Journal of Stored Products Research 61 (2015) 102–107

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Effectiveness of the egg parasitoid *Trichogramma evanescens* preventing rice moth from infesting stored bagged commodities

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ARTICLE INFO

Article history:
Received 26 September 2014
Received in revised form
29 December 2014
Accepted 3 January 2015
Available online 14 January 2015

Keywords:
Corcyra cephalonica
Jute
Paper
Bag
Trichogramma evanescens
Hymenoptera
Trichogrammatidae

ABSTRACT

Experiments were carried out in the laboratory with the aim of accessing the effectiveness and parasitism by *Trichogramma evanescens* to prevent *Corcyra cephalonica* from infesting rice in paper and jute bags. Eight small jute or paper bags filled with 5 kg of organic rice grains were prepared and the openings sealed. Sentinel egg cards were prepared with thirty fresh eggs of *C. cephalonica* glued onto small pieces of paper cardboard. Eight sentinel egg cards were introduced into a plastic box measuring 60 × 40 × 21 cm, i.e four cards on top surface of the bag and the box bottom, respectively. Approximately 500 adults of *T. evanescens* were released 10–30 cm away from the egg cards. The control boxes contained no parasitoids; there were five replicates for all treatments and controls. Two experimental conditions were tested, i) placing a single *T. evanescens*-release unit with sentinel egg cards placed every 3–4 days without any further replacement of the release unit for three weeks, ii) both new host eggs and *T. evanescens* release units were replaced every 3–4 days. Mean emergence of *C. cephalonica* was significantly ($p > 0.001$) suppressed by the release of *T. evanescens*. There was statistically no significant difference on the number of emerged moths on paper bag compared to jute bag. All sentinel egg patches were visited by *T. evanescens*. There was no correlation between the distance (10–30 cm) at which the sentinel egg cards were placed away from the *T. evanescens* release point and the number of parasitized *C. cephalonica* eggs. There was no decrease in parasitism over time. The results demonstrate that *T. evanescens* has the potential for host-location ability and parasitism of *C. cephalonica* both on paper and jute bags. This parasitoid could be a promising candidate for the biological control of moth pests in bagged stored products.

1. Introduction

Rice is the most important staple food for a large part of the world's human population, especially in the tropics (FAO, 2004a, 2004b) and provides 20% of the dietary energy supply in the world (FAO, 2004c; FAOSTAT, 2013).

The rice moth *Corcyra cephalonica* (Stainton) is a major pest of durable stored produce throughout the world causing considerable losses to cereals, grain legumes and other high value crops such as cocoa beans and dried fruits (Haines, 1992; Sedlacek et al., 1996). The control of stored-product moths in bagged commodities is difficult because the developmental stages of the moths are protected by the bagging material from control measures such as the application of contact insecticides. World-wide control of storage pests is primarily dependent upon continued application of synthetic contact insecticides and fumigants, mainly phosphine (Arthur and Peckman, 2006; Walter, 2006). Although effective, their indiscriminate use for decades has disrupted biological control by natural enemies and led to outbreaks of various insect species, development of resistance to the chemicals, undesirable effects on non-target organisms, and environmental and human health concerns (Rajendran and Narasimhan, 1994).

Small quantities of bio-organic rice are stored in paper bags especially in Europe and in jute bags in developing countries like

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http://dx.doi.org/10.1016/j.jspr.2015.01.002
0022-474X/

Africa and are sold in bio-market and departmental stores. The paper and jute bags easily get infested with stored product insects like *C. cephalonica* and *Plodia interpunctella* (Hübner) which affect the quality for human consumption (Adarkwah et al., 2014; Schöller et al., 2006; Flinn and Schöller, 2012). The increasing concern about the adverse effects of pesticides has highlighted the need for the development of more selective insect-control alternatives that are less harmful to humans and environmentally friendly (Mbata, 1989; Subramanyam and Hagstrum, 1996; Schöller, 2010; Adarkwah et al., 2012, 2014; Trematerra, 2013; Kaur et al., 2014).

