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Short communication. Efficacy of phosphine as a fumigant against *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) in palms

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Abstract

The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera, Curculionidae), the major pest of palms in the Mediterranean, has mainly spread through movement of infested plants. The aim of this work was to determine the possibility of using aluminium phosphide in palms as a safe quarantine treatment against this pest. Laboratory tests were carried out on eggs, young and old larvae, pupae and adults of *R. ferrugineus* with different doses of Eurofume® and mortality was assessed. Fumigations of infested canary palms were carried out in a hermetic container and subsequently palms were carefully dissected and all *R. ferrugineus* specimens found, either dead or alive, were counted. Results suggest that a dose of 1.14 g aluminium phosphide m^{-3} for 3 days is enough to kill all stages of *R. ferrugineus* in an infested palm tree. Provided that applied dose of aluminium phosphide is not phytotoxic for palms, this treatment could significantly reduce at low cost the enormous risks that palm imports suppose worldwide.

Additional key words: *Phoenix canariensis*, quarantine, red palm weevil.

Resumen

Comunicación corta. Eficacia de la fosfina como fumigante contra *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) en palmeras

El picudo rojo de las palmeras, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera, Curculionidae), la principal plaga de las palmeras en el Mediterráneo, se ha extendido básicamente a través de palmeras infestadas. El objetivo de este trabajo fue determinar la posibilidad de usar fosfuro de aluminio en palmeras como un tratamiento de cuarentena contra esta plaga. Se realizaron ensayos de laboratorio sobre huevos, larvas jóvenes, larvas maduras, pupas y adultos con diferentes dosis de Eurofume® y se evaluó la mortalidad. Se hicieron fumigaciones de palmeras canarias infestadas en un contenedor hermético y seguidamente se diseccionaron las palmeras y se contaron todos los *R. ferrugineus* vivos o muertos encontrados. Los resultados sugieren que una dosis de 1,14 g m^{-3} de fosfuro de aluminio durante 3 días es suficiente para matar a todos los estados de *R. ferrugineus* en una palmera infestada. Si la dosis de fosfuro de aluminio a aplicar no resulta fitotóxica para las palmeras, este tratamiento podría, a bajo coste, reducir significativamente el enorme riesgo que supone la importación de palmeras en todo el mundo.

Palabras clave adicionales: cuarentena, picudo rojo de las palmeras, *Phoenix canariensis*.

Currently, the invasive weevil *Rhynchophorus ferrugineus* (Olivier) (Coleoptera, Curculionidae) is the major pest of palms in the Middle East and the Mediterranean (Faleiro, 2006; Llácer *et al.*, 2009) and may become a serious pest in the Americas (EPPO, 2009). This species feeds on soft succulent tissues of most Palmaceae but *Phoenix canariensis* Hort. ex Chabaud palm is the preferred host of *R. ferrugineus* in the

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Abbreviations used: a.i. (active ingredient), EU (European Union), RH (relative humidity).

Mediterranean Basin (Dembilio *et al.*, 2009a). Larvae are the most destructive stage of the weevil as they can penetrate deep in the stem damaging its internal tissues. Up to three complete generations can take place inside one single adult palm (Abe *et al.*, 2009; Dembilio *et al.*, 2009b) and this process can take as long as one year. Only at the end of this period, external symptoms become obvious and the palm eventually dies.

An important problem associated to *R. ferrugineus* is the difficulty of detection of the early phases of infestation (Nakash *et al*., 2000). Because recently infested palms can be easily mistaken as pest-free, this species has been passively spread by inadverted movement of infested plants. This situation has lead to the extremely fast dispersal of *R. ferrugineus* along the Mediterranean Basin in the latest years (EPPO, 2008). In 2007 the European Union (EU) established emergency measures to prevent the introduction into and the spread within the Community of this pest (OJ, 2007). However, these measures did not include any quarantine treatment. Such a treatment routinely applied to imported palms could dramatically improve the control of this pest.

The main procedures for eliminating arthropod pests from a commodity are classified broadly as chemical and physical treatments. Chemical treatments include fumigants, such as methyl bromide (CH_3Br) and phosphine (PH_3) that penetrate the commodity and are toxic to pests. Methyl bromide was the most commonly used postharvest and quarantine treatment worldwide. However with the phasing out of ozone-depleting substances in accordance with the United Nations Montreal Protocol it is currently forbidden as a postharvest treatment and its use will be not permitted after March 2010 in the EU, even for quarantine treatments (OJ, 1991). Fumigation with phosphine will continue for the immediate future as an important and economically viable fumigant for widespread use against insect infestation (Bell, 2000; Donahaye, 2000).

