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RESEARCH ARTICLE

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Electrocution and containment methods to reduce the activity of red palm weevil (*Rhynchophorus ferrugineus*, Ol.)

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Abstract

Aim of study: To evaluate the susceptibility of the *Rhynchophorus ferrugineus* to electric discharges, to eventually use this application in synergy with other methods as part of an integrated control strategy.

Area of study: Worldwide.

Material and methods: Three different electric voltage intensities were applied to *R. ferrugineus* to investigate the insect's susceptibility in both adult and larval stages. The transmission and diffusion of electricity within a portion of the palm tree was tested. In addition, the suitability of containment materials for handling and transportation of plant residues was tested.

Main results: The results of adult test showed that the total number of eggs laid per mating couple and the number of larvae born were about 1.5 times higher in the control compared to the electrified samples. The duration of the electric discharge on larvae had a small impact on the mortality rate, while the electric voltage showed a strong impact on mortality (87% for 10 V and 99% for 15 V). The presence of a significant electric current was observed in a viable portion of stem (distance from the source of electricity 10 cm) providing a direct current with 250 V tension. Among the materials tested for the transportation of palm residues, the aluminium net sheet showed the best results in the containment of both larval and adult individuals.

Research highlights: Electrocution has proven to be a system which can help in the control of red palm weevil reducing the use of synthetic chemicals.

Additional key words: integrated pest management; date palm; Canary palm; pesticides; principal coordinates analysis.

Abbreviations used: AC (alternating current); DC (direct current); EPPO (European and Mediterranean Plant Protection Organization); HSD (honestly significant difference); IPM (integrated pest management); IR (infrared radiation); PCoA (principal coordinates analysis); PVC (polymerizing vinyl chloride); RH (relative humidity); RPW (red palm weevil); UV (ultraviolet).

Authors' contributions: All the authors equally contribute to the writing of the paper and to its content.

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Introduction

Rhynchophorus ferrugineus Olivier (Coleoptera, Dryophthoridae), also known as red palm weevil (RPW), is an oligophagous invasive weevil pest, affecting more than 20 species of palms in the family Arecaceae (Murphy & Briscoe, 1999). Native to South-Eastern tropical Asia, due to global trade, in recent years *R. ferrugineus* has become the most destructive pest of palm trees in the world. Since 2015, RPW is on the A2 European and Mediterranean Plant Protection Organization (EPPO, 2015) list for quarantine pests, being a major pest of coconut, oil palm, sago palm and date palm. In the Mediterranean Basin and in the Middle East it is particularly destructive for *Phoenix canariensis* Hort. ex Chabaud. (Giblin-Davis *et al.*, 2013; Sardaro *et al.*, 2018). The spread of RPW in the newly colonized environments can endanger many species of palm tree, with a risk of extinction at least in urban centres (Manachini *et al.*, 2012; Sardaro *et al.*, 2018). A key feature in the widespread invasion by the RPW is its high reproductive capacity, which was widely investigated in earlier studies (Esteban-Durán *et al.*, 1998; Kaakeh, 2005; Ju *et al.*, 2011). A single female can lay up to 300 eggs at the base of the leaf petiole in a shallow hole drilled by the insect, which is cemented after laying. The newly emerged larvae feed on the surrounding tissue, burrowing tunnels in the tissues that first weaken the crown and then the trunk of the trees (Wattanapongsiri, 1966; Murphey & Briscoe, 1999; Kaakeh *et al.*, 2001). In a severely infested palm tree, the stem or crown sometimes break off from the tree (Abraham *et al.*, 1998).

RPW has a big economic impact both in countries where palms are exploited for subsistence or trade (date and oil palms) and in those where palms are grown for ornamental purposes, such as *P. canariensis*. RPW infestation on *P. canariensis* in anthropic habitats also raises serious safety concerns for people and for things in surrounding areas, not to mention the high costs required to remove dead palms and for their disposal (Fedrizzi *et al.*, 2013; Sardaro *et al.*, 2018). As part of an integrated control strategy, several containment methods have been proposed (Faleiro, 2006), including the felling infested palm trees and their disposal by incineration, the use of insecticides and the use of pheromone traps (Giblin-Davis *et al.*, 2013; Dembilio & Jaques, 2015).

Early detection of infested palms, eventually followed by curative treatments, is crucial to avoid the death of the trees and can be considered the key to the success of any integrated pest management (IPM) strategy (Massa *et al.*, 2017). Several early detection methods have been proposed, such as the use of trained dogs or gas sensors to detect chemical volatiles emitted by infested palms (Nakash *et al.*, 2000). Other techniques include the use of bioacoustic sensors (Mielle & Markuis, 1999) or thermal imaging methods and, more recently, nano-technologies (El-Sayed, 2020). However, until now these methods appear expensive, poorly effective or labor consuming. From a practical point of view, simple visual detection represents the only reliable procedure (Al-Dosari *et al.*, 2016). The symptoms of RPW infestations in palms are difficult to detect in the early stages, while the preventive strategies are crucial. If palms are treated in the early stages of attack they can recover after treatment, but all forms of the insect (larva and adult) must be eliminated from the plant (canopy and stem) to assure full recovery (Massa *et al.*, 2017). To this end, accurate pruning can allow the gradual removal of infested tissues for complete removal of insects without compromising the vegetative apex that produces new vegetation.

