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Research

Commodity Treatment and Quarantine Entomology

Light Traps in Shipping Containers: A New Tool for the Early Detection of Insect Alien Species

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Abstract

Insects are one of the most successful groups of invasive species, and the number of new introductions has been increasing in the last decades. Insect invasions are affected mainly by the increase in international trade, as most of them travel across the world inside shipping containers. The effectiveness of sticky light traps was tested for the interception of alien pests inside the containers. The tested hypotheses were that light traps have a valuable broad-spectrum attraction and their trapping performance differs between empty or loaded containers. The optimal trap density in a container was also investigated. Trapping tests were conducted on four model species: *Cadra cautella* Walker (Lepidoptera: Pyralidae), *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), *Sitophilus zeamais* Motschulsky, and *Ips typographus* L. (Coleoptera: Curculionidae). Insects were released within a standard shipping container, in either empty or loaded conditions, where sticky light traps were deployed for 15 h. Traps were tested with light on (activated) or off (control). Activated traps captured more Lepidoptera and Diptera than control ones, with no differences between empty and loaded container. Instead, Coleoptera were rarely caught, probably because of their ability to escape from traps. Results show that higher trap density in the container (from 1 to 8) increases the probability of insect capture. In conclusion, positive results on *C. cautella* and *D. melanogaster* suggest a possible application of sticky light traps against some small Lepidoptera and Diptera species flying in containers and infesting seeds, grains, and fruits, while traps need improvement for application against beetles.

Key words: early warning, biological invasion, survey, innovative tool, interception

Arthropods are one of the most successful groups of invasive species in the world and the number of new introductions is increasing worldwide (Seebens et al. 2018). In Europe, the number of new species introduced annually is also increasing exponentially (Hulme 2009). Between 2000 and 2008, an average of 19.6 alien species have been established in Europe every year, while 10.9 were introduced between 1950 and 1974 (Roques 2010). In 2009, alien insects registered in Europe were about 1,300 species (Roques et al. 2009), but only 10 yr later, there were more than 3,000 non-native species of terrestrial invertebrates in Europe, and about 2,500 of these were insects (European Commission 2019). Biological invasions of arthropods are mainly and positively affected by the increase in speed and volume of international trade (Levine and D'Antonio 2003, Westphal et al. 2008, Hulme 2009) and, on a global scale, the historical accumulation curves of alien species introductions show an increasing trend (Brockerhoff and Liebhold 2017, Seebens et al. 2017). Furthermore, global warming assures insect survival also for tropical species arriving in temperate regions and affects their chances of settling permanently (Walther et al. 2009).

ISO standard shipping containers are largely used in international trade and are now considered one of the main drivers of economic globalization in the 20th century (Bernhofen et al. 2016). Containers on ships carry about 90% of global trade (IMO 2012). In the last 40 years, world maritime trade volumes tripled, and in 2015, they reached about 10 billion tons per year (UNCTAD 2016). Global containerized trade increased annually by 6.4% in 2015–2017, and future previsions for seaborne trade are still positive (UNCTAD 2018). With such a large volume of commodities transported in containers all around the world, even minimal percentages of container contamination can represent a serious risk of introductions of new alien pests. In this respect, shipping containers are well-known to easily lead to the introduction of alien species in new territories. For instance, in 1,174 containers inspected in Australia in the period between February and August 1996, more than 7,400 insects were found, belonging to 18 orders and at least 114 families, and 19% of them were still alive (Stanaway et al. 2001). In New Zealand, the Ministry of Agriculture and Forestry conducted a survey of about 11,200 containers arriving at four of their ports in 2001/2002. Live

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insects, mainly belonged to Coleoptera, Psocoptera, Hymenoptera, and Hemiptera orders were found in 4.1% of loaded containers and in 3.6% of empty ones (MAF 2003). In general, the insect orders most commonly found in containers are Coleoptera, Diptera, and Lepidoptera, and they can be found in different life stages, from eggs to adults (Meurisse et al. 2019).