Trichogramma evanescens Westwood (Hymenoptera: Trichogrammatidae) is a polypahgous egg parasitoid of several lepidopteran species (Wajnberg and Hassan, 1994). *T. evanescens* is commercially applied in the retail trade and the food processing industry in Central Europe to control stored-product moths, mainly *P. interpunctella*, *Ephestia kuehniella* Zeller and *Ephestia elutella* (Hübner); mass-rearing and storage of this species is well established (Prozell and Schöller, 1998; Schöller, 2010). *T. evanescens* is known to be capable of developing successfully in *P. interpunctella* (Schöller and Fields, 2003) and to forage on various types of food packages (Ambrosius et al., 2006), but no information is available on the control of *C. cephalonica* in bagged stored rice by this parasitoid. The present study examined for the first time the potential of using egg parasitoid *Trichogramma* for preventing infestations of *C. cephalonica* in bagged rice typical for tropical or subtropical countries, and also examined the influence of packaging of different surface texture on parasitoid effectiveness.

2. Materials and methods

2.1. Culturing of C. cephalonica and parasitoids

The rice moth *C. cephalonica* was obtained from the permanent rearing cultures of the Federal Research Centre for Cultivated Plants – Julius Kühn-Institut, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin, Germany. The tested strain of *T. evanescens* was originally obtained from eggs of *Helicoverpa armigera* (Hübner) (Lep.: Noctuidae) in Egypt in 1981. The strain is arrhenotokous, with a sex ratio of 1.47:1 females: males. The original population of *T. evanescens* was obtained from the Biologische Beratung Ltd. Berlin, Germany. *C. cephalonica* were reared in 1-l glass jars filled with 150 g of organic rice with a moisture content of 14% to which organic rice germs were added to make up 5% of the total. The jars were placed on a mechanical roller to mix the content properly. Two hundred and fifty *C. cephalonica* eggs were added to each jar and were kept in the culturing room. *T. evanescens* were reared on UV-sterilised eggs of *Sitotroga cerealella*. Both species were reared in a growth cabinet maintained at 65 ± 5% relative humidity (r.h.), a constant temperature of 25 °C.

2.2. Host location of T. evanescens on paper bags and jute bags

Experiments were carried out in the laboratory to assess the host location and parasitism of *T. evanescens* for biological control of *C. cephalonica* on paper and jute bags, by evaluating the efficacy of this beneficial for bagged stored rice. The jute bags used were prepared from pieces of industrial bagging material originating from Côte d'Ivoire through the importation of cocoa beans to the Port of Hamburg by cutting the original bags into different pieces. The bags were re-sewn with a needle and rope thread obtained from the Ghana Cocoa Board, Accra, Ghana. The mean mesh width of the jute fabric was 2.5 mm. The experimental paper bags used for the bioassay were also prepared from pieces of industrial bagging material obtained from the organic bakery Märkisches Landbrot GmbH, Berlin, Germany through the packaging of spelt flour Type 812 by cutting the original bags into different pieces. The organic Langkorn Spitzenreis "Thaibonnet-variety" was purchased from Hofladen Domäne Dahlem, Berlin, Germany. The rice was kept at −15 °C for two weeks to kill any living insects (Fields, 1992). After this period, the grains were removed and kept under experimental temperature and humidity conditions for 1 week before being used in the experiments. The rice grain moisture content used for experiment after the grain was kept at −15 °C was 12–14%. The moisture content was determined by using Pfeuffer Mess-