Chemical control used as a preventive method requires many applications per season (Dembilio & Jacques, 2015; Dhouibi *et al.*, 2017), causing environmental pollution and posing a risk to human health. In public and/or anthropic areas, special care should be taken during the treatments (localized foliage operations and low-pressure applications, injections in proximity of the apical bud) (Dhouibi *et al.*, 2017; Massa *et al.*, 2017). Endotherapy with injection of systemic insecticides has also been proposed for infested palms (Metwaly, 2010), but they are too expensive and labour consuming for large-scale applications, while the diffusion of bioactive ingredients inside palm tissues often remains problematic (Di Ilio *et al.*, 2018). Furthermore, the development of pesticide resistance in RPW has been reported in recent years (Wakil *et al.*, 2018). Other techniques include biological and biotechnological methods (Soffan *et al.*, 2016).

The recommendations of the European Commission have provided a strong impetus for the identification of new techniques and action plans for sustainable pest control, including: entomopathogenic organisms (fungi or nematodes) (Mazza *et al.*, 2014), sterile insect technique (Musmeci *et al.*, 2017), use of bait-stations (attractants in combination with chemicals) (Sayed *et al.*, 2016), or the screening and implementation of physical methods. Among these, the use of microwaves has received great attention, specifically as electromagnetic fields in the frequency range 300 MHz-300 GHz. Microwave radiation should induce a temperature rise in the insect body up to a lethal level, but without damaging plant tissues (Massa *et al.*, 2011, 2017).

Research programs on insect physiology could allow the identification of alternative approaches which, from an integrated management point of view, could help develop new environmentally friendly control systems. For this reason, it may be convenient to take advantage of possible physiological alterations, such as the response to thermal stimuli. The insect's sensitivity to heat has been suggested by the presence of areas on the body that function as infrared radiation (IR) receptors, both on larvae and adults (Ragaei & Sabry, 2013). RPW larvae pupate inside cocoons placed in the exterior portion of the crown in *P. canariensis* or in hidden spots at the base of branches in *P. dactylifera*. Brush-shaped sensors on the cuticle of larvae, pupae and adult wings act as thermo-receptors and help RPW pupae to absorb IR heat in the early stages of development (Ragaei & Sabry, 2013).

The electrical properties of plants have been studied for a long time and the existing literature dates back to the beginning of the last century (Stone, 1903; Stiles & Jorgensen, 1914). Most of these studies regarded forest trees, often for phytopathology issues (presence of pathogens or decay), or the response of plants to atmospheric events like lightning. Such electrical properties are defined at the cellular level. Based on the literature, Gora & Yanoviak (2015) listed the relationships between cellular components and electrical properties: cell and organelle membranes behave as microcapacitors because they oppose the flow of electric current and store electric energy;

on the other side the extracellular and intracellular fluids have low resistance and minor capacitance; the intracellular connections (plasmodesmata) may be considered as electric bridges between cells, decreasing membrane resistance. However, being based on structural and physiological characteristics, the extent of the electrical properties may differ among the plant tissue (leaf, stem or root) and the species in both magnitude and patterns (Gora & Yanoviak, 2015; Bar *et al.*, 2019).

Ragaei (2010) conducted a study on RPW adult elytra and hind wings to verify their conductivity characteristics. The results of this study showed the presence of potassium (K) in the elytra and silicon (Si as SiO_2) and phosphorus (P as GaP) in the hind wings. Silicon is used to create most of the commercially available semiconductors, that conduct electricity under specific conditions (White *et al.*, 1998; Leech & Ressel, 2003). The conductance depends on the current or voltage applied to a control electrode or on the intensity of irradiation with IR, visible light, ultraviolet (UV) or X-rays. This would prove that elytra and hind wings have semiconductor characteristics, with composition and functions compatible with solar absorption systems. These biophysical characteristics support the possibility of examining new physical systems for the control and containment of the insect pest.

In a previous study, Niamouris & Psirofonia (2014) proposed the use of electricity as a method of RPW containment. In their work, the flow of electricity was induced using hollow electrodes. The system was quite efficient in controlling larvae (84.8% mortality) and pupae (96.5% mortality), while adults showed no sensitivity.

Electrocution can be regarded as an interesting system, due to its environmental sustainability, whose physical principle has been widely applied in electrified grids used for the control of insects at home and in work environments (Urban & Broce, 2000; Matowo *et al.*, 2016).

The main goal of this work was to specifically evaluate the insect's susceptibility to electric discharges applied to adult and larval stages, in order to eventually use electricity in synergy with other methods as part of an integrated control strategy. These preliminary results are intended to confirm the hypothesis that electrocution can be used as a control method, not alone but in combination with other existing methods.