According to the European Council Directive, phytosanitary inspectors of the National Plant Protection Organizations have to check all cargos arriving from non-UE countries or suspected to contain quarantine pests. Nevertheless, no common and optimal survey strategy between all European member states exists yet (Surkov et al. 2008). Moreover, inspectors can sample only a small volume of total consignments of commodities arriving in the international ports (Everett 2000, Surkov et al. 2008). This problem does not just affect Europe; e.g., it is estimated that only 2% of all maritime cargos entering the United States is inspected, and at most 54% of insect species are detected (Work et al. 2005). Inspectors often use historical records from the interception databases to select shipments to be inspected, but this procedure reduces the number and types of new routes (pathways) checked, increasing the risk of new entries (Bacon et al. 2012). For example, most of the main insect alien species entering Australia in 1986–2005 went unnoticed by phytosanitary controls in the points-of-entry (Caley et al. 2015).

Given the wide variety of alien insects that can easily be introduced in new areas through international trade and the gaps occurring in border phytosanitary controls, new early-detection tools helping inspectors' surveillance are badly needed. The development of nonspecific broad-spectrum traps to be used within shipping containers during the cargo travel could be a simple and effective way for prompt early detection of alien species at the points-of-entry. Species captured during travel, in fact, can help to determine in advance if the load is infested, to direct most efforts only on the lots deemed as riskier. The aim of this study was to test the effectiveness of a sticky light trap to capture different orders of insect pests inside shipping containers. We wanted to verify 1) if light could be an effective broad-spectrum attractant for pests belonging to different insect orders, and 2) if container status (empty or loaded with goods) affects the number of captures. We also wanted to investigate if there was an optimal traps density to maximize captures.

Materials and Methods

Tested Traps

The experimental trials were conducted using sticky light traps (TransTrap, Alpha Scents Inc., West Linn, OR) developed to capture pests potentially occurring inside shipping containers (Mangan and Chapa 2013). This trap model consists of a carton box (15 × 23 × 4 cm) made attractive to flying and walking insects by an LED (Light Emitting Diode) light powered by a long-life AA battery. These LEDs emit light that have two peaks, the main at 465 nm (indigo) and a second more broadband included between 525 and 600 nm (between green and yellow) (Alpha Scents Inc., personal communication 2020). The light is positioned in the center of a removable yellow sticky card fixed to the bottom of the box. In our experiment a second yellow sticky card was applied, attached to the inside of the box lid to increase the sticky surface and trap performance (Fig. 1). This trap model is simple to use, easily manageable, potentially attractive to a large number of different insect species, and does not require additional lures.

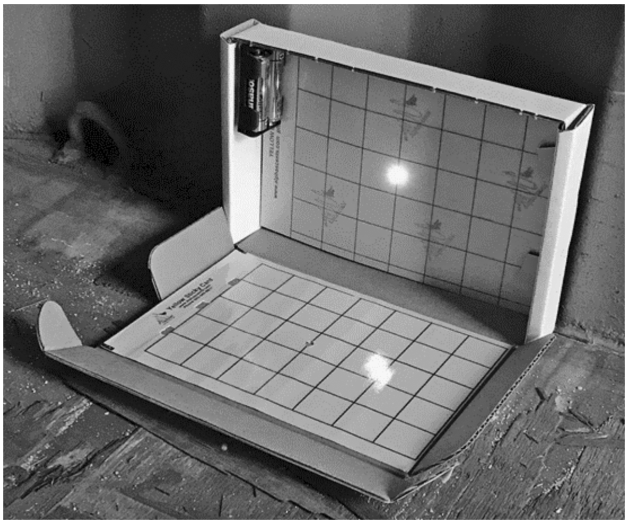

Fig. 1. The trap used for the experiment.

Model Species

The tests were conducted on four model species, belonging to three different orders of insects. The almond moth, *Cadra cautella* Walker (Lepidoptera: Pyralidae), is a stored food products pest with larvae developing on cereal grains and flour, beans and other dried seeds and fruits (Sedlacek et al. 1995). *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), is a common insect associated with fruits and vegetables (Mallis 1954, Birmingham et al. 2011). The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is considered one of the major pests of stored maize (Erenso and Berhe 2016, Nwosu 2018). The European spruce bark beetle, *Ips typographus* L. (Coleoptera: Curculionidae, Scolytinae), is the main European spruce pest developing in stressed or recently dead trees (Wermelinger 2004), and travels in containers used for the international spruce timber trade. These model species were chosen because beetles (Coleoptera), flies (Diptera) and moths (Lepidoptera) are the most common insect orders found inside shipping containers used in international trade (Meurisse et al. 2019).

