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SHORT COMMUNICATION

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Short and long-term efficacy and phytotoxicity of phosphine against *Rhynchophorus ferrugineus* in live *Phoenix canariensis* palms

Óscar Dembilio and Josep A. Jaques*

Universitat Jaume I (UJI). Unitat Associada d'Entomologia Agrícola UJI-IVIA, Departament de Ciències Agràries i del Medi Natural, Campus del Riu Sec. 12071, Castelló de la Plana. Spain ** Formerly J. A. Jacas*

Abstract

The red palm weevil, *Rhynchophorus ferrugineus*, is a palm borer native to South Asia which has spread mainly due to the unintended movement of infested planting material. As a result, this species has become the most destructive palm pest in the world. The difficulty of detecting the early stages of infestation due to its cryptic life cycle has led many countries to implement, strict pre- and post-entry quarantine regulations to prevent further spread. However, there are no quarantine protocols to ensure that palm material for planting is free of *R. ferrugineus*. The aim of this study has been to determine the efficacy of aluminium phosphide as a safe quarantine treatment against different stages of *R. ferrugineus* and the possible phytotoxic effects on live *Phoenix canariensis* palms. Our results confirm that a dose of 1.14 g/m^3 for 2 days is enough to kill all stages of *R. ferrugineus* in live palms with no phytotoxic effects on treated palms for up to one year after the treatment. This procedure, which could be easily applied in sealed containers used for palm trade, could drastically reduce risks associated to palm movement worldwide.

Additional key words: red palm weevil; Canary Islands date palm; quarantine; aluminum phosphide.

Abbreviations used: EU (European Union); RPW (Red Palm Weevil).

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Correspondence should be addressed to Ó. Dembilio: odembili@uji.es

The invasive red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), has become the most destructive pest of palms in the world (Dembilio *et al.,* 2014). This species native to Southeast Asia and Melanesia, has enormously increased its geographical range to the Middle East, the Mediterranean Basin, and the Caribbean in the last decades as the result of multiple unintended introductions through planting material (EPPO 2008, 2009; Rugnam-Jones *et al.,* 2013). This borer has been reported on 26 palm species belonging to 16 different genera (Dembilio *et al.,* 2009; Malumphy & Moran, 2009). During this colonization, *R. ferrugineus* has become a lethal pest for *Phoenix canariensis* Hort. ex Chabaud, an endemic palm to the Canary Islands commonly used as ornamental worldwide. A serious problem coupled with *R. ferrugineus* is the difficulty of detecting the early stages of infestation due to its cryptic life cycle (Dembilio & Jacas, 2011). As a consequence, strict pre- and post-entry quarantine regulations to prevent further spread have been put in place by affected countries. For instance, the European Union (EU) requires complete physical protection for palms imported from infested areas during one year depending on the origin of the palms subsequent to their arrival into the EU. Only when these palms prove healthy during this confinement period, palm movement within the EU is permitted (OJEU, 2007, 2008, 2010). However, the efficacy of this procedure remains controversial and would greatly increase if accompanied by a suitable quarantine treatment. Yet, a quarantine protocol to ensure that palms for planting are free of *R. ferrugineus* is not available with one exception. A report from Saudi Arabia (Al-Shawaf *et al.,* 2013) recommends dipping date palm offshoots in 0.004 % fipronil for 30 min before transporting to ensure complete mortality of the hidden larval stages, if any, and to complete certification and transport of the treated offshoots to the new planting site within 72 h. However, this treatment cannot be applied to *P. canariensis* as it does not multiply by offshoots. Furthermore, fipronil is currently registered for some uses in the EU different from those indicated by those authors because of its effects on bees (MAGRAMA, 2015a).