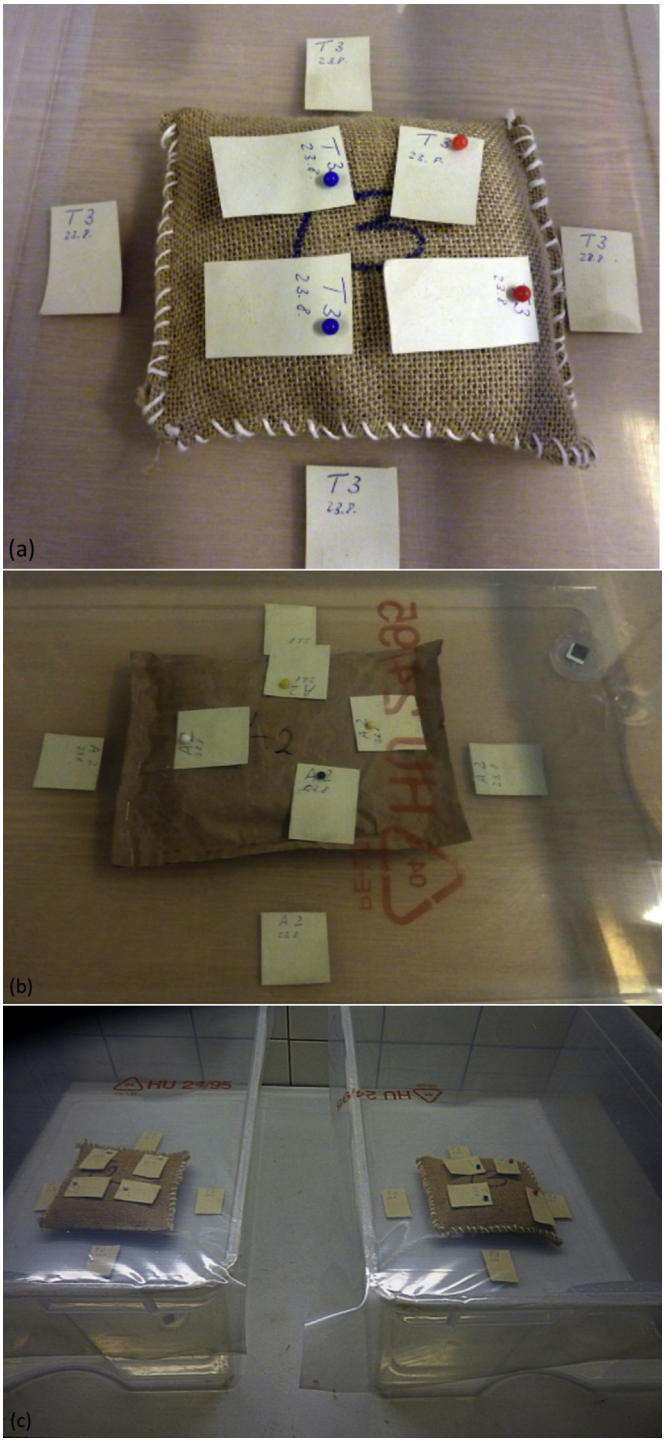

Fig. 1. Sentinel egg cards placed (a) on the surface of jute and (b) paper bags, and (c) plastic boxes containing the experimental set up.

Prüfgeräte HOH-Express HE 50 and obtained from Pfeuffer GmbH, Kitzingen, Germany. The paper bags were re-made with the help of silicon glue by fixing tightly all edges of the bag to form a 1 kg sized paper bag filled with rice. A total of eight small 1 kg paper and jute bags containing organic rice were prepared. The openings of the paper bags were sealed with the silicon while the jute bags were sealed with the needle and rope thread as above.

A total of 30 freshly laid eggs of *C. cephalonica* were counted with the help of a stereo-microscope (AHAH 475052-9901, Zeiss Germany) and placed inside a Petri-dish. The eggs were glued on to pieces of paper cardboard measuring 5 cm × 3.5 cm by means of Tragant (Merck, Germany), a commercial glue based on a plant compound which is known to be harmless to *Trichogramma* (Schöller and Hassan, 2001). The sentinel *C. cephalonica* egg cards were stacked separately on either the paper or jute bags surface or at the bottom of the container (plastic boxes measuring 60 cm × 40 cm x 21 cm diameter), i.e. a total of 8 egg cards per bag per container being incubated. Four of the cards were pinned on the top surface at the four corners of each bag. The other four sentinel egg cards were secured with double-sided sticky adhesive tape at the bottom edges inside the incubator (Fig. 1a and b). Approximately 500 adults of *T. evanescens* aged 0–6 days old were released at least 10–30 cm away from the bags in the incubator this was achieved by cutting a 1 cm square of the *Trichogramma* egg card obtained from Biologische Beratung Ltd. The commercial *T. evanescens* card had approximately 3000 immature *T. evanescens*. Double-sided sticky sellotape was first used to coat all the rims of the vertical parts of the plastic box and a transparent high density polythene sheet was cut into a piece of 65 × 45 cm and then used to tightly cover the plastic box (Fig. 1c). This was done to avoid parasitoids from escaping the box, and other insects from entering the box. The boxes were placed in a growth cabinet and maintained at 65 ± 5% r.h., a constant temperature of 25 °C and in constant darkness.