Ips typographus adults were captured by Theysohn slot-traps (Salzgitter, Germany) set up in clear-cut areas of natural spruce forests of central Alps (Trentino, Italy) infested in 2019. Traps were installed at about 15–20 m from the forest edge, and baited with pheromone dispensers specific to *I. typographus* (Superwood Serbios, Italy). Traps were checked and emptied every second day, and all trapped adults of *I. typographus* were stored in darkness at +4°C in plastic jars containing wet paper and small pieces of spruce bark. The other species (*Cadra cautella*, *Drosophila melanogaster* and *Sitophilus zeamais*) were bought from a company (Entostudio s.r.l., Padua, Italy) specialized in rearing insects of various species and for different uses. Adults of *Cadra cautella* were bred in 5-liter glass jars measuring 16 cm in diameter and 25 cm in height. The jars were positioned upside down with the opening covered of a 2 mm mesh net. The jar was placed above a transparent plastic cup (12 cm in diameter and 6 cm in height) to collect the eggs. These eggs were moved daily into transparent plastic cup (11 cm in diameter and 9 cm in height) that contained a mixture of wheat and corn flour, oat, bran, dry fruit, glycerol, honey and yeast, where larvae can develop. Adults who emerged in these boxes were taken and put inside glass jars. The insects were reared at 25 ± 1°C and 50 ± 5% R.H. The exposure to light lasted 12 h during 24 h and the light intensity was

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300 lux at 6,000 K. Adults of *Sitophilus zeamais* were bred in plastic cups measuring 12 cm in diameter and 6 cm in height, closed by a fine net, at 25 ± 1°C and 50 ± 5% RH. The photoperiod lasted 12 h at a solar spectrum artificial light of 6,000 K and 300 lux intensity. Insects were fed with grain. The colony originated in 2014 with insects collected in the field. Adults of *Drosophila melanogaster* were bred in BugDorme cages measuring 32.5 × 32.5 × 32.5 cm. The food and oviposition substrate consisted of a mixture of water, pieces of potatoes and fruit, powdered milk and sugar. The insects were reared at 25 ± 1°C and 50 ± 5% of RH. The photoperiod lasted 12 h at a solar spectrum artificial light of 6,000 K and 300 lux intensity.

All insects were tested in the trials only once and within 2 d from their emergence (or trapping) to ensure the highest vitality. We used insects without discriminating between males and females and assuming a sex-ratio 1:1. The reared species *C. cautella*, *D. melanogaster*, and *S. zeamais* reproduce sexually, producing a sex-balanced offspring (Santos et al. 1994, Danho et al. 2002, Soffan et al. 2012). An aggregation pheromone was used to capture *I. typographus*, which attracts both males and females with a sex-ratio slightly unbalanced in favor of females (Faccoli and Buffo 2004).

Trials in Container

A blue ISO standard shipping container 1CC (interior size: 5.8 m length, 2.3 m wide, 2.3 m height) with a volume of 32 m^3 (ISO-668 2013) was used for the experiments. The container was placed in a square of the Agripolis Campus, University of Padua (Legnaro, Italy), without any shelter from sun and rain. Specific tests were then conducted between June and August 2019 with the container both empty and loaded.

Empty container. Each model species was tested singly through seven tests, on seven consecutive days. In each test 50 individuals per species were released inside the container provided with two sticky traps: one with the light on (activated trap) and the other with the light off (control trap). Insects were put inside a plastic cup with a lid resting on the top and placed at the bottom of the container. With a rope tied to the cup and stretched to the door of the container, it was possible to overturn the cup, releasing the insects and closing the container doors before they escaped. The two traps were placed in corners of the door side of the container, on the floor. Each test, i.e., each repetition, lasted about 15 h (from 6:00 p.m. to 9:00 a.m.). At the end of each daily trial, before starting a new one, we ventilated the container for many hours and we made sure no survivor was left inside.