Llácer & Jacas (2010) set the basis for developing a quarantine protocol against *R. ferrugineus* using phosphine as a fumigant. These authors demonstrated that phosphine fumigation diffuses quickly and penetrates deeply into recently cut *P. canariensis* palm crowns and a dose of 1.14 g aluminium phosphide/m^3 for 3 days was enough to kill all stages of *R. ferrugineus*. However, these authors did not report possible phytotoxic effects from phosphine and evidence from additional studies with small potted plants of different palm species (*Chamaerops humilis*, *P. canariensis*, *P. dactylifera*, *Trachycarpus fortunae* and *Washingtonia* spp.) showed that this type of damage could occur (same authors, unpublished results). These effects, which seem to be caused by ammonium carbonate or urea impurities in the formulated product rather than by the fumigant itself, are not rare when phosphine is used in fresh vegetables (Horn & Horn, 2004). As a consequence posphine has not been widely applied for fumigation of fresh commodities (Llácer & Jacas, 2010; Zhang *et al.,* 2012). As aluminium phosphide, which is not currently registered as a fumigant in EU but still authorized in Spain as a molecide and insecticide against stored insect pests in Spain (MAGRAMA, 2015a), is one of the few fumigants commercially available, we decided to evaluate whether reduced exposure time to the same dose of phosphine used by Llácer & Jacas (2010) could still result in complete mortality of *R. ferrugineus* with no phytotoxic effects on palms up to one year after the treatment.

Experiments were carried out at Institut Valencià d'Investigacions Agràries (IVIA, Montcada, Spain) in 2013 and 2014. Commercial 8-year-old potted *P. canariensis* palms were used in our assays. The stipe of these palms was around 45 cm high and 40 cm wide. They were planted in 50-L containers and were watered every other day. These palms were conveniently enclosed together depending on the treatment (see below: control, presumably infested, subjected to fumigation) in separate cages in a mesh house.

Adult weevils collected in the province of Valencia in traps baited with ferrugineol (Pherosan Rhinchoforus®, *R. ferrugineus* aggregation pheromone) and plant kairomones (ethyl acetate and pieces of palm fronds) were used to infest palms (Dembilio *et al.,* 2009).

The commercial product Gastoxin-B® (a.i. 57 % aluminium phosphide in 0.6 g pellets, Roca Defisan S.L., Massanassa, Spain), which is registered in Spain against rodents and insects infesting stored commodities (MAGRAMA, 2015b), was used. We started our experiments with the same dose as Llácer & Jacas (2010) (2.0 g/m^3 Gastoxin-B®) and reduced exposure time from 72 to 48 h, the shortest treatment time according to technical advice. Because of the results obtained (see below), we did not study any additional exposure time. The treatment took place in a 33.20 m^3 hermetic container (6 × 2.4 × 2.6 m) heated by a hydraulic system to 25.0 ± 2.6 °C. The container was carefully sealed to avoid gas leaks. After exposure, the container was opened and ventilated.

Assays targeting *R. ferrugineus* larvae. Twelve palms were used in each assay, which was repeated three times (starting in June, July and September 2013, and finishing one year later). For each assay, nine palms were infested by releasing four adult *R. ferrugineus* (three females and one male) per palm for one week. We will refer to these palms as "RPW-exposed" (Red Palm Weevil-exposed). The remaining three palms per assay will be referred to as "healthy". Fourteen days after infestation, six presumably infested (likely containing larvae of different ages up to the VII larval instar; Dembilio & Jacas, 2011) and three healthy palms were moved to the hermetic container for fumigation. The three remaining RPW-exposed palms were kept in the same mesh enclosure where infestation had taken place as a control. On completion of phosphine exposure, three RPW-exposed treated and three control treated palms were carefully dissected and inspected for the presence of *R. ferrugineus* (Fig. 1). These numbers were compared using a *t*-test. The remaining three RPW-exposed treated and three healthy palms were kept in the mesh enclosure for one extra year where they were regularly checked for symptoms of phytotoxicity (burnt and discolored fronds, dead growing tip) and infestation. At the end of the assay, one year after exposure to phosphine, these palms were also dissected and checked for presence of *R. ferrugineus*.

Assays targeting *R. ferrugineus* pupae. Following the same procedure as before, infested palms were treated 50 days after infestation to make sure that *R. ferrugineus* pupae were present in the palms when treated. As the objective of this assay was to check efficacy against pupae and phytotoxic effects had already been assessed in the previous assays, only three RPW-exposed and three control palms were used per assay. The assay was also repeated three times in August, September and October 2014 and finished

