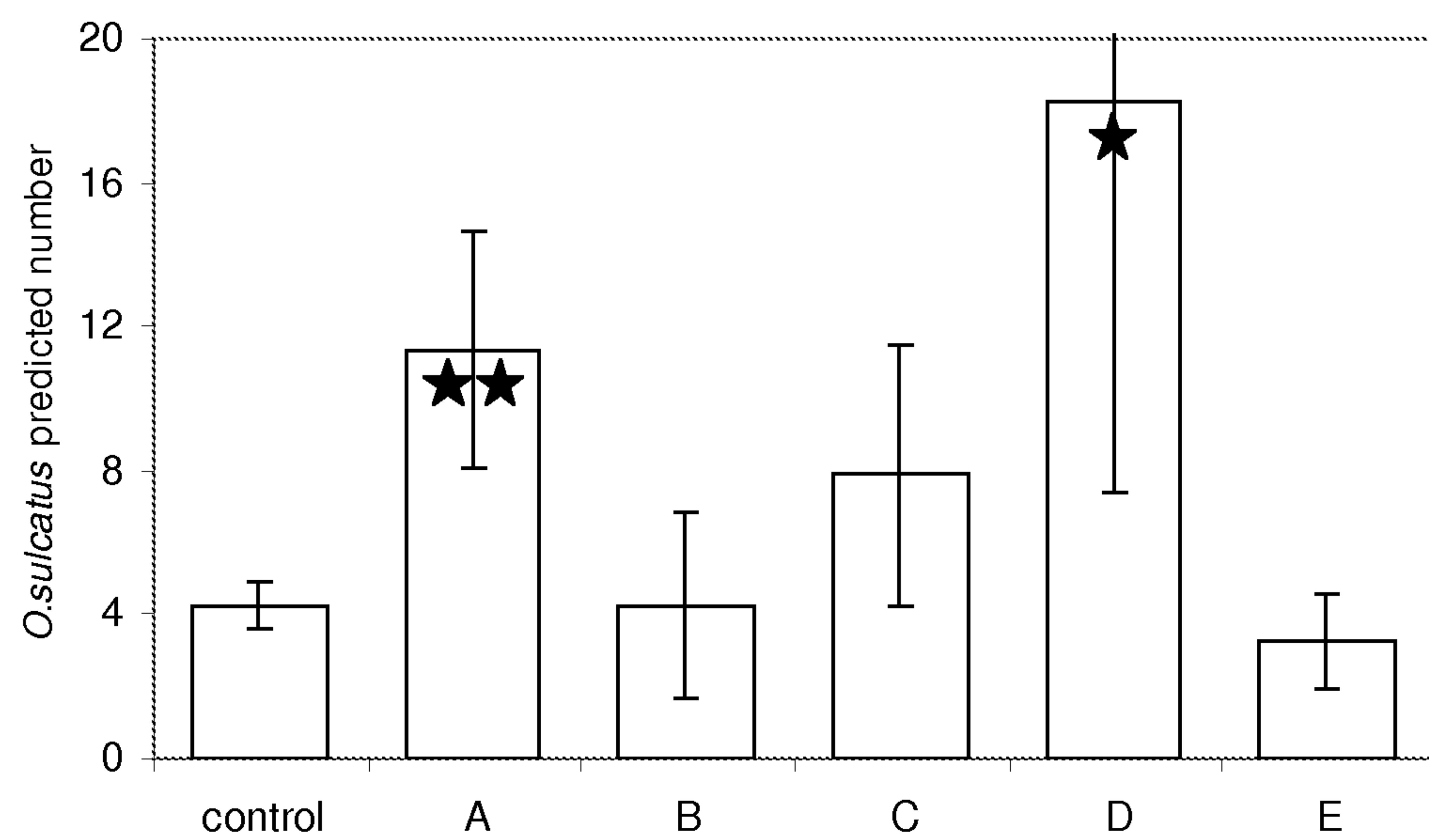


FIG. 2