Loaded container. The same tests as those in the empty container were conducted in containers filled with empty cardboard boxes simulating a cargo. In this second group of tests only *C. cautella* and *D. melanogaster* were used (seven tests per species with 50 individuals released per species). We verified that the *S. zeamais* were able to escape the traps and decided not to use them in the following tests, while the *I. typographus* were not used because we did not have enough specimens. Each test lasted about 15 h (from 6:00 p.m. to 9:00 a.m.).

Optimal Traps Density

Optimal trap number maximizing insect catches in the container was also tested in September, on one of the two species that had recorded the best number of catches in previous tests. The captures of *C. cautella* were recorded in loaded containers with four different trap densities, using one, two, four, or eight traps set up in the same container (Fig. 2). For each trap density, five tests of 50 insects each were conducted on five consecutive days. Each test lasted about 15 h, with an unlit trap used as a control.

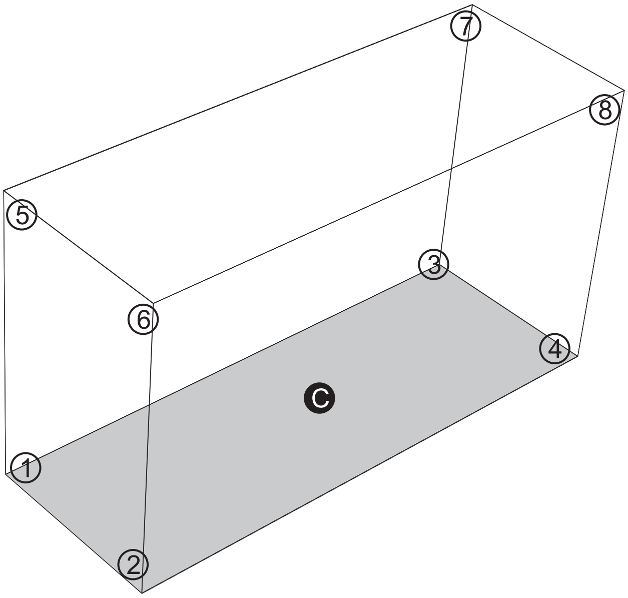

Fig. 2. Position of the traps inside the container (doors were on the left side). One-trap trial: 1. Two-traps trials: 1–2. Four-traps trials: 1–4. Eight-traps trials: 1–8. C is the control trap, always present.

During each test in the container, air temperature was recorded every 15 min with three data loggers (RC-5 model, Elitech LTD, London, UK) one placed outside and two inside the container, one on the bottom and one at the top.

Escape Test

After the first tests on *S. zeamais* in the empty container, given the few specimens captured, the hypothesis was tested that the insects could escape from the trap. Therefore, 10 living *S. zeamais* were placed in each of five traps, marking the insect positions on the sticky card with a circle. Two tests were conducted at 16 and at 26°C constant temperature inside climatic chambers. After 18 h traps were checked, looking for number and position of the insects placed on the sticky card.

Statistical Analysis

Statistical analysis was conducted using R software, version 3.6.1 (R Core Team 2019). Mean catches of *C. cautella* and *D. melanogaster* with activated and control traps were compared using Poisson mixed-effect model, with trap type (activated or control) as fixed variable and tests as random variable. The model was fitted using the ‘glmer’ function in the lme4 package (Bates et al. 2015). For *S. zeamais* and *I. typographus*, for which the use of this model was impossible because catches in control traps were nil, the Wilcoxon test was therefore applied using the ‘wilcox.test’ function in the stat package (R Core Team 2019). Catches made with activated traps in the empty and loaded container were also compared for each single species using Poisson mixed-effect model and, in this case, the container status (empty or loaded) was the fixed variable while the tests were the random variable.