For this reason, a basic study was conducted to observe the behaviour of the insect in an electrified substrate and the effect of different intensity and duration of the electric discharge on insect viability and vitality, also on the transmission and diffusion of electric current through palm tissues. Furthermore, this technique could be used during the disposal of dead plants, in order to suppress larval development and reduce the spread of the pest. In fact, in Italy as well as in other countries (FAO, 2017), specific laws prescribe mandatory phytosanitary measures, demanding the removal and disposal of plant residues after cutting down highly infested palms or after pruning in the attempt of tree recovery. In addition, to prevent insect escape during the transportation of infested plant material, a study on containment materials was conducted to evaluate the use of different types of nets and sheets.

Material and methods

All experimental tests were carried out with RPW individuals, reared on replacement substrate (apple) as described in Arnone *et al.* (2014) and under controlled conditions (T=27°C; relative humidity RH=60%; L:D=16:8).

Adult test

— Choice test. A choice test was performed by applying an electric current differential on the food and oviposition substrate to verify any repellent or deterrent effects of the electric current towards the target insect pest (Shackleton *et al.*, 2005). The test involved five independent trials, each including five newly emerged adult couples. For each couple it was previously established that mating had taken place.

Each couple was placed in a plastic parallelepiped box (size 20 × 30 × 16 cm) and two halves of an apple (cv. *Golden Delicious*) were provided as a substrate for feeding and oviposition (Fig. 1a). One half of the apple was kept under voltage (30V) during the test, while the other was used as the control substrate without electricity applied. The apples were replaced every two days.

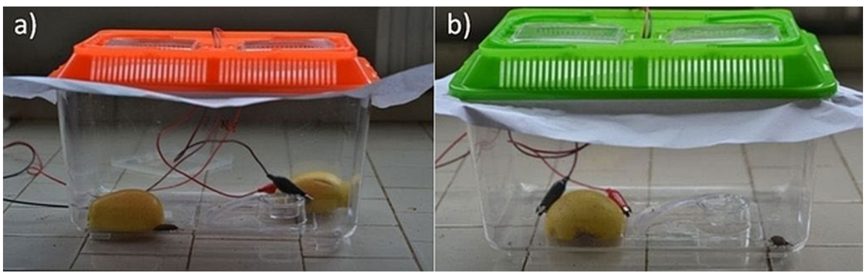

Figure 1. Choice test (a) and no-choice test (b).

The electric circuit was created using a voltage generator, terminals and steel electrodes. The latter were introduced in reverse orientation into the apple to close the circuit. The magnitude of the voltage was purposely chosen *a priori* to avoid any possible risk for the operators and because of its hypothetical large-scale application. The position inside the box of the electrified half-apple was exchanged every second day, to avoid any environmental disturbances.

During the experiment the number of eggs laid was recorded. The eggs were progressively collected and placed separately inside the wells of the Multiwell Petri 24 plates, sealed and humidified by a moistened filter paper inserted between the bottom and the lid of the plate (T=27 °C; L:D = 16:8). Records were taken to verify over time the number of eggs per couple, the number of hatched larvae, the hatching rate for each of the two apple parts, and to detect the presence of any interference with egg hatching due to the electric induction.

— No-choice test. In the corresponding "no-choice test", the same operating conditions described for the "choice test" were maintained, but the apple substrate under voltage and the control were placed in separate boxes (Fig. 1b). A weevil couple was placed in each box, counting the number of eggs per couple, the number of hatched larvae, and the rate of hatching. The no-choice test was replicated three times.

Electrocution test on larvae

Electrocution tests were carried out on RPW larvae, applying current by contact to larvae belonging to two different weight groups: <0.2 g and between 0.2-0.5 g. The larvae were treated using direct current at different voltages of 5, 10 and 15 V from a power supply (Eventek KPS305D). The voltage intensity was verified through a digital multimeter (IP-67 Marcucci Lafayette) connected in parallel to the Eventek power supply cables. Each voltage was applied for two durations of time (5 and 15 min), using aluminium plates as conductors (Fig. 2). The larvae were placed at the intersection of the two aluminium plates and their movement was limited using plastic separators. In this way, ten larvae were treated simultaneously with the same voltage.

After the treatment, each larvae was separately placed in a box (size 10 × 10 × 10 cm) on a replacement substrate (apple). Periodic checks were made after 1 hour, and after 1, 5 and 10 days from the start of the test, measuring the weight of the larvae and recording mortality rates. A total of 40 larvae was used for each test: they were divided into three groups (10 larvae each) and exposed to the specific voltage-time combinations; while the remaining 10 larvae were used as the control group (not treated with electric current).

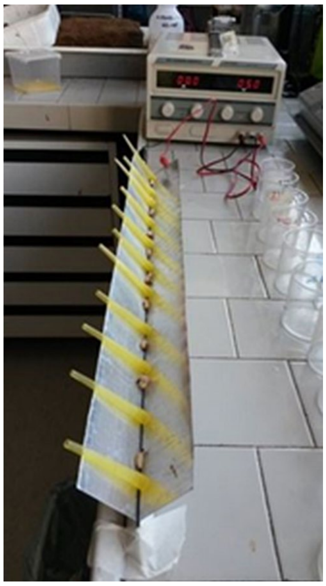

Figure 2. Larvae electrocution tests on aluminium plates and with plastic separators.