Two experimental conditions were tested to elucidate; i) how long parasitism will occur when placing a single *T. evanescens*-release unit. New sentinel egg cards were placed every 3–4 days in the box treated with *T. evanescens* without any further replacement of the *T. evanescens*-release unit until week 3, ii) both new host eggs and *T. evanescens* release units were replaced every 3–4 days. The untreated control boxes contained no parasitoids. Treatments were replicated five times. The parasitoids were allowed to oviposit for 3–6 days in the treated boxes. *T. evanescens* adults still present on the surface of the host egg cards were removed, and the host egg cards were transferred singly into Petri-dishes. The samples were placed under the same climatic conditions as above. After this period, parasitized black host eggs, shrunk eggs (mortality), and transparent eggs indicating moth larval emergence were recorded under the stereo-microscope. Emerged F_1 *T. evanescens* were recorded and sexed as well.

2.2.1. Statistical analysis of data

Statistical analyses were performed using the software package SIGMASTAT 3.1. Treatments were considered significantly different at the 5% level. Means obtained by the emergence of *Trichogramma* offspring and sex ratio as well as mortality of moths both on paper and jute were compared using one way-analysis of variance (ANOVA) on Ranks. Kruskal–Wallis One Way ANOVA on Ranks was applied in case the test for normal distribution of data failed. The correlation between the distance at which the sentinel egg cards were placed away from the parasitoids release point and the number of parasitized *C. cephalonica* eggs in the box was analysed using Linear Regression. Mean percentage of *T. evanescens* parasitized *C. cephalonica* eggs on jute and paper depending on time were analysed with One Way Repeated Measures ANOVA. Comparisons between means for jute and paper bags, i.e. independent data, were analysed using the t-test set at 95.0% confidence interval.

3. Results

3.1. *C. cephalonica* larvae emerged on paper bags and jute bags vs control

Analysing the survival combined with the two materials, jute and paper, mean emergence of *C. cephalonica* was significantly suppressed by the release of *T. evanescens* (Kruskal–Wallis One Way ANOVA on Ranks, $P < 0.001$, $H = 44.115$, $DF = 1$). A mean of 0.86 ± 1.18 and 25.00 ± 3.25 larvae emerged in the treated and the untreated, respectively (Fig. 2). Mean mortality of *C. cephalonica* eggs (shrunk eggs) in the *T. evanescens* treated trials was significantly higher (5.18 ± 3.26) compared to the untreated (3.06 ± 2.08) (Kruskal–Wallis One Way ANOVA on Ranks, $P = 0.004$, $H = 8.516$, $DF = 1$).

Significantly less *C. cephalonica* larvae emerged in the *T. evanescens* treated boxes both containing the jute bags (Kruskal–Wallis One Way ANOVA on Ranks, $H = 21.302$, $DF = 1$, $P < 0.001$) and the paper bags (Kruskal–Wallis One Way ANOVA on Ranks, $H = 22.710$, $DF = 1$, $P < 0.001$) compared to the untreated control. In the untreated control boxes with jute bags, a mean number ± Std. Dev. of 26.38 ± 3.02 larvae emerged, while only 1.15 ± 1.37 emerged in the treated boxes. The untreated boxes with paper bags had 23.63 ± 3.02 larvae, while in the treated boxes the lowest number of 0.58 ± 0.88 emerged *C. cephalonica* larvae were recorded (Fig. 3). No difference was detected between the mean number of *C. cephalonica* larvae emerged from the treated jute and paper bags, respectively (Kruskal–Wallis One Way ANOVA on Ranks, $H = 2.935$, $DF = 1$, $P < 0.087$).

3.2. Comparison of parasitism on paper bags and jute bags

The emergence of *C. cephalonica* was significantly suppressed in the trials with *T. evanescens* released, both on the jute and paper surfaces of the bags (Fig. 4a), and on the egg patches placed directly on the box (Fig. 4b). Natural mortality in the untreated trials ranged between 8.3 and 12.5%. There was no significant difference in the