Solid preparations made of aluminium phosphide are commonly used for phosphine fumigation of farm stored grains and tobacco (Imai and Harada, 2006; Daglish and Pavic, 2008). When aluminium phosphide comes into contact with the moisture in the air, it releases PH_3 toxic gas. Since PH_3 diffuses rapidly and penetrates deeply into materials, it may be a useful tool to control *R. ferrugineus* inside its palm hosts. Indeed, Rao *et al.* (1973) and Muthuraman (1984) successfully used slow release phosphine tablets placed in sealed holes in the palm stipe against *R. ferrugineus*. However, such a use is considered unsafe and is not allowed under EU regulations. In recent years, imported palms in containers arriving at the Port of Valencia (Eastern Spain) have been sometimes subjected to a phosphine treatment as a safeguard measure against quarantine pests such as *R. ferrugineus*. These palms were inspected months later in the nursery and no phytotoxic effects were detected (J. Roca, Roca Defisan S.L., personal communication). However, the effectiveness of these treatments, if any, has never been measured. Therefore, the aim of this work has been to determine the possibility of using aluminium phosphide as a safe quarantine treatment against *R. ferrugineus* in palms.

The assays reported in this study were carried out at the Institut Valencià d'Investigacions Agràries (IVIA) with the commercial product Eurofume® (a.i. 57% aluminium phosphide in 0.6 g pellets, Roca Defisan S.L., Alboraia, Spain). This product can be used against rodents and insects which infest stored commodities (MARM, 2009).

Experimental insects: Laboratory tests were carried out on eggs, young and old larvae (15- and 60-d old, respectively), pupae and adults of *R. ferrugineus*. To obtain enough individuals of these stages, wild adults were captured in the province of Valencia in traps baited with ferrugineol (male aggregation pheromone) and plant kairomones (ethyl acetate and pieces of palm fronds). These adults were kept in plastic boxes and offered thin slices of red apple (*Malus domestica* Borkh.) cv. 'Starking delicious' both as food and as oviposition substrate. Wild adults were replaced monthly. After hatching neonate larvae were reared on an artificial diet (Martin and Cabello, 2006) until they reached the development stage required for our assays. To promote cocoon formation, 70-d old larvae were individually introduced in 100 mL vials half filled with esparto grass (*Stipa tenacissima* L.) fibers. All of individuals were reared in a climatic cabinet at 25 ± 2°C; 80 ± 10% RH and a photoperiod of 16:8 (L:D).

Laboratory assays: Treatments were performed in plastic 1 m^3 cube-shaped hermetic containers located inside a climatic cabinet at 25 ± 2°C and 80 ± 10% RH Eurofume® was applied at doses 0.0, 0.2, 1.0, 1.6 and 2.0 g Eurofume® m^{-3}. Immediately after the application, containers were sealed and left undisturbed for three days. This time was established based on the experience of the manufacturer with other commodities. Starting with the lowest dose, four replicates of 10 individuals per dose and stage were considered. A control treatment was included in each test. Eggs and larvae of each replicate were kept together in either 50 mL vials or

250 mL plastic boxes, respectively. In both cases, the containers were half filled with *R. ferrugineus* artificial diet. Both pupae and adults were individually kept in 100 mL vials. Phosphine concentration was monitored during the assays with a fumiscope gas analyzer (Spectros PM200, Spectros Instruments, Inc., Hopedale, MA, USA). Upon completion of the exposure time, the containers were opened and ventilated for 3 additional days. Subsequently, *R. ferrugineus* mortality was assessed. Specimens were considered dead when no movement was observed after gently shaken with a fine brush. Percent mortality was corrected (Abbott, 1925), transformed into probits and when possible the corresponding probit line fitted (LeOra Software Inc.). A chi-square test was used to prove the goodness-of-fit.

Assays with palms: Sixteen presumably infested canary palms (*Phoenix canariensis* Hort ex. Chabaud) obtained from infested foci in the province of Valencia were appropriately transported to IVIA. These palms (n = 16) had a crown 0.85 ± 0.35 m high (mean ± SEM) and 1.25 ± 0.20 m in diameter. Each palm was considered as a replicate. Treatments were carried out in a 33.20 m^3 hermetic container (6 × 2.4 × 2.6 m) heated by a hydraulic system to 27.5 ± 2.5°C. Palms were individually introduced into the container. Eight of them were exposed to a dose of 2.0 g m^{-3} Eurofume® for 72 h. The other eight palms were left undisturbed within the container for 72 h and used as a control. On completion of the exposure, the container was opened and ventilated. Subsequently, palms were carefully dissected and inspected for the presence of *R. ferrugineus*. All specimens found, either dead or alive, were counted.