Plant tissue conductivity

A laboratory-scale test was performed to verify the ability of plant tissue to conduct electricity and to measure the electric voltage to which the larva would be exposed by applying electricity directly into the stem. A portion of stem (height 1.40 m, diameter 0.45 m) was cut from a palm trunk (*P. canariensis*), 24 hours after it had been felled. This segment was placed vertically on a rubber insulating base and used for the test. A plastic grid, having 16 square slots, with sides measuring 5 cm, was positioned on the top face of the vertical stem (perpendicular to the longitudinal axis and coinciding with the cutting surface). The grid was used as a reference frame to take the measurements concerning the propagation of electricity in the plant tissues (Fig. 3). The tests were carried out using both direct (DC) and alternating current (AC), using five different voltages: 50, 100, 150, 200 and 250 V. Stainless steel nails measuring 5 mm in diameter and 80 mm in length were used as electrodes to allow the electric current to pass through the tissues. The two electrodes were inserted on the sides on the trunk, at increasing distances from the top cross-section (cutting plane), moving downwards with 5 cm steps. The different positions of the electrodes used in the numerous tests are summarized in Table 1. The electrodes were inserted into the wood up to a depth of 50 mm.

The electricity was supplied by an electronic transformer that could operate in the 40 to 260 V range in both AC and DC. For each combination of electrode positions (distance along the cutting plane of the palm stem), 16 measurements were taken corresponding to 16 areas of the trunk surface (Fig. 3). A Lafayette digital multimeter (model DMB-97) was used to measure the voltage. The two tips of the multimeter were placed in contact with the surface of the palm

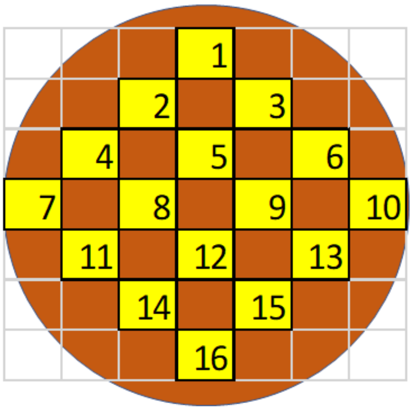

Figure 3. Areas of the stem portion tested for conductivity

stem, keeping them 10 mm apart. This distance was established considering the average diameter of the tunnels made by the insect excavating inside the plant tissues.

Containment tests

The Regional Directorate for Agriculture, Technical and Scientific Services Area, Regional Plant Health Service of the Lazio Region (Rome, Italy), with the regulation "D.D. C0472" dated 03/03/2010, in relation to the mandatory felling of infested plants and the subsequent disposal of infested wood, established specific procedures to avoid the dispersion of the pest. The first step consists in laying out plastic sheets underneath the trees to collect the residues. After cutting down the palm tree, all the infested residues collected on these sheets must be gathered and sealed with wrapping packaging (insect nets or plastic sheets with adequate thickness and strength). Subsequently, this material must be destroyed on site, or alternatively it must be taken away to a disposal site, using closed or tarpaulin trucks for transportation, to prevent accidental dispersion.

To assess the appropriateness of the materials used for the containment and transportation of plant residues, confinement tests were carried out using four different low-cost and easily retrievable materials: anti-aphid net, polymerizing vinyl chloride (PVC) sheet (thickness 0.20 mm), aluminium net sheet (thickness 0.25 mm, mesh 1.63 mm, 1.17 mm), polyethylene tarpaulin sheet with eyelet (density 110 g m^{-2}). Their sealing capacity was tested in parallelepiped-rectangular boxes. Each material was shaped into a package in which five larvae at the pre-pupal stage were reared on an apple for a 21-day period (Table 2). The larvae were provided with coconut fibre, to simulate the conditions of an infested palm and to provide the mature larvae with the fibres required to spin the cocoon. The packages were sealed with nylon cable zip ties. For each material we considered five replications. After 3 weeks, the packages were opened to establish for each material tested the number of adults emerged, insects escaped from boxes, dead individuals inside the boxes and holes in the packaging. The same tests were repeated with individuals in the adult stage. In this case, the test was performed in duplicate. Each repetition was prepared as described above but placing three adult males and two adult females inside each package.

Table 1. Combinations of the distances (cm) between anode and cathode tested in the plant tissue conductivity test

Anode (+)	Cathode (-)			
	5	**10**	**15**	**20**
5	X	X	X	X
10		X	X	X
15			X	X
20				X

Statistical analysis

All the statistical analyses were performed using the software PAST, vers. 2.15 (2012). Differences in the choice and no-choice tests were analyzed by analysis of variance (ANOVA). Data were checked for normality by Shapiro-Wilk test, and in case of deviation the non-parametric tests were used: the Wilcoxon test for non-independent samples (choice), the Mann-Whitney for the independent ones (no-choice) and the Kruskall-Wallis test for non-parametric ANOVA. Differences among the means were tested according to the Tukey's honestly significant difference (HSD) test.

A principal coordinates analysis (PCoA) was performed to explore and visualize the containment systems tested in this study. The PCoA is a multivariate technique that shows the objects (in this case the containment systems) in an ordination plane. The positions of the objects represent their similarities. Two very similar objects tend to position themselves close together in the reduced ordination plane. On the contrary, two very different objects are projected in more distant and opposite positions (Montanari, 2012).