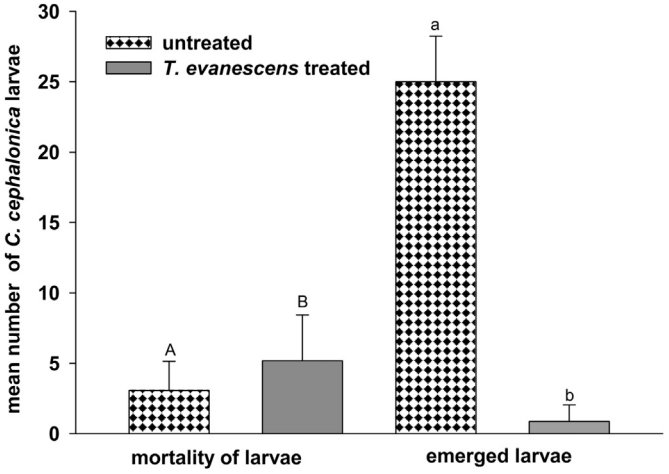

Fig. 2. Mean number (±SD) of *C. cephalonica* larvae emerged or dead (excluding parasitism) from sentinel egg cards placed on jute and paper bags in untreated samples and after release of *T. evanescens*. Means followed by the same lowercase letter (mortality of larvae) or uppercase letter (emerged larvae) do not differ significantly at $P < 0.001$ (Kruskal–Wallis test).

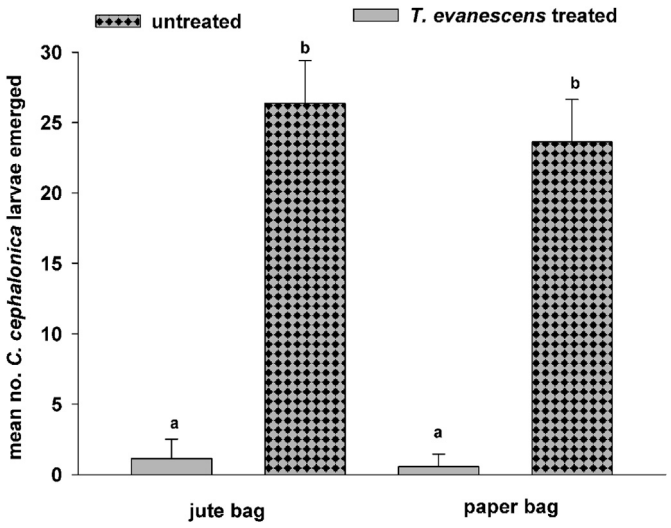

Fig. 3. Mean number (±SD) of *C. cephalonica* larvae emerged from sentinel egg cards in boxes containing jute or paper bags, *T. evanescens* treated or untreated control. Means followed by the same lowercase letter do not differ significantly at $P < 0.001$ (Kruskal–Wallis test).

the box (Linear Regression, R = 0.125, Rsqr = 0.0156, $P = 0.269$) (Fig. 5). Over all distances, a mean of 22.7 *C. cephalonica* eggs were parasitized per egg patch.

3.4. Parasitism depending on time

Parasitism was ranging between 65% and 92% during the 18 days shelf life of the release unit tested. Mean percentage of *T. evanescens* parasitized *C. cephalonica* eggs on jute and paper statistically showed no significant differences (One Way ANOVA, $P = 0.375$, $F = 0.883$, DF = 4), with a total mean of 73.17 ± 4.11 and 78.17 ± 11.17 on all egg patches on paper and jute bags, respectively (Fig. 6). There was no decrease in parasitism over time, the highest mean percentage parasitism was observed in the eight egg patches exposed from day 15–18 with a mean of 78.8 and 90.8 parasitised eggs on jute and paper, respectively.

3.5. Emergence of Trichogramma offspring and sex ratio

In all treatments combined, the mean + SD percent of females was 76.47 ± 10.03. Significantly more female *T. evanescens* emerged compared to males ($P < 0.001$, H = 39.168, DF = 1, Kruskal–Wallis