Percentage egg hatching in control boxes was 73.4 ± 8.2 (n = 20), a similar rate (78.8 ± 4.9) (n = 4) was obtained by the same authors in another assay (Llácer *et al*., 2010). All control neonate, 15-d and 60-d old larvae, pupae and adult were alive (0% mortality) when inspected after the phosphine treatment (Fig. 1). Fifteen-d old larva and egg were the most susceptible stage tested and 0.2 g m^{-3} Eurofume® (equivalent to a mean concentration of 96.4 ± 3.8 mg L^{-1} of PH_3 during the 3-d exposure period, as measured with the fumiscope) were enough to cause 100 and 98.1% larval and egg mortality, respectively, which were not significantly different. These stages were followed by adults as 100% mortality was observed at 1.0 g m^{-3} Eurofume® (equivalent to 813.4 ± 24.5 mg L^{-1} PH_3). Sixty-d old larvae required at least 1.6 g m^{-3} Eurofume® (1,040.5 ± 37.2 mg L^{-1} PH_3) for complete mortality. Mortality data for 60-d old larvae could be satisfactorily fitted to a probit line

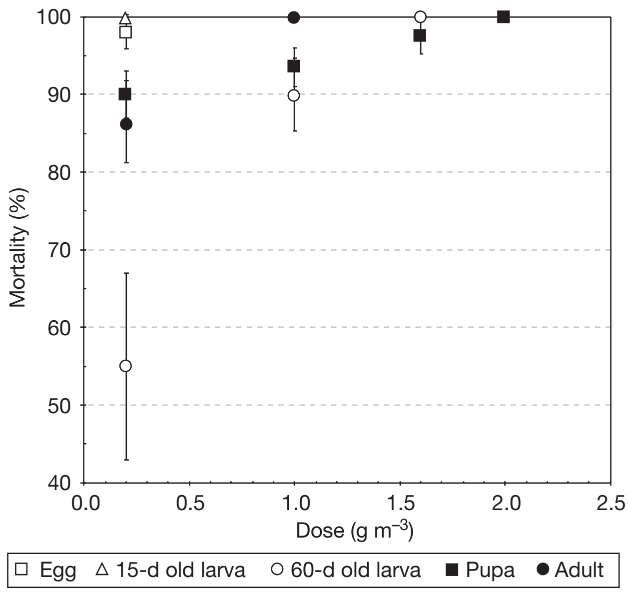

Figure 1. Mortality (%) of *Rhynchophorus ferrugineus* at different developmental stages when exposed to different doses of Eurofume® (a.i. 57% aluminium phosphide) for three days.

and a LC_{99} of 1.790 g m^{-3} Eurofume® could be estimated (95% fiducial limits FL = 1.272 – 2.975 g m^{-3} Eurofume®; mean = 2.378 ± 0.345; $\chi^2 = 4.661$; index of significance for potency estimation $g = 0.08101$). The pupa was the least susceptible stage tested and complete mortality was observed only at 2.0 g m^{-3} Eurofume® (1,624.9 ± 57.6 mg L^{-1} PH_3).

The sixteen palms used in the assays were infested and all developmental stages were present (means of 12.94 ± 7.25, 28.88 ± 10.72 and 78.00 ± 37.19 specimens per palm for adults, pupae and larvae, respectively). The eight palms treated with PH_3 had from 4 to 581 specimens (mean 89.25 ± 70.71) and control ones from 5 to 607 (mean 150.38 ± 70.48). These values were not significantly different ($F = 0.37$, $df = 1, 14$; $P = 0.5502$). All insects found in treated palms (714 in total) were dead after the treatment. Only 15.3 ± 10.8% of the specimens in control palms (1,203 in total) were dead. Therefore efficacy was 100%.

Pupae and eggs of grain pests are often the most difficult stages to kill with PH_3 (Annis, 2000). Likewise, pupae of *R. ferrugineus* required the highest dose tested for complete mortality.

Our results suggest that a dose of 2.0 g m^{-3} Eurofume® (1.14 g m^{-3} of aluminium phosphide) for 3 days is enough to kill all stages of *R. ferrugineus*. This dose coincides with the standard dose used for food commodities, although in that case application time is 7 days

(Reddy *et al.*, 2007), but it is lower than that used by Muthuraman (1984) in date palms against *R. ferrugineus* (1.71 g aluminium phosphide per palm). Apart from the evidence from the palms exposed to PH_3 in the Port of Valencia, there is no information on the tolerance of palms to PH_3. When present, phytotoxic effects in plants seem to be caused by ammonium carbonate or urea impurities in the formulated product rather than by the fumigant itself. Pure PH_3 applications (700-3,500 mg L^{-1}) can control the main pests of fresh fruit without damaging fruit quality (Horn and Horn, 2004). Similarly, tests carried out on tomato and poinsettia plant cuttings (MacDonald and Mills, 1995) with 2 mg L^{-1} of phosphine and 2-36 h of exposure time showed no detrimental effects to plants. Although specific trials aimed at ascertaining the possible phytotoxic effects of PH_3 on palms are needed before PH_3 fumigation can be recommended, the results from the palms exposed to PH_3 in the Port of Valencia are indicative that this treatment could be feasible. In this experiment, the use of a PH_3 fumigation applied to infested canary palms produced complete mortality of *R. ferrugineus*. Provided that a dose of 1.14 g m^{-3} of aluminium phosphide for 3 d is not phytotoxic for palms, such a treatment could be easily applied in sealed containers used to prepare and ship palms overseas. Its application would result in a low cost treatment, but could significantly reduce the enormous risks that palm imports suppose at this moment worldwide.

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