Results

Adult test

Results did not reveal any significant statistical difference between electrified and control substrates in terms of number of eggs, hatching rate and larval survival, for both choice and no-choice test conditions (Table 3). The number of eggs laid in the control treatment and their

Table 2. Duration of containment tests

Date	Containment materials			
	PVC sheet	**Aluminium net sheet**	**Polyethylene tarpaulin sheet**	**Anti-aphid net**
02-June				
03-June	S			
10-June	C1	S	S	
17-June	C2	C1	C1	S
24-June	E	C2	C2	C1
01-July		E	E	C2
08-July				E

S: starting day. C1 and C2: check. E: ending day

hatching rate were always higher than the respective numbers in the electrified treatment.

Electrocution tests on larvae

For the electrocution tests, since the normality test failed for all the datasets, the statistical analysis was carried out with the Kruskal-Wallis test (a non-parametric test similar to ANOVA which, by comparing the medians of the groups, makes it possible to determine whether or not the samples come from the same population).

By applying the test to the different factors of voltage, of discharge duration and to their interaction (Table 4), it was observed that the duration factor does not have a specific incidence on the mortality rate, while the voltage and the interaction of voltage × duration showed a significant effect on larval mortality.

Discharges of 10 and 15 volts can induce a particularly high mortality rate (Fig. 4), equal to 87% (10V) and 99% (15V), while for the 5 volts treatment the rate is just over 45%. The effect of voltage is much greater than the duration of treatment because, limited to each discharge intensity, the application for 5 or 15 min showed a slight effect only at the highest voltage (Fig. 5).

Plant tissue conductivity

As a general comment, the palm stem showed a low conductivity (and, conversely, a high resistivity) to electric current (Fig. 6). Notwithstanding, the application of DC showed some interesting results at the dosage of 250 V and with the electrodes inserted at 10 cm from the cutting plane (Fig. 6A). In this configuration, the maximum intensity of electric voltage was recorded on almost the entire surface, able to affect insect viability. Greater depths did not show any activity even at the highest voltage. On the contrary, the use of AC had almost no effect on stem tissues and a weak activity was observed only near the surface when 250 V voltage was used (Fig. 6B). Such results could be encouraging, but it must be taken in consideration that the best results were limited to a 10 cm far from the source of electricity.

Containment tests

— Larval containment. To evaluate the tests carried out on the larvae, the data were analysed through the PCoA technique. In relation to the effect of the different materials on RPW larvae containment, the aluminium net sheet

Table 4. Statistics of the Kruskal-Wallis test on the mortality of the RPW larvae.

Source of variation	H	*p*	Significance
Voltage	10.19	0.004	**
Time	0.4817	0.475	ns
Volt × Time	10.67	0.045	*

Table 3. Results of the choice and no-choice test on the main mating parameters (mean±SD) and results of the Wilcoxon test (*p* value).

	Choice test			No-choice test		
	Apple		*p* value	Apple		*p* value
	Electrified	**Control**		**Electrified**	**Control**	
Eggs per couple (n)	4.8±1.0	5.3±0.8	0.893	7.5±1.9	12.1±1.9	0.376
Hatched larvae (n)	19.8±4.2	24.4±4.7	0.686	33.7±9.4	56.7±9.3	0.190
Rate of hatching (%)	84.0±5.4	89.7±5.5	0.465	88.7±3.7	93.8±1.0	0.662

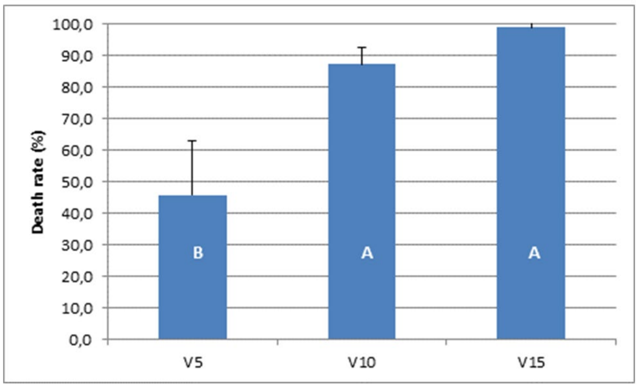

Figure 4. Average mortality (±SD) of larvae exposed to three voltage levels. Averages with different letters are significantly different for $p<0.01$.

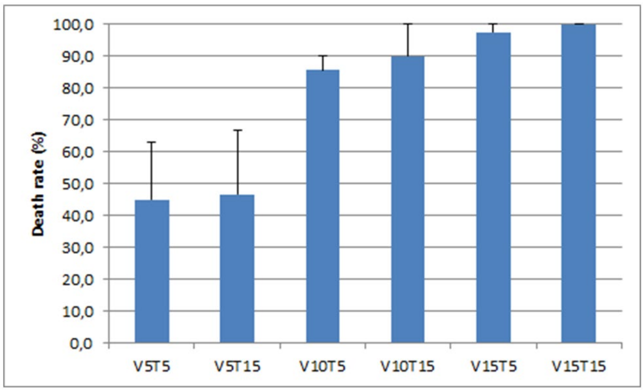

Figure 5. Average mortality (±SD) of larvae in the combination voltage per treatment time.

and the polyethylene tarpaulin sheet behaved practically in the same manner. These results were similar to those achieved with the use of the PVC sheet (Fig. 7). On the other hand, the anti-aphid net was clearly separated from the other materials.