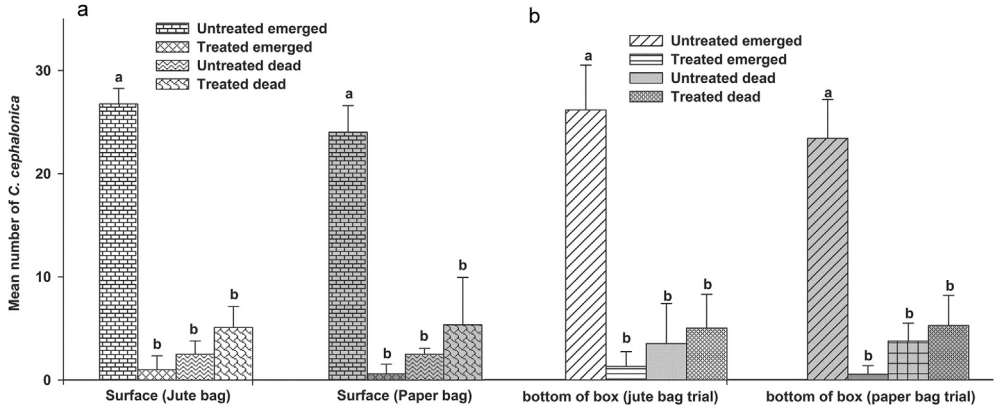

Fig. 4. The mean (±SD) number *C. cephalonica* eggs emerged or dead on (a) the jute and paper bag surfaces, or (b) the bottom of the experimental boxes. Means followed by the same lowercase letter do not differ significantly at $P < 0.001$ (Kruskal–Wallis test).

number of dead *C. cephalonica* eggs within the untreated or treated trials for all treatments (Kruskal–Wallis One Way ANOVA on Ranks, H = 247.66, DF = 23, $P < 0.001$). However, the percentage of dead eggs was always higher in the *Trichogramma*-treated trials, ranging from to 16.7–17.3 %.

Parasitism of the eggs placed on the jute and paper bags resulted in a mean number of 21.95 ± 3.41 and 23.1 ± 4.62 black eggs, respectively. No significant difference in the number of *T. evanescens*-parasitised black eggs was detected (T-test, $P = 0.38$, $t = -0.94$). From the eggs placed on the bottom of the plastic boxes, a mean number of 21.95 ± 3.41 and 23.8 ± 4.62 were parasitised in the trials with jute and paper bags, respectively. No significant difference in the number of *T. evanescens*-parasitised black eggs was detected (T-test, $P = 0.22$, $t = -1.35$) as well.

3.3. Parasitism depending on distance from release unit

In all the distances tested, *C. cephalonica* eggs were parasitized. There was no the correlation between the distance at which the sentinel egg cards were placed away from the release point of *T. evanescens* and the number of parasitized *C. cephalonica* eggs in

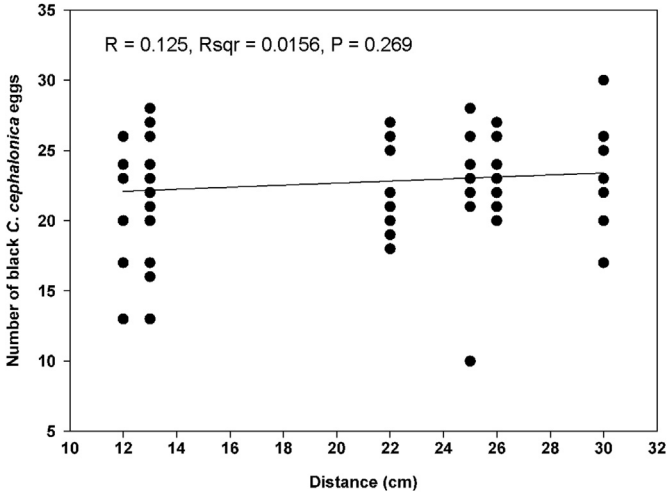

Fig. 5. The number of parasitized *C. cephalonica* eggs depending on the distance at which the sentinel egg cards placed away from the release point of *T. evanescens*.

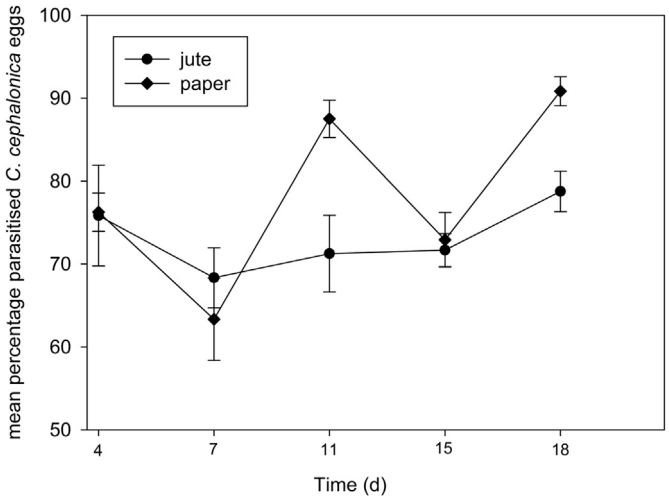

Fig. 6. Mean percentage number and (±SD) of parasitized *C. cephalonica* eggs out of 30 sentinel eggs per patch exposed every 4 days on jute or paper bags depending on time after placing the *T. evanescens* release unit.