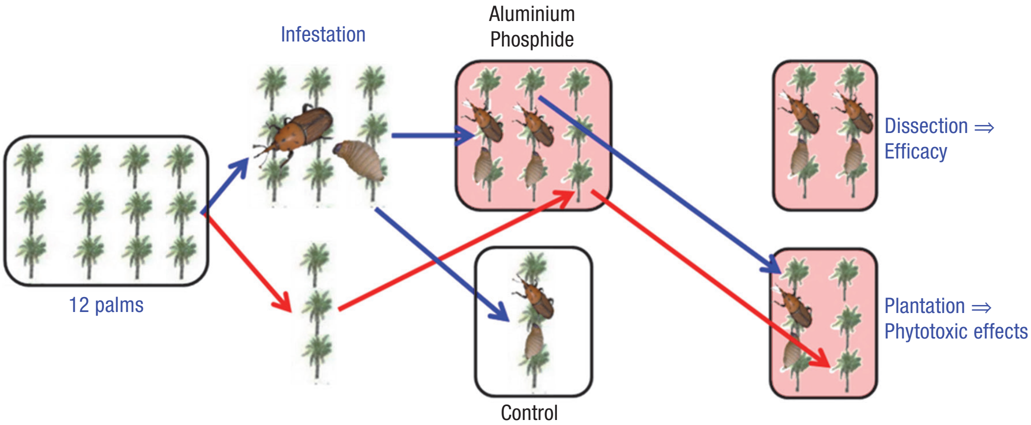

Figure 1. Diagram representing the experimental process on the efficacy of phosphine against the RPW and its possible phytotoxic effect on *P. canariensis* palms. This assay was repeated 3 times.

when palms were dissected after exposure to phosphine.

Assays targeting *R. ferrugineus* larvae. The three RPW-exposed palms per assay exposed to phosphide for 48 h had from 2 to 17 larvae (mean 8.7 ± 5.0; n = 9) and the three RPW-exposed control ones had from 2 to 23 (mean 10.1 ± 7.0; n = 9). These values were not significantly different (t = 0.503; p = 0.622). All larvae in treated palms (78 in total) were dead after the treatment whereas no larval mortality was observed in control palms. Therefore efficacy against larvae was 100%.

Assays targeting *R. ferrugineus* pupae. The three RPW-exposed palms per assay exposed to phosphide for 48 h had from 4 to 9 pupae (mean 6.5 ± 1.7; n = 9), the same as RPW-exposed control ones (mean 7.1 ± 1.7), as these values were not significantly different (t = 0.702; p = 0.493; n = 9). All pupae in treated palms (58 in total) were dead after the treatment except one which was taken to the laboratory, where it died within the following 24 h. No pupal mortality was observed in control palms. The additional 61 mature larvae and 19 adults found in treated palms were dead. Therefore, efficacy was 100% as before.

No phytotoxic effects were observed immediately after exposure and up to one year after treatment in treated palms, either RPW-exposed (presumably infested) or not (control). Furthermore, no *R. ferrugineus* specimens were found after dissection of both presumably infested and control treated palms.

In agreement with previous work (Llácer & Jacas, 2010), our results demonstrate that a dose of 2.0 g/m^3 Gastoxin-B® (1.14 g/m^3 phosphine) is enough to kill all stages of *R. ferrugineus* in live *P. canariensis* palms. Remarkably, we obtained 100 % efficacy with an exposure one day shorter than that reported by those authors. This reduction may have been the key to avoid the problems of phytotoxicity previously observed when palms were treated for 3 days (same authors, unpublished results). Indeed, our results demonstrate the harmlessness of a 2-day exposure to phosphine for live *P. canariensis* palms, a pre-requisite for any in-transit quarantine treatment against this pest (Al-Shawaf *et al.,* 2013). This requirement had not been taken into account by Llácer & Jacas (2010) who used already sectioned palm crowns in their assays. Interestingly, our dose is also lower that that used by Muthuraman (1984), who applied 1.71 g aluminium phosphide per palm in the field. Our assays, where infestation was controlled, allowed the use of palms with a sufficient number of specimens (2-23 individuals per palm) for results to be statistically sound and not too seriously damaged that any conclusion about phosphine diffusion within the palm tissues could be questioned. The use of phosphine fumigation at 25 °C for 2 days may have great potential due to its convenience at destination, right before complete physical protection during one year as enforced by EU legislation (OJEU, 2007, 2008, 2010). All RPW-exposed palms were successfully recovered as pest-free one year after treatment. Besides, the same procedure might be applicable to *P. dactylifera* offshoots (the most common way to multiply this palm species in the field) provided that they are not more sensitive to phosphine than *P. canariensis*. As this type of treatment could be easily applied in sealed containers used for palm trade, its application would result in a relatively low cost and easy treatment which could significantly reduce the huge risks associated to palm exchanges in our global world.

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