The anti-aphid net showed the highest percentage of dead larvae and the lowest percentage of cocoons, compared to the other containment systems (Fig. 8). However, from a safety point of view, the other materials (in particular the aluminium net sheet and the polyethylene tarpaulin sheet) are considered more suited for use in transportation because, within these containment packages, the individuals were present mainly as non-active stages in the form of cocoons (80%) and dead larvae (8%), while the remaining living forms showed no significant mobility. Although PVC, aluminium net and polyethylene tarpaulin sheets were all perforated, no larval escapes were recorded. On the contrary, the anti-aphid net was found perforated in a single test, causing the escape of two larvae.

— *Adult containment.* In general, adult insects showed a better skill than larvae in making holes in the containment material and escaping (Table 5): in the first test all the materials were perforated; in the second test only the anti-aphid net and the polyethylene tarpaulin sheet were perforated, while the aluminium net sheet and the PVC sheet remained intact. From the perforated material, the adults escaped in percentages ranging from 20% (polyethylene tarpaulin sheet in the second test) to 100% (PVC sheet in the first test).

Finally, it should be noted that the containment test was carried out using active larvae and adults, which had not previously been treated by electrocution. Therefore, the containment values could be higher if the insects are previously treated with electric discharges.

Discussion

In recent years a major effort has been made to find an efficient, inexpensive and environmentally friendly method to control the RPW, but to date no solution seems to be completely satisfactory. Probably the simultaneous combination of different techniques could increase the success rates of the containment. This work was intended as a preliminary contribution to a better understanding of how the application of electricity can influence the viability, vitality and behaviour of the different life stages of the RPW. This was done through a number of tests designed to verify and understand the potential effects of electricity on the survival and mobility of RPW larval and adult stages (preference and electrocution tests on aluminium plates). Generally, the number of eggs laid in the control treatment and their hatching rate were always higher than the respective numbers in the electrified treatment. This trend should be further investigated as the lack of significance could be attributed to sampling problems. An example of this is given by the values obtained in the no-choice tests, where the number of eggs laid per couple and the respective hatching rate in the control is more than 1.5 times higher than in the electrified treatment. Moreover, when the couples were exposed in the no-choice test conditions, the number of eggs laid was almost double and the number of larvae born was more than double the number observed in the choice-tests. This could be due to the presence of two substrates in the choice test with respect to one. Therefore, the influence of this factor should be reviewed.

At the same time, the intensity of the electricity required for propagation inside the tissues of the palm tree stem was studied. In this sense, the rationale of the work and its results should be considered as a first approach to set the baseline for the use of the electrocution in combination with other IPM methods. As reported by El-Mergawy & Al-Ajlan (2011), the IPM of RPW is very difficult to achieve due to the intrinsic characteristics of the tree and the life cycle of the pest. Some successful strategies

