

NEW BIOLOGICAL CONTROL STRATEGY AGAINST BLACK WEEVIL (*COSMOPOLITES SORDIDUS*) IN BANANA CROPS

 TECNOLOGÍA PATENTADA

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RESUMEN

The *Phytopathology* research group of the University of Alicante has discovered seven volatile organic compounds (VOCs) naturally present in entomopathogenic and nematophages fungi that act as repellents of the black weevil (*Cosmopolites sordidus*) in a selective, specific and very effective way.

By incorporating these compounds, alone or in combination, into any formulation, the biological control of the black weevil plague is achieved in a sustainable and environmentally friendly way.

These VOCs can be obtained from fungi (*Beauveria bassiana*, *Metarhizium anisopliae*, *Pochonia clamydosporea*) or by chemical synthesis -which it enables a very economic production cost and, therefore, an interesting way of marketing worldwide-.

These compounds can be impregnated in slow release devices, and they have their direct application in the field of agrobiotechnology, agriculture and horticulture, both for biological control of adult black weevil insects and to prevent infections in banana crops.

It is looking for companies interested in acquiring this technology for commercial exploitation.



INTRODUCCIÓN

With a world production of 114 million tons per year, bananas are the most consumed and cultivated fruit in the world.

Among the main organisms affecting banana trees, the black weevil (*Cosmopolites sordidus*) stands out as the cause of the greatest economic losses in all areas of production, that is to say, it is the key pest of banana plantations with losses that can range between 30% and 90%.

The black weevil is an insect native to the Indo-Malayan region, although it is currently found in all the regions where banana is grown. For example, in Spain, it is mainly present in Canary Islands.

The black weevil obtains resources for its growth, development and reproduction thanks to antennas that function as specialised primary chemo-receptor and mechanoreceptor organs. These precise mechanisms of environmental assessment are crucial to ensure their survival and reproduction.

Antenna chiomireceptors are able of detecting volatile chemical compounds. These compounds generate alerts in the insect about the presence of possible pairs, food, suitable places to deposit eggs or dangers to avoid. Therefore, any chemical that can interrupt and/or modify the behavior of the black weevil in the search for the host (banana trees), provides a very useful tool for sustainable management.

Volatile organic compounds (hereinafter VOCs) are compounds with a carbonate base, in a solid or liquid state, which enter to gaseous phase by vaporization at room temperature (20°C).

Fungi, naturally produce and release a broad spectrum of VOCs with different chemical groups that perform essential ecological and physiological functions, including control or communication between microorganisms and their environment.

Fungal VOCs derive from primary and secondary metabolism and can be spread through the atmosphere and soil, thus acting as mediators in interactions between organisms.

The production of fungal VOCs is biologically dynamic, i.e. the profile of a particular species or strain varies according to substrate or nutrients, incubation time, temperature and other environmental parameters.

There is currently no integrated plan to control the black weevil: only pheromone traps are used to capture and count them, thus controlling potential outbreaks and eliminating overly infected plants, making this method inefficient and quite expensive.

Due to the high incidence of the black weevil on banana crops, and increased national and European restrictions related to the use of chemically synthesized insecticides for these pests, there is a need to develop new compounds derived from natural biological control agents to mitigate damage caused by the black weevil, as well as to eliminate the damage caused to the environment when chemically synthesized insecticides are used.

DESCRIPCIÓN TÉCNICA

The present invention solves the problems described above, as it provides **new volatile organic compounds** (VOCs) derived from primary and secondary metabolism of entomopathogenic and nematophages fungi to **specifically repel black weevil** (*Cosmopolites sordidus*).

Seven VOCs (C1-C7) have been described (see Figure 1), that alone or in combination, they have a high ability to selectively repel black weevil.

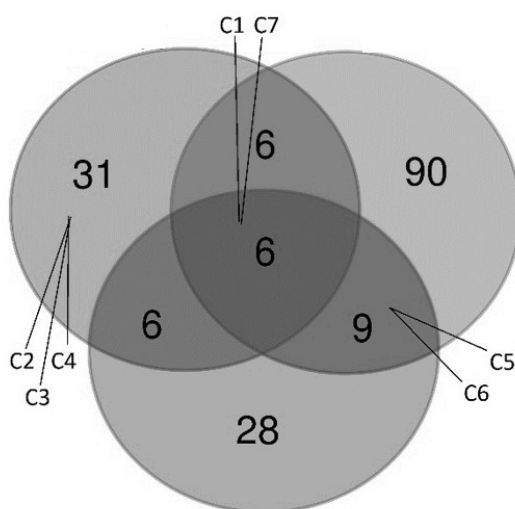


Figure 1: Venn diagram representing VOCs identified by GC/MS-SPME in the samples.

These compounds come from the following fungi:

- **Entomopathogens:** *Beauveria bassiana* and *Metarhizium anisopliae*.
- **Nematophagous:** *Pochonia clamydosporia*.

These VOCs are solid and/or liquid carbonated chemical substances that appear as intermediates or end products of the metabolic pathways of the fungi mentioned above, although they can also be obtained by **chemical synthesis**.

These compounds can be used in **solid, liquid** (impregnated in a matrix) or **gel form**.