Results

Tests in Empty Container

Activated traps captured significantly more individuals of *C. cautella* ($P < 0.001$, z-value = 6.68) (Fig. 3), *D. melanogaster* ($P < 0.001$,

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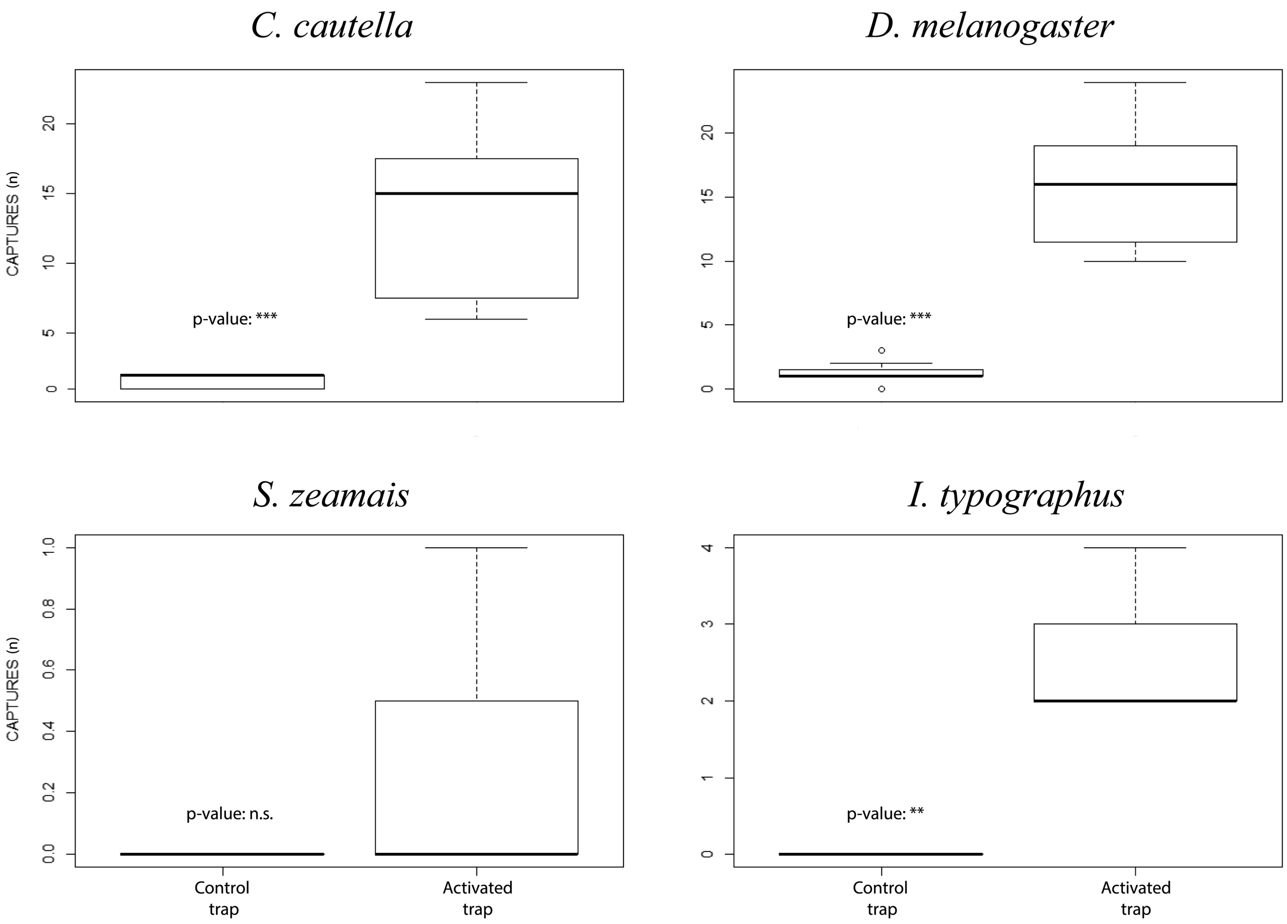

Fig. 3. Catches (±SEM) of activated and control traps for the four model species tested in the empty container. Significant results are displayed within each box (**$P < 0.01$; ***$P < 0.001$).

z-value = 7.27) (Fig. 3), and *I. typographus* ($P < 0.01$) (Fig. 3) than control traps, while for *S. zeamais* activated and control traps showed similar captures ($P = 0.173$), with only a very few specimens in activated traps and nil in control ones (Fig. 3). No abnormal temperature trends were found during the tests, which remained similar during each repetition. The average temperatures recorded during the trials inside the container ranged between 20 and 25°C, with no significant differences between tests.