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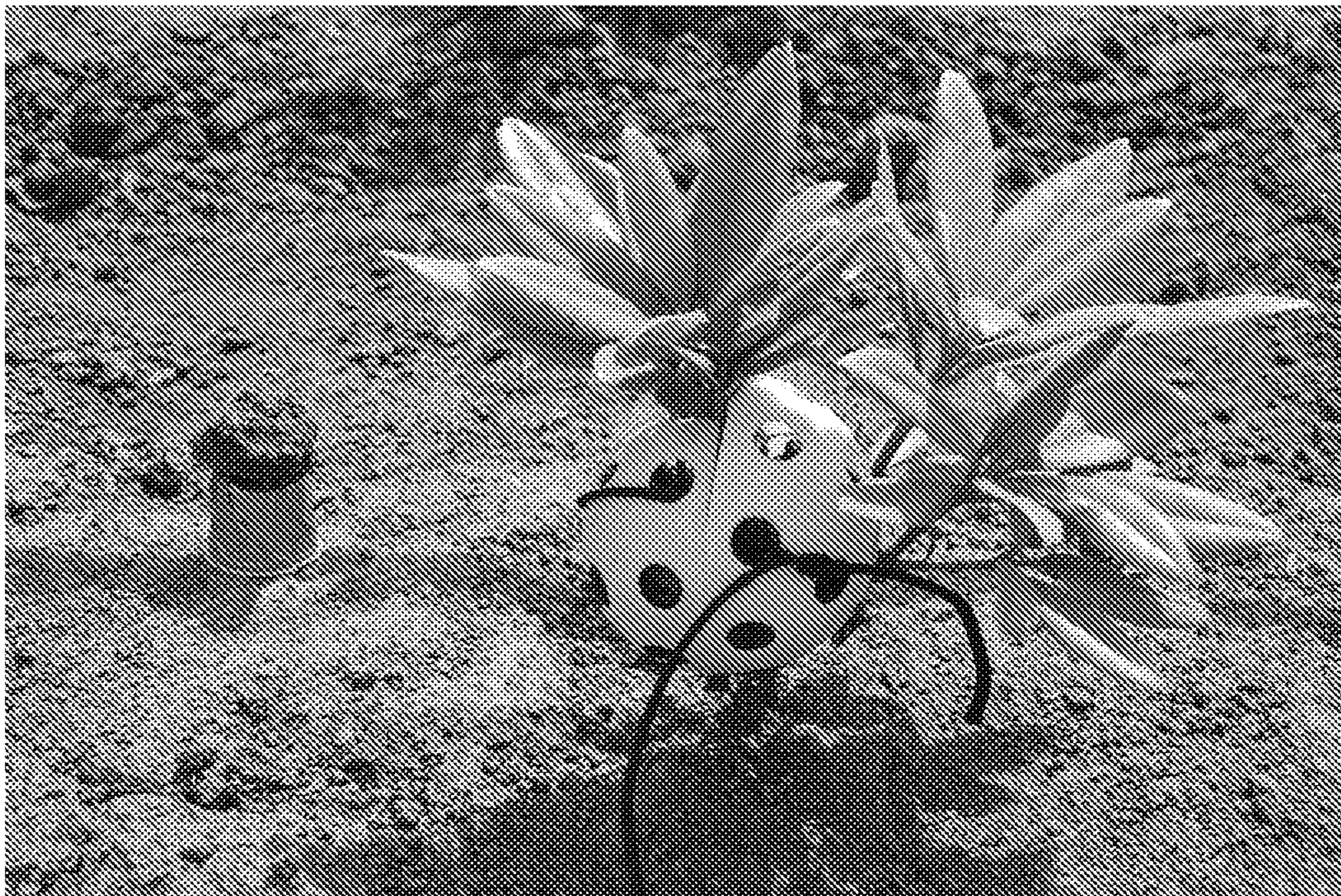


