Electroantennogram technique for rapid and convenient screening procedure as a new approach for the red palm weevil, ***Rhynchophorous ferrugineus***, semiochemicals

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1. Introduction

Date palms are commonly infested with various insect pests. The red palm weevil, ***Rhynchophorous ferrugineus*** F. is considered as one of the most destructive one. Although different methods of management have been applied against this serious insect pest, severely infested palms should be cut and burnt. The most important problem in this respect is the difficulty of discovering the early stages of infestation, which may lead to serious damage of infested palms before the appearance of visual symptoms. At such stage, pesticides do not give satisfactory results. Also, the attention to environmental contaminants such as pesticide residues in food and ground water were much of concern. Moreover, the tendency of insect resistance to pesticides rises as a serious problem in the field of insect pest control. With this background of concern, much research grants have been directed towards developing environmentally friendly alternatives such as: microbial pesticides, beneficial insects, hormonal pesticides, naturally occurring pesticides and semiochemicals.

The aim of the fore-mentioned information and techniques is to attract the attention of the research organizations responsible for red palm weevil control to invade such field of research. Establishing electrophsiological set up devote for studying the behavior and pharmacology of such pest, will lead definitely to much improvement in the way to cop with the dangerous problems caused by the red palm weevil.

2. Semiochemicals and its role in integrated pest management

2.1. Definition

Semiochemicals are known as the chemicals that involve in insect communication. They send a message that is transmitted as a signal in coded form to a receiver target insect. It is therefore is registered by sense organs and code is interpreted according to the context, providing a particular "**meaning**". As a result, the concept of channels of communication arises. It is similar to radio or television channels, with a narrow frequency band carrying different kinds of information.

2.2. Aggregation pheromone:

This pheromone is used for mass trapping of such insect pest. The attraction of this weevil has been found to be greatly enhanced when the aggregation pheromone is used in combination with host plant volatiles for mass trapping (Chinchilla ***et al.***, 1993; Oehlschlagar ***et al.***, 1993). Trap catch, in addition to being influenced by the design of the trap and attractant source, is affected by two other factors; trap placement (height, position and density) and the biology of the target pest.

2.3. Anti-oviposition or oviposition-deterrent pheromones:

These pheromones are known to present in various insects belonging to orders Lepidoptera and Diptera. They are also known as **epidietic** pheromones, in reference to their effects of reducing intraspecific competition. This pheromone is host-marking pheromone that is deterring other females of the same species from oviposition on the same fruit. A formulation of anti-oviposition pheromone of cherry fruit fly applied to whole cherry trees has been shown to reduce the number of fruits attacked by the same insect pest by up to 90% (Katsoyannos & Boller, 1980).

Oviposition-deterring pheromones have been also shown in other insect orders; e.g. the spotted cowpea bruchid, ***Callosobruchus maculatus*** (Messina ***et al.***, 1987). This field of investigation will be taken into consideration to reduce the oviposition patterns of the economic insect pests.

When the population of insects such as the bark beetles reaches a sufficiently high level, the attracted beetles begin to release an anti-aggregation pheromone that interrupt the response of other attacking beetles to the attractive semiochemicals. An obvious survival advantage to this mechanism might be resulted in by preventing the build up of beetles any particular tree and, in return, minimizing their reproductive capacity. Synthetic versions of these chemicals have potential as a means of preventing colonization of high value trees, protecting infested stands from attack or alternatively reducing infestation levels in stands that have already suffered some attack (Borden, 1989).

3. Proposed electrophysiological techniques as a powerful research tool:

Electrophysiology is a very powerful and direct method of measuring the aspects of the signal to which the insect response. In the study of pheromone communication, its value lies in identifying the communication channel. The most common assays of this field of study involve recording from the whole antenna or from individual sensory cells.

3.1. Electroantenno technique

Electroantennography (EAG) is considered the most practical form of electrophysiology. EAG can be practiced either with an excised antenna or with the antenna attached to head. Basically, the difference between potentials of antennal tip and base is recorded as a puff of odor passed over the antenna (Fig. 1). If antennal receptors are stimulated, their summed electrical activity is a slow DC depolarization of around 1-20 millivolts. The summed changes in potential of numerous receptor neurons, not the summed action potential, are recorded. The EAG potential is a relative measurement of the number of chemoreceptors stimulated by the presence of the odor molecules.