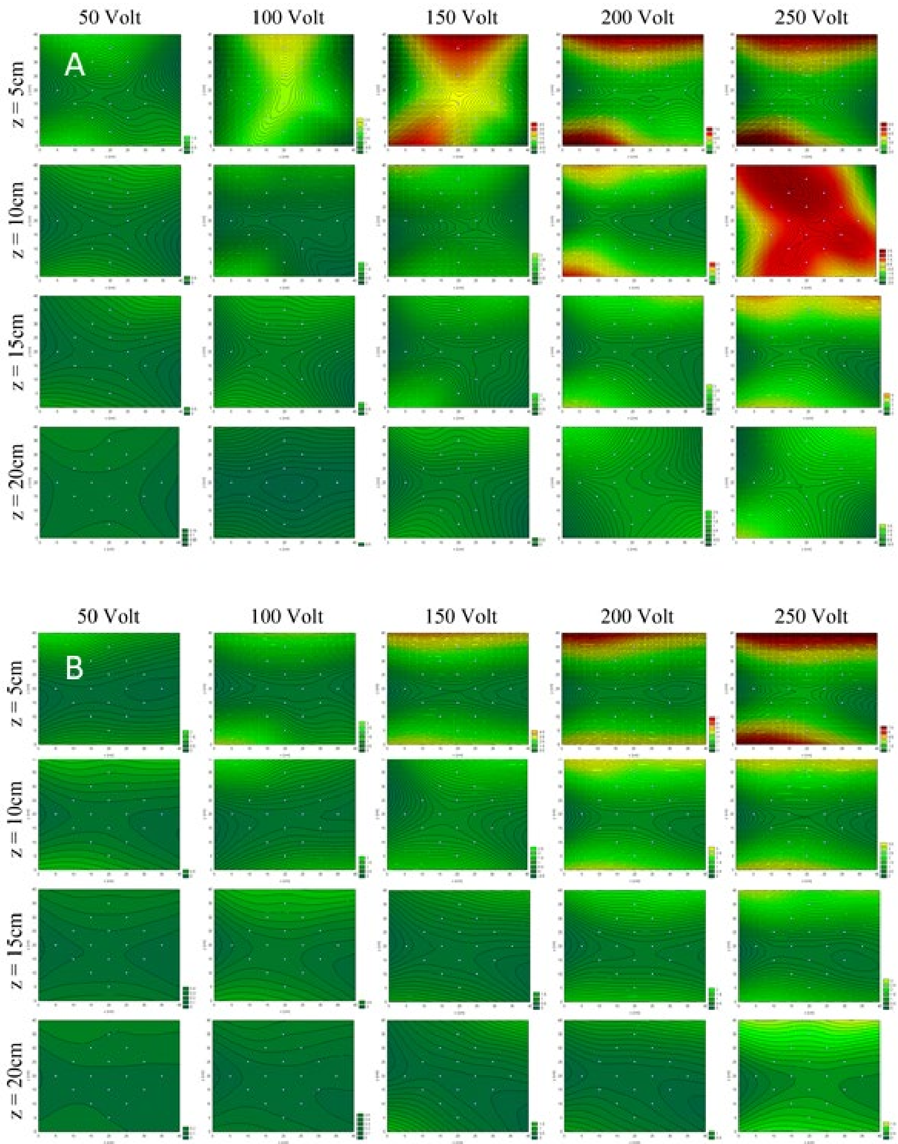

Figure 6. Pattern of the electrical signal intensity inside a portion of the palm tree after providing different electric voltage (*i.e.*, 50, 100, 150, 200 and 250 V) for direct current (A) and alternating one (B). The signals were registered by the electrodes inserted at different depth from the cutting plane.

have been applied on coconut and Canarian palm trees (Faleiro, 2006). If this work has a limit (which, however, can also be considered a stimulus), it can be found in the lack of field validation for testing the application of electricity under different conditions and in combination with other control methods. The application of the electricity/plant relationship at lab-scale has been previously studied by Kaimoyo *et al.* (2008), showing that electric current appears to be a general elicitor of secondary plant metabolites. These molecules are synthesized in large quantities in response to microbial infection. Japanese researchers have carried out several studies in recent years, investigating the electrostatic-based entomological behaviour (Kakutani *et al.*, 2012; Matsuda *et al.*, 2015 and 2020). One of the main outcomes of such lab-tests is that for a wide range of insect pests the electrostatic devices may have either a capture or repulsion function. While the first depends on an attractive force of the electric field, the second may depend on the insect's perception of this force as a potential risk signal (Matsuda *et al.*, 2020). Beyond the scientific and ethological interest of such behaviour, their observations can have important practical consequences both for use in trap devices to reduce the number of individuals (*e.g.*, electric field screen) and for the implementation of prevention strategies to contrast the massive colonization of plant tissues.

Even though the present work shows some weaknesses linked to the limited number of samples (*e.g.*, in the adult containment tests), some indications showed that they may constitute the starting point for future developments

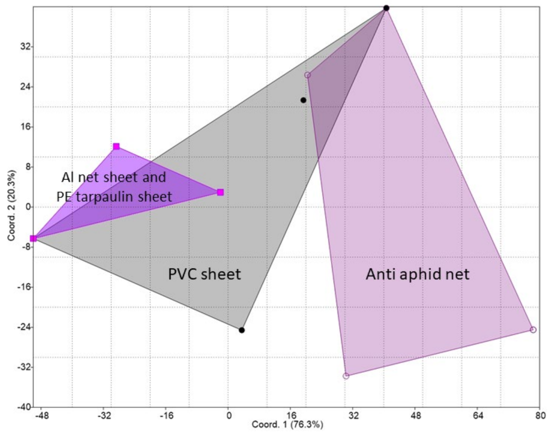

Figure 7. Principal coordinates analysis (PCoA) performed on the dataset of the different containment materials used for larvae (*i.e.*, PVC sheet, aluminium net sheet, polyethylene tarpaulin sheet and anti-aphid net).

in the application of electrocution. The RPW pest has characteristics, both at larval and adult stages, that would allow the conduction of current. The application of relatively high voltages (15V) necessary to achieve significant mortality levels and the intensity of the current, rather than the duration of treatment, seems to be the key factor determining the success of the system. Nonetheless electrocution appeared to limit and reduce the insect's mobility and viability. The tests carried out in this work showed an interesting potential, but the transition from laboratory bioassays to practical applications will require further testing to improve the lethal effect of the system and to further verify the capacity to reach all the adults burrowing into the palm tree.