Tests in Loaded Container

Activated traps captured significantly more individuals than control traps, for both *C. cautella* ($P < 0.001$, z-value = 5.27) (Fig. 4) and *D. melanogaster* ($P < 0.001$, z-value = 6.81) (Fig. 4). Furthermore, catches of the activated traps were similar in both the empty and loaded container, with no significant differences for either *C. cautella* ($P = 0.237$, z-value = 1.18) (Fig. 5) or *D. melanogaster* ($P = 0.424$, z-value = 0.80) (Fig. 5). Average temperatures recorded during the trials inside the container were about 22°C, with no significant differences between tests.

Optimal Trap Density

Densities of one, two, and four activated traps per container showed mean catches with no significant differences ($P = 0.556$), whereas with eight traps per container the number of trapped insects more than doubled. Captures of the control traps were not affected by trap density, although they were negatively correlated with captures in the activated traps ($P < 0.01$). The catching trend of the activated traps increases with trap density, but starts to flatten with eight traps (Fig. 6). The average temperatures recorded during the trials inside the container were around 19–25°C, with no significant differences between tests.

Escape Test

Considering the two temperatures separately, the mean proportion of *S. zeamais* escaped from sticky cards were 42 and 62%, for trials at 16 and 26°C, respectively.

Discussion

Results show that the tested trap model is effective in catching *C. cautella* and *D. melanogaster*, in both empty and loaded containers. For Coleoptera, instead, and in particular for *S. zeamais*, results are not satisfactory as beetles are able to escape from the sticky card of the trap.

Although results concern only one model species for each tested order, we can assume that similar results would be expected for other species and genera belonging to the same family and having similar size and behavior. In fact, several researches demonstrate the effectiveness of light as an attractant both for Pyralidae (Kanno et al. 1985, Loganathan et al. 2001, Sambaraju and Phillips 2008) and other Lepidoptera families like Crambidae (Keszthelyi and Sáringer 2003, Haihua et al. 2016), and Hyblaeidae (Loganathan et al.

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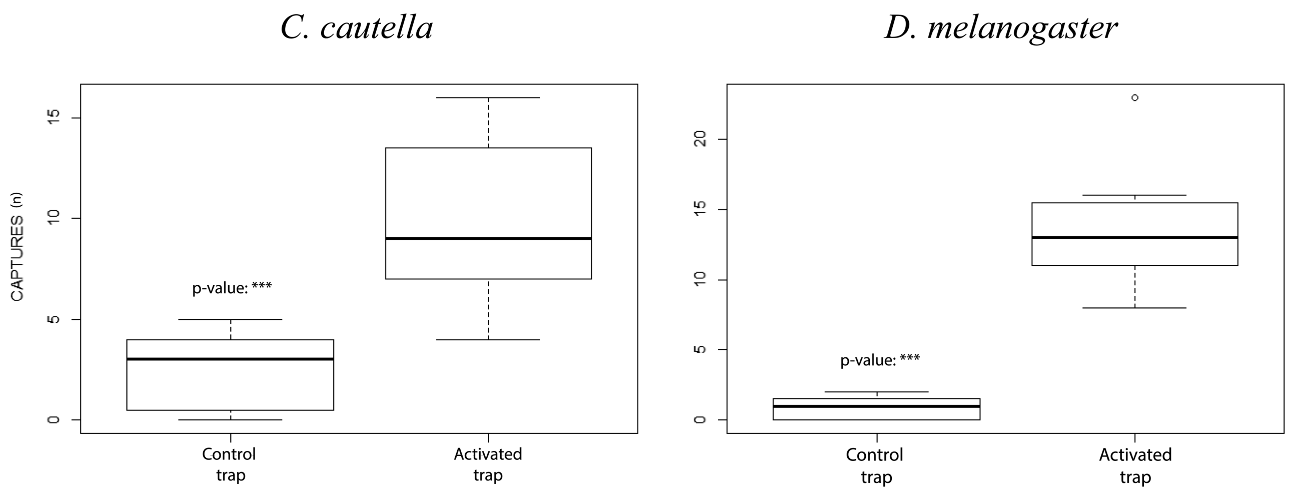

Fig. 4. Catches (±SEM) of activated and control traps for the two model species in the loaded container. Significant results are displayed within each box (***$P < 0.001$).