FIG. 3

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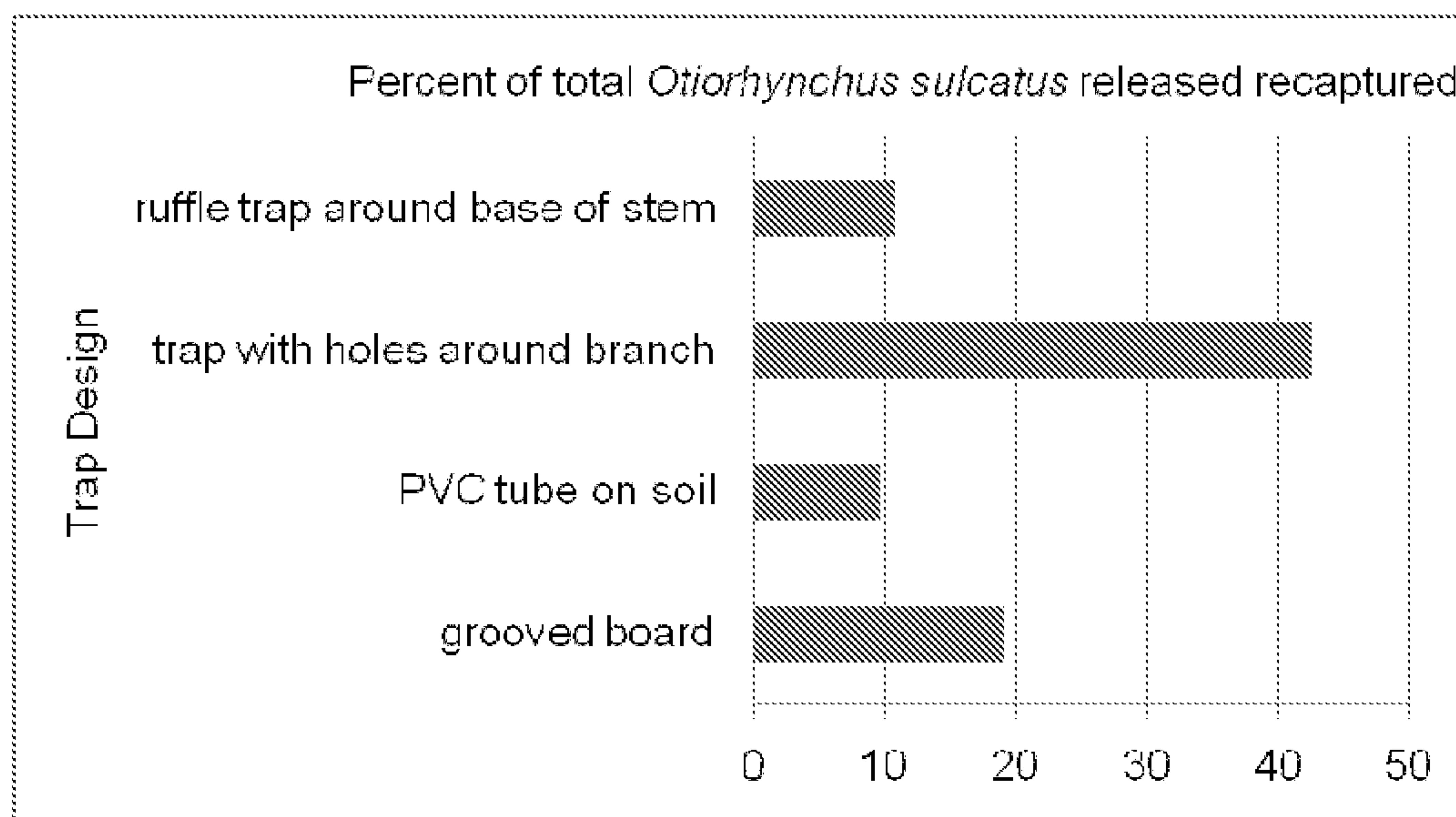


FIG. 4