ANALYSIS OF VOCs PRODUCED BY INVERTEBRATE FUNGAL PARASITES:

GC/MS (Gas Chromatography-Mass Spectrometry) with SPME (Solid Phase MicroExtraction) has been used to analyze both fungal samples and control.

BEHAVIORAL BIOASSAYS:

Two-way olfactometers (or "Y" tubes) have been made (see Figure 2) to evaluate the behavior of black weevil subjected to the

action of different olfactory stimuli (attractants and/or repellents).

Bioassays were performed by placing a black weevil in the center of the straight arm, and these had 10 minutes to move and choose, or not, a stimulus. Ten different bioassays (with a total of 120 healthy black weevil individuals in each bioassay) were conducted under different physiological (hunger vs. satiety) and environmental (light vs. dark) conditions using commercial samples of volatile organic compounds (C1-C7).

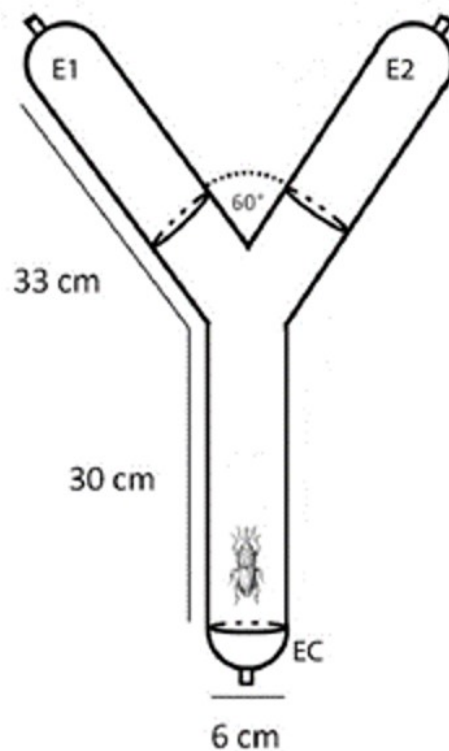


Figure 2: Diagram of the stimuli placed in the olfactometer. E1 represents the individuals who have chosen the attractants; E2 represents the individuals who have resorted to the repellent of the evaluated candidates or, alternatively, to the absence of stimuli; EC indicates the individuals who have chosen not to move.

With this test, the “movement indices” are obtained, and these allow to explain in a fast and simple way the behaviour of the black weevils in the tested laboratory conditions.

In this sense, nocturnal habit of the black weevil makes the insect more mobile. In addition, starvation condition stimulates the weevils in their search for food and gives them greater mobility.

CONCLUSION:

A total of **seven VOCs** (C1-C7) have been described that they have a **clear repellent action of the black weevil**, significantly decreasing its mobility respect to the control.

VENTAJAS Y ASPECTOS INNOVADORES DE LA TECNOLOGÍA

ADVANTAGES OF THE TECHNOLOGY

- New **selective** and **specific** treatment against black weevil (*Cosmopolites sordidus*) pest.
- It allows the execution of a **new control strategy** based on repellent properties.
- These are **natural compounds** for **biological control**.
- Source for obtaining these active principles (VOCs) come from metabolism of entomopathogenic and nematophagous fungi, so it is an **environmentally friendly technology**.
- Another source of VOCs is chemical synthesis, so the **cost of producing** these repellent compounds is **very economical**.
- A new formulation can be developed to **effective, efficient** and **sustainable control** of the pest.
- The formulation **prevents the invasion** of the black weevil in banana crops (*Musa sp*).
- These compounds allow **application** and **dosage** on a **large scale**.

INNOVATIVE ASPECTS OF THE TECHNOLOGY

A **novel repellent composition** for the black weevil of banana crops has been developed from volatile organic compounds

identified in entomopathogenic and nematophages fungi.

This invention is characterized because **it is not necessary to use the entire microorganism**, but simply **any of its seven metabolites** (VOCs), alone or in combination, which it simplifies the production process of the formulated, as they can be obtained by chemical synthesis at a **very low cost**.

In addition, it is an environmentally friendly **biological control** strategy **specifically** against the black weevil.

ESTADO ACTUAL DE LA TECNOLOGÍA

Experiments have been successfully carried out at **laboratory, greenhouse and field level**. To do it, entomopathogenic and nematophagous fungi have been used, as well as healthy adults of black weevil (*Cosmopolites sordidus*) collected on the island of Tenerife (Canary Islands).

Thanks to the experiments carried out, it can be concluded that the **repellent effect** of the VOCs (C1-C7) tested against the black weevil is **greater than 80%**.

APLICACIONES DE LA OFERTA

This technology is framed in the field of **agrobiotechnology, agriculture and horticulture**. In particular, it refers to a new formulation containing volatile organic compounds such as **black weevil repellents** (*Cosmopolites sordidus*).

Therefore, this invention finds its application in the **biological control and sustainable management** of adult black weevil insects in banana crops.

COLABORACIÓN BUSCADA

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

DERECHOS DE PROPIEDAD INTELECTUAL

This invention is protected through **patent application**:

- Title of the patent: "Uso de compuestos orgánicos volátiles de hongos parásitos de invertebrados como repelentes del picudo negro de la platanera (*Cosmopolites sordidus*)".
- Application number: P201930831.
- Application date: 25th September, 2019.

SECTORES DE APLICACIÓN (3)

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