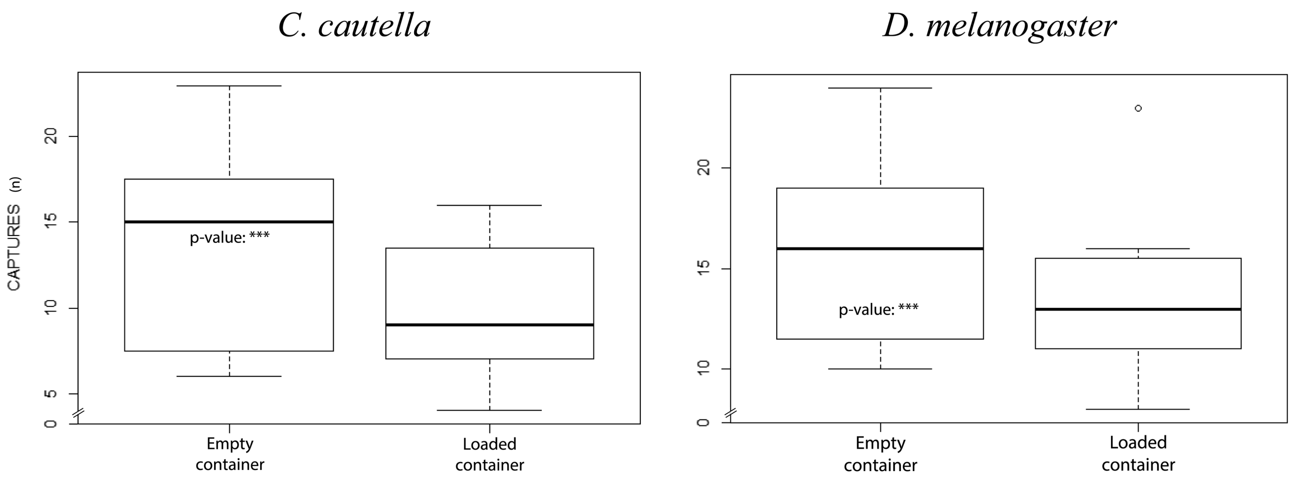

Fig. 5. Catches (±SEM) of activated traps for the two model species in empty and loaded container. There are no significant differences.

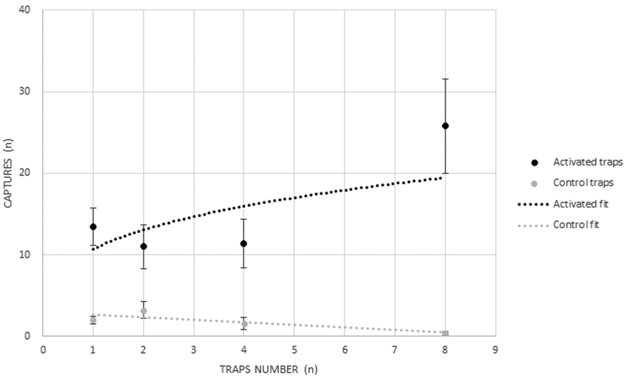

Fig. 6. Captures of *C. cautella* recorded in each test with increasing trap density.

2001). Light traps are already widely used to capture Diptera like Chironomidae (van Grunsven et al. 2014), Culicidae (Burkett et al. 1998, Silva et al. 2019b), Psychodidae Phlebotominae (Cohnstaedt et al. 2008, da Silva et al. 2019a), and other 14 families (Ndengué et al. 2019). Moreover, this light trap has already been tested on other orders, like *Diaphorina citri* (Hemiptera: Liviidae) (Mangan and Chapa 2013).

