NOVEL ECOLOGICAL AND SELECTIVE REPELLENT AGAINST RED PALM WEEVIL (RHYNCHOPHORUS FERRUGINEUS)

PATENTED TECHNOLOGY

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ABSTRACT

The Phytopathology research group of the University of Alicante has discovered two volatile organic compounds (VOC1 and VOC2) that act as repellents of the red palm weevil (*Rhynchophorus ferrugineus*) in a selective, specific and highly effective way. By incorporating these compounds into any formulation, biological control of the pest is achieved in a sustainable and environmentally friendly way.

Among the different sources for obtaining VOC1 and VOC2 are the entomopathogenic fungus *Beauveria bassiana*, as well as chemical synthesis, which allows a very economical production cost of VOC1 and VOC2. These compounds can be integrated in fast or slow release devices, and they have applications in agrobiotechnology, agriculture and horticulture, both for the biological control of adult red palm weevil insects, and to prevent infections in palm orchards or gardens with palm trees as ornamental species. Companies interested in acquiring this technology for its commercial exploitation are sought.

INTRODUCTION

The red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae), is one of the most serious pests of palm trees (especially date palms), as it has a high flight capacity, and it is able of infecting palms of great height and size, which entails a significant environmental and heritage cost.

The red palm weevil has an efficient search mechanism based on specialised chemical and mechanical receptors located on its antennae, which allow it to make an environmental assessment of its surroundings to ensure its survival and reproduction.

The chemoreceptors on the antennae are able of detecting a wide variety of volatile chemical compounds. These compounds alert the insect to the presence of food, mates, suitable egg-laying sites, and even hazards to avoid.

Volatile chemical compounds are molecules with different chemical structures (e.g. ketones, lactones, alcohols, esters, etc.) that occur as intermediates or end products in various metabolic pathways in any organism.

Fungi produce numerous volatile chemical compounds that are involved in different biological processes, such as biological control or communication between micro-organisms and their environment (insect-fungus biological interactions).

The existence of volatile organic compounds (VOCs) with both insecticidal and insect repellent activity has been described in the scientific literature.

Moreover, the profiles of these VOCs have been correlated with different levels of pathogenicity of the entomopathogenic fungi

Beauveria bassiana and *Metarhizium anisopliae* as biocontrol agents to reduce termite populations.

TECHNICAL DESCRIPTION

The present invention consists of two VOCs (hereinafter **VOC1** and **VOC2**), obtained from the entomopathogenic fungus *Beauveria bassiana*, although they can also be chemically synthesised, which have a **highly effective** and **specific use** as **repellents** of the **red palm weevil** (*Rhynchophorus ferrugineus*). This composition comprises, at least, one of these two VOCs.

Both VOC1 and VOC2 can be found in **solid, liquid, gel**, or even as part of a liquid **formulation impregnating a solid matrix**.

BEHAVIOURAL BIOASSAYS

Using a Y-tube olfactometer in which a control arm (no sample) was used against different stimuli: 1) uninoculated substrate; 2) substrate inoculated with the entomopathogenic fungus *Beauveria bassiana*; 3) fresh palm petioles. It was concluded that healthy adult females of the red palm weevil showed a significant repulsion towards the entomopathogenic fungus *Beauveria bassiana*, with respect to the control (*see Figure 1*).

The time of the visits of these red palm weevil females to the solid formulation of *Beauveria bassiana* was negligible, as 80% of the females chose the uninoculated substrate. Thus, it can be concluded that **female red palm weevils avoid the VOCs of the entomopathogenic fungus *Beauveria bassiana*** passively released into the air.

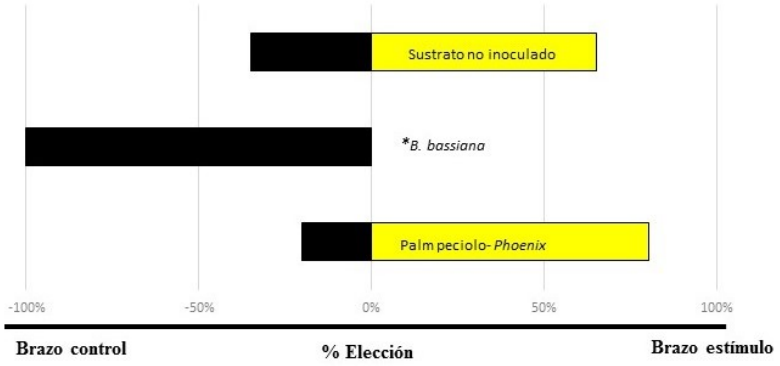

Figure 1: Response of Rhynchophorus ferrugineus females (%) in an olfactometer ("Y" tube) (10 minutes), when given a choice between ambient air (control), and olfactory stimuli (uninoculated substrate; entomopathogenic fungus Beauveria bassiana formulation; or palm fragments). N=20 individuals per trial.

ANALYSIS OF VOCS PRODUCED BY THE ENTOMOPATHOGENIC FUNGUS *Beauveria bassiana*

For the Y-tube bioassays, VOCs were separated in a Gas Chromatograph (GC) and identified by Mass Spectroscopy (MS). GC-MS analysis identified a total of 22 compounds from the *Beauveria bassiana* formulation. After performing a Venn diagram analysis to identify the VOCs that are only present in the *Beauveria bassiana* sample, VOC1 and VOC2 were identified as high and low volatile compounds, respectively (*see Figure 2*).