Our results partially confirmed those obtained by Niamouris & Psirofonia (2014) who applied the electrocution *in vivo* at a lower current intensity (220 V) with the addition also of water with electrolytes in the stem of the palm tree to improve the electrical flow. The basic assumption was that the high moisture of the infested parts of the stem

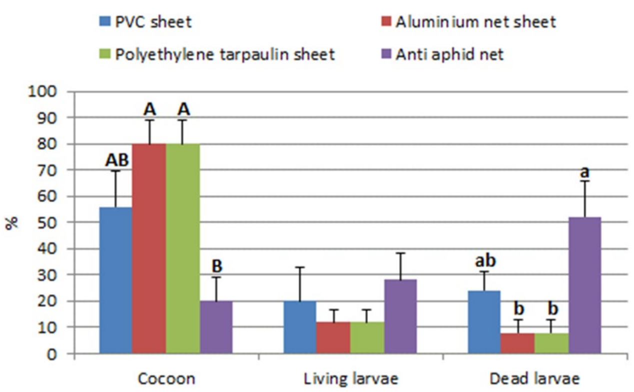

Figure 8. Percentage distribution of the larvae (average ± SD) in the different containment materials. The averages of each group (cocoon, live larvae, dead larvae) with different letters are significantly different for $p<0.05$ (lowercase letters) or $p<0.01$ (uppercase letters).

could increase electric conductivity enough to kill the insect. The application of electric current directly to the tree of *P. canariensis* achieved high mortality levels for larvae (84.8%) and pupae (96.5%), but the effectiveness on adults was lower (3.6%). Notably, the voltage applied (220V, 0-12 Amp, 60 min approximately, 50–60 Hz using various combinations of the electrodes) and the supply of water to facilitate the flow of electricity led to the death of five out of ten palms. However, the heavy effect on the plant vitality reported by the authors should warn about the real possibility of envisioning the system *in planta.*

Our data showed that, in general, without the supply of water, the transmission of the electrical impulse inside the stem was not easy (high resistivity). Anyway, working on different combinations and increasing the intensity it is possible to obtain results (high electrical signal with 250 V in at a distance of 10 cm from the source of electricity). Still it has to be established if the tree can tolerate this shock. In addition to the work of Niamouris & Psirofonia (2014), some tests were undertaken on herbaceous plants by Kaymoyo *et al.* (2008). The application of electrical current (10 mA) for 3 h to 3-to 4-weeks-old pea plantlets, grown hydroponically, did not cause damage over the next 8 weeks of culture. However, although the *in vivo* application as an exclusive control method appears questionable, proof of interest in this system is provided by the patenting activity based on this approach. Two years after the work of Niamouris & Psirofonia (2014), a patent application was filed for a system that uses electrocution to remove PRW adults and larvae (FR3050358A1, 2017) and at least three others were requested based on electrocution as a method to control tree pests [DE19753437 (A1); KR101117161 (B1); JP2004141127 (A)]. In any case, the effectiveness of electric current on the larval mortality, as emerged in our work, suggested that electric current applications could be adopted together with future and more reliable early detection strategies, and could be more effective especially in the early stage of infestation when the young larvae have not yet reached the inner core of the palm.

At the national or regional level, local administrations have developed defence protocols that may support different actions. Within the Lazio region (central Italy), the mandatory phytosanitary measures indicated for RPW infested palms require the adoption of several containment procedures (Determination no. C0472 03/03/2010). These include, among others: i) removal and confinement of all infested waste in packaging (those examined in this study); ii) timely suppression of all stages of the insect, which could be accidentally released in the surrounding area, by any suitable means (manual collection with confinement in closed containers activated with insecticide, elimination by mechanical compression, burning with gas burners); iii) transport of infested material (if it cannot be destroyed on site) to a site for disposal using appropriate packaging materials (anti-aphid nets or plastic tarpaulins of adequate

Table 5. Results of containment tests on adults.

Test	Material	Adults (%)		Holed	Escaped insects (%)
		Dead	Alive		
1	Aluminium net sheet	40	60	yes	60
	Anti-aphid net	40	60	yes	60
	Polyethylene tarpaulin sheet	0	100	yes	40
	PVC sheet	0	100	yes	100
2	Aluminium net sheet	60	40	no	0
	Anti-aphid net	20	80	yes	80
	Polyethylene tarpaulin sheet	0	100	yes	20
	PVC sheet	60	40	no	0

thickness and resistance) and/or using a closed or tarpaulin truck for transport, to prevent accidental dispersion. Thus, after having studied the effects of electrocution on insect viability, we also considered the need to evaluate the most suitable containment strategy for the safe transportation of infested plant residues to prevent the spread of the pest, both adults and larvae. From this point of view, in contrast to the observations made on the larvae, the results show that adults have a great ability to escape all the containments materials examined. However, among the materials tested, the aluminium net sheet obtained the best results in the containment of larvae and adults. On the other hand, the anti-aphid net was clearly separated from the other materials. This difference can be explained both by the effect that the anti-aphid net had on the development and mortality of the larvae and by the degree of resistance offered against the escape of the pest. In particular, at the pupation stage, the aluminium net sheet seemed to be quite interesting because it blocks the development in the cocoon (a protective structure formed during pupation), reducing the number of live emerging adults and it provided the highest mortality rate and partial resistance to perforation.

Overall, the results of the present study and the indications obtained regarding a practical application suggest that electrocution could be considered a "clean" approach and represent an additional control tool. In a perspective view, electrocution could complement and support the other control methods when the reduction of the mobility of larvae and adults is required. Based on the results of our study, the future studies could involve a more in-depth analysis of the application of electrocution in an integrated approach to manage the spread of RPW by testing outside, *in vivo* conditions.

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