Beetles show different results. Although the captures of *I. typographus* in activated traps were very low (only 5% of released insects were captured) they were significantly higher than those recorded in the control traps (no insect). Positive light-responses were also recorded in other scolytines where ethanol baited traps activated with green or UV light are more attractive to *Xylosandrus crassiusculus* than normal traps (Gorzlancyk et al. 2013, 2014). *Sitophilus zeamais*, lastly, shows no significant difference between activated and control traps, with only two insects trapped by activated ones and no capture in control traps over a total of seven replicates (i.e., 350 insects). The low trapping performance of beetles is probably related to the ability of these insects to escape from the traps, verified by the appropriate test showing that 42 and 62% of *S. zeamais* escape from sticky cards at 16 and 26°C, respectively. In this context, therefore, it is not clear if the low beetle captures are related to a non-attraction to the light or to their ability to escape. However, light traps are already used for catching beetles, like Tenebrionidae (Duehl et al. 2011), or Curculionidae, Pselaphidae, Silvanidae and other 33 families (Ndengué et al. 2019), and the attractiveness of light—in particular red wave-length (625 nm)—has

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also been verified for *S. zeamais* in a double-choice test (Park and Lee 2017). The difference between moths/flies and beetles is likely due to their landing strategies. Moths and flies are glued by the wings whereas beetles are somewhat able to avoid wing contact and walk away. To check if the reduce trap performance in catching beetles is related to the ability of these insects to escape from traps, more powerful glues should be tested or the sticky card could be sprayed with contact insecticides to prevent insect's escape after their capture.

It was very difficult to check what happened to insects not captured by traps. We suppose that some of them died during the test, and some others remained alive but undetectable inside the container, which was ventilated and cleaned before running a new test.

In our experiment, the container status (empty or loaded) does not affect the number of captures of the light sticky trap. Trials conducted in the empty container recorded about 27 and 32% of captures versus 21 and 28% in the loaded container for Lepidoptera and Diptera respectively, with no significant differences. This is one of the most interesting results from this study, suggesting the useful application of the light sticky trap also in containers loaded with commodities and, hence, exposed to a major risk of movement and introduction of alien species across countries and continents.

Tests conducted on trap density in the container show that, although using eight traps (the highest number of traps during this study), the rarefaction curve built on the number of catches per number of traps has not yet reached flattening. So, the more traps that are used the more insects would be expected to be captured. However, the aim of the light sticky trap is not to capture as many insects as possible, but to capture the maximum number of alien species potentially traveling inside the container. In this way, traps could provide information on the status of cargo infestations and allow pre-delivery quarantine measures to prevent the introduction of new alien species in non-native countries. On the other hand, increasing the number of traps also increases the probability of catching species present in low numbers. However, placing a large number of traps inside a container loaded with cargo could be problematic logistically, and considerably increase the survey costs. In this respect, results show that the mean number of captures is similar among one, two, and four traps per container. For this reason, one or two traps per container seems to be a sufficiently high number to discover small and flying alien species traveling with the commodities.

This trap technology needs some improvement and more extensive testing, but the preliminary results are very encouraging, especially for small species of Diptera and Lepidoptera infesting seeds, grains, and fruits exported internationally in containers. Although in our tests only a white LED lamp was used, the type of light used to activate the trap could be an important variable to test, as the spectral composition is important to determine the attractiveness of the light to insects (van Grunsven et al. 2014). Insects sensitivity to UV, blue, and green light spectrum is well-known (Briscoe and Chittka 2001, Cohnstaedt et al. 2008) and, in some cases, also to red light (Peitsch et al. 1992, Park and Lee 2017). In particular, different studies demonstrate the major effectiveness of UV light for catching many different insect species (van Grunsven et al. 2014, Infusino et al. 2017). For example, insects of about 480 species belonging to 10 different orders were captured in a survey conducted in South Korea using UV light (Thein and Choi 2016).

Finally, new tests will be required during real shipments. Tests conducted up to now were in controlled conditions, which simulated reality. However, it is necessary to verify the effective performance of these traps in real situations, where weather, environmental conditions and species involved can be very different from those tested in our trials. The duration of the shipment can also play a key role; we successfully used the light for 1 mo without interruption, so we are pretty sure that this trap is suitable for prolonged use in a container during shipment. In conclusion, light traps set up in containers represent a potentially effective tool for border surveillance and early detection against biological invasions. This study represents only a first preliminary work dealing with the early detection of alien species potentially traveling with commodities in containers. Further and deeper tests about light source and glue type are needed to improve trapping performance and the potential applications of this novel tool of pest interception.

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