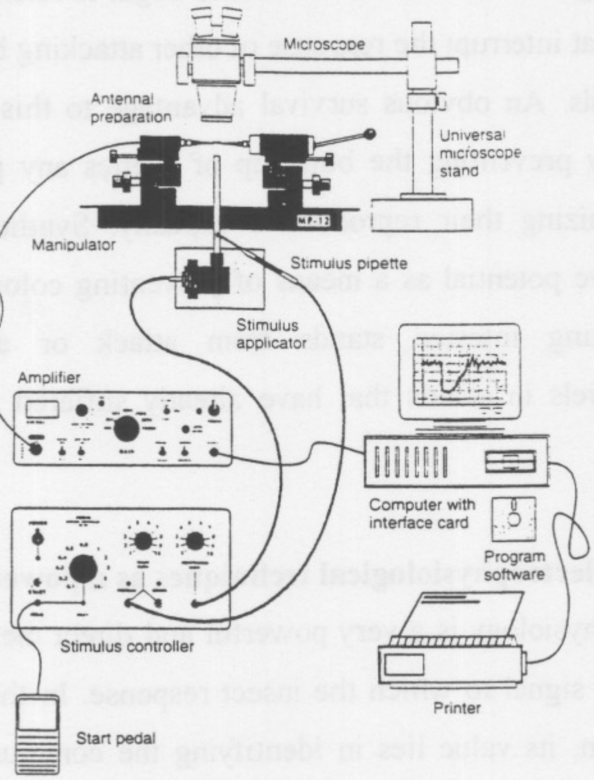

Fig. 1. An apparatus for recording EAGs. (From Howse *et al.*, 1998).

Antennal preparations are positioned between electrodes mounted on micromanipulators. Odor stimulus is delivered in a controlled air stream produced by an applicator containing a solenoid. The potential change between electrodes is amplified by a DC amplifier and the output is stored in a computer or fed into a suitable printer.

Positive peak is always associated with compounds, which are repellent in behavioral tests, while negative peaks are associated with attractants (depending on the concentration of such compounds). In general, the values obtained by EAG technique can rapidly test large numbers of compounds to eliminate those having no apparent effects on insects.

Techniques exist for coupling an EAG recording set-up with the output from a gas chromatography (GC). The gas eluting from the column is divided into two streams by a splitter (Fig. 2). One stream goes to the detector of the

GC and the other is introduced into a clear air stream, which is passed over the antenna. In this way, GC peaks can be correlated with EAG responses to determine the effectiveness of such compounds. Extracted mixed can, thus, be screened rapidly without the necessity for fractionization and separate testing of fractions. Peaks that provoke no electrical response can, therefore, be ignored. Alternatively, the GC can be coupled to a single-cell recording apparatus, or even to an actography for recording changes in the activity of insects (Hummel and Miller, 1984).

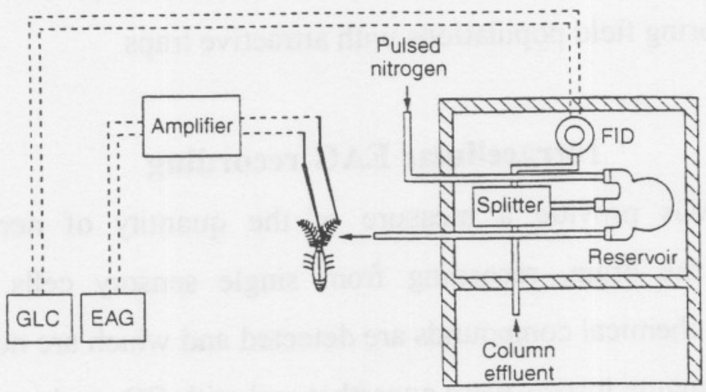

Fig. 2. Splitter assembly for coupled GLC-EAG. (From Howse ***et al.***, 1998)

Mobile EAG apparatus has been designed for use in the field or in wind tunnels as a sensitive means of detecting pheromone. Baker and Haynes (1989) used such an apparatus to monitor the detection odor filaments in a pheromone plume as a mounted male oriental fruit moth was moved upwind towards the pheromone source.