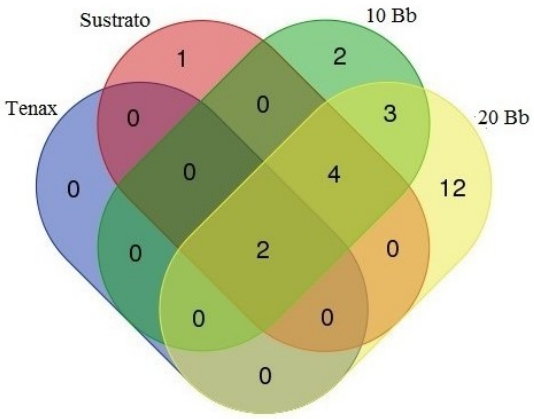

Figure 2: Venn diagram representing the VOCs identified by GC-MS in the samples: Tenax (blue); uninoculated substrate (red); 10 grams of substrate with Beauveria bassiana (green); 20 grams of substrate with Beauveria bassiana (yellow); 10 grams of substrate with Beauveria bassiana (green); 20 grams of substrate with Beauveria bassiana (green); 20 grams of substrate with Beauveria bassiana (yellow).

BEHAVIOURAL BIOASSAYS USING VOC1 AND VOC2

An experiment was carried out on **commercial samples** of **VOC1** and **VOC2** to measure the ability to generate repellency on the red palm weevil.

For this purpose, **two methods** were tested:

1) Free evaporation: *rapid release* of VOC1 and VOC2 by injecting a certain amount of these VOCs into the chemical stimulus arm of the Y-tube.

Adult female red palm weevils showed **repulsive behaviour** towards both VOC1 and VOC2 chemical stimuli when exposed to them for 10 minutes.

In both cases, females **immediately moved away from both chemicals** towards the control arm, and also **decreased their activity** at the end of the test.

2) Matrix impregnation: *slow release* of VOC1 and VOC2 by impregnating a certain amount of these VOCs into a matrix.

Adult female red palm weevils showed **repulsive behaviour** towards both VOC1 and VOC2 chemical stimuli when exposed to them for 10 minutes.

In both cases, females **moved away from both chemicals** towards the control arm (**repulsion effect**), and they **moved slowly** compared to the fast release technique.

ADVANTAGES AND INNOVATIVE ASPECTS

ADVANTAGES OF THE TECHNOLOGY

- Novel **selective** and **specific** treatment against the adult pest of the **red palm weevil** (*Rhynchophorus ferrugineus*).
- **Effective** and **efficient** compounds against this pest as a **repellent**.
- These are **natural compounds** for the **biological control** of the red palm weevil.
- A formulation can be developed that allows **effective** and **sustainable control** of the pest.
- One of the sources for obtaining the active ingredients (VOC1 and VOC2) corresponds to the **entomopathogenic fungus *Beauveria bassiana***, so it is an **environmentally friendly technology.**
- Another source of the active ingredients (VOC1 and VOC2) is chemical synthesis, so the **production cost** of these repellent compounds is **very low**.
- The composition **prevents the invasion** of the red palm weevil **in palm species** (palm orchards or other palm groves that are World Heritage Sites, and public or private gardens).
- Allows **large-scale application** and **dosage**.

INNOVATIVE ASPECTS OF THE TECHNOLOGY

A **novel red palm weevil repellent composition** has been developed based on volatile organic compounds detected in the entomopathogenic fungus *Beauveria bassiana*.

This invention is characterised by the fact that *it is not necessary to use the microorganism* in its entirety, but **simply two of its metabolites** (VOC1 and VOC2), which **simplifies the production process of the formulation**, since they can be obtained by chemical synthesis at a **very low cost**.

Moreover, it is an **environmentally friendly biological control** strategy **specific** to the pest *Rhynchophorus ferrugineus*.

CURRENT STATE OF DEVELOPMENT

Laboratory experiments using the entomopathogenic fungus *Beauveria bassiana* and healthy adult females of the red palm weevil (*Rhynchophorus ferrugineus*) **have been successfully carried out**. A **prototype is available for demonstration**.

From the experiments, it can be concluded that the **repellent effect** of VOC1 and VOC2 against adults of the red palm weevil is **more than 80%**.

The research group has the **knowledge**, **experience** and **technology** necessary to reproduce the experiment in a pilot study at **greenhouse** or **field level** to validate its real efficacy, as well as its technical and economic feasibility.

MARKET APPLICATIONS

This technology is framed in the field of **agrobiotechnology**, **agriculture** and **horticulture**. In particular, it concerns a novel composition containing volatile organic compounds as **repellents** of the **red palm weevil** (*Rhynchophorus ferrugineus*).

This invention finds its **application** in:

- **Biological control** of adult insects of the red palm weevil.
- **Prevention** of infections of red palm weevil in palm orchards or gardens.

COLLABORATION SOUGHT

It is looking for companies interested in acquiring this technology for **commercial exploitation** through **patent license agreement**.

INTELLECTUAL PROPERTY RIGHTS

The present invention is protected through **patent granted with examination**:

- *Title of the patent: "Compuestos orgánicos volátiles del hongo entomopatógeno Beauveria bassiana como repelentes de insectos".*
- *Application number: P201631534.*
- *Application date: 30th november, 2016.*

MARKET APPLICATION (3)

Agri-food and Fisheries
Molecular Biology and Biotechnology
Pollution and Environmental Impact