Electroantennographic (EAG) responses of the male pea moth, ***Cydia nigricana***, to a range of saturated and unsaturated straight chain alcohols and acetate were examined (Wall ***et al.***, 1976). Their manuscript provides further example of the value of the EAG technique in selecting synthetic compounds for field evaluation and has led to the discovery of two potent sex attractants, which can be used in field trapping (Lewis & Macaulay, 1976) before natural sex pheromone is identified. The authors concluded that there is a relation between EAG responses and field behavior. This agrees with the results of Roelofs & Comeau (1971) and Roelofs ***et al.***, (1971) who found a similar relation between EAG response and field behavior in ***Argyrotaenia velutinana*** (Walker) and ***C. pomonella***. The same results were given by Minks ***et al.***, (1973) on ***Adoxophyes arana*** (F. V. R.) and ***Clepsis spectrana*** Treitschke.

From the previous research activities, it is possible to produce large consistent catches of insects for several weeks and thus provides a practical basis for monitoring field populations with attractive traps.

Intracellular EAG recording

While EAGs provide a measure of the quantity of nerve impulses transmitted to the brain, recording from single sensory cells (SCR) tells precisely which chemical compounds are detected and which are not.

In this technique insects were anaesthetized with CO_2 and immobilized on a block of plasticine so that one antenna could be pinned out across the surface without damage. A glass microelectrode (resistance 10-20 MΩ) filled with physiological Saline was inserted through the cuticle of one of the first three segments and grounded, and a similar electrode, carried on a Bioelectric PAD 1probe, was carefully advanced until it penetrated the cuticle of the fourth, fifth or sixth segment from the tip of the antenna. The top electrode was considered correctly placed when the total resistance at the input had decreased to 35-50MΩ.

Conventional recording methods were used. The output from the probe and control unit was monitored with a pre-amplifier and oscilloscope; pen recorder with DC6 pre-amplifier provided a permanent record. The smallest detectable signal was about 0.1 mV (Baker and Haynes, 1989).

Insect selective toxins as biological control agents

Electrophysiological studies provided the researchers with new discoveries about the neurotransmitters of the nervous system (CNS). Such transmitters may be of great importance in pesticidal industry. Insect CNS has provided a major source of inspiration and a fair share of frustration for the industrial scientists which to exploit this tissue as a target for novel pesticides (Usherwood ***et al.***, 1980). This comment made, at the first **Neurotox' 79** (1979) symposium of the UK Society of Chemical Industry, remains true to this day. Since 1979, considerable progress has been made in understanding the properties of cholinergic synapses and acetylcholine receptors of insect CNS.

Now it is well known that glutamatergic synapses are present in insect CNS, although their functiona properties are still not well characterized (Usherwood ***et al.***, 1980; Giles and Usherwood, 1985). For example, many of the motor neurons innervate the selected musculature of the locust, ***Locusta migratoria*** also make synaptic connections with other neurons in the CNS of this insect. Since the motorneurons release L-glutamate at their neuromuscular synapses, it seems reasonable to assume that their control synaptic contacts are also glutamatergic (Sombati and Hoyle, 1984). Support for this assumption has been provided by the immuno-histochemical studies of Bicker ***et al***. (1988) and Watson (1988), in which L-glutamate antiserum was applied to sections of honeybee and locust (***L. migratoria***) CNS. The presence of immuno-reactivity was demonstrated not only in motorneurons but also in certain interneuron populations. The notion that L-glutamate is a central transmitter in insects received further support when this amino acid was iontophoresed onto regions in the locust (***L. migratoria***) metathoracic ganglion where motorneuron projections are located and the nerve cells, with which these motorneurons might be expected to synapse were depolarized (Sombati & Hoyle, 1984 and Dubas, 1991). A major difference between GluR of CNS and skeletal muscle may be the representation in the former of Kainite-sensitive GluR [Kain R], (Ultsch ***et al.***, 1992 and Wafford ***et al.***, 1992), which do not seem to occur peripherally in insect skeletal muscle (Daoud and Usherwood, 1975).

Neurobiologists in the industrial settings investigate the ways that insects differ from vertebrates and the ways that neural components of one particular insect species differ from another. When these differences are understood, the hope is to develop highly specific agents that will selectively target the pest species and only the pest species. Towards this end, insecticidal companies are using the toxins found in venom of animals that prey on the pests of row crops as probes for insect-specific differences. It is hopped that these differences are, what these differences may answer the following questions: how voltage and ligand-gated channels operate and how they are modulated.

One likely source of toxins that attack neural elements, which are specific to insects is the venom of arachnids and insect predators. These predators are able to evolve venom and one might expect this venom to contain toxins to be directed against targets. These toxins are insect-specific. The discovery of such toxins will help neurobiologists to understand and get better knowledge about the functional aspects of neuronal excitability as well as tools to protect crops from pest attack.

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