One Way ANOVA on Ranks). A mean ± SD of 83.92 ± 24.35 percent *T. evanescens* offspring emerged from the parasitized black eggs per host egg patch.

4. Discussion

Egg parasitoids in the genus *Trichogramma* are applied for biological control of various pest of Lepidoptera in field crops like corn or apple (Hassan et al., 1998; Parra and Zucchi, 2004).

They are polyphagous and accept eggs of many Lepidoptera (Wajnberg and Hassan, 1994) including all stored product moths of economic importance (Brower, 1983). Immature *Trichogramma* sp. are glued on cardboard release units, the adults emerge continuously for several weeks. Nowadays, release units are available that provide activity of *T. evanescens* for 3 or even 4 weeks (Schöller, 2010).

Trichogramma spp. walk on surfaces rather than flying while foraging. They were shown to walk distances of at least 15 m on smooth surfaces within 1 h, equalling 30,000 times a wasp's body length in 1 h, comparable to a vehicle 2 m in length driving at 60 km/h (Quednau, 1958). However, for *Trichogramma* spp., the surface structure of woven cloth like jute is comparable with hairy leaf surfaces. Hairy leaf surfaces were shown to reduce the foraging activity of *Trichogramma* sp., e.g. on tomato leaves (Wührer, 1996). In order to test if a large surface will reduce the effectiveness of released parasitoids on cloth, Zimmermann (2005) placed cloth (25 cm × 45 cm) in cages (100 cm × 50 cm x 65 cm). He compared 3 types of cloth: (1) Finely woven cloth 1.5 mm in thickness without long distant strands (fibres) (2) medium-finely woven cloth ca. 3.0 mm in thickness with long distant strands and (3) tanned sheepskin rug ca. 2.5 cm in thickness. Five sentinel egg cards were placed 10 cm, 20 cm, 30 cm and 40 cm from the release point as baits. Fresh host eggs were provided on day 2, 3 and 5 after release of *Trichogramma* individuals. The numbers of *Trichogramma* individuals on the egg baits were recorded as well as parasitism by counting black host eggs. The number of female *T. evanescens* active on the cloth increased with an increasing number of parasitoids released and decreased with increasing thickness of the cloth. Based on these results we expected a better host finding on paper bags compared to jute bags. However, this was not the case, indicating the number of *Trichogramma* released compensated for potential differences due to the bagging material, and for distance from the release point. The increased mortality in the treated trials compared to the untreated also supports the assumption of a large number of parasitoids finding the host-patches, because parasitoid-induced mortality like host feeding and superparasitism typically increase the mortality of the hosts (Hansen and Jensen, 2002). We followed the current recommendation of 1000 *T. evanescens* per m^2 and week for empty room treatment with *T. evanescens*, and our data support the validity of this recommendation. The parasitism did not change over the three-week period, indicating a continuous emergence of the parasitoids from the release unit.

We might have overestimated the host-finding success by exposing patches of grouped host eggs rather than exposing single moth eggs. Grieshop et al. (2010) found *Trichogramma* spp. to parasitise more eggs when egg density was high compared to single-egg patches, suggesting that host-finding occurred in a density-dependent manner. On the other hand, in none of the trials were the moth eggs placed by natural oviposition. *Trichogramma* spp. are known to be arrested when encountering adult moth scales (Lewis et al., 1972), which act as kairomones. The presence of these kairomones might facilitate host-finding in stored product environments as well. Alternatively, structures like bag stacks and shelves present a more complex habitat in practice, and increased habitat complexity was shown to affect *Trichogramma* foraging success in the laboratory (Andow and Prokrym, 1990; Lukianchuk and Smith, 1997; Gingras and Boivin, 2002; Gingras et al., 2002). These questions have to be addressed in future semi-field trials.

The control of stored-product moths in bagged commodities is one of the most difficult control situations in stored product protection, because the developmental stages of the moths are protected by the bag material from e.g. contact insecticides or diatomaceous earths. Consequently the bagged products have to be unpacked in order to treat the stored product directly, or methods like freezing or fumigation have to be applied. These techniques are labour intensive or require special equipment and storage rooms, respectively. Consequently the release of *Trichogramma* spp. would be an alternative easy to apply, and due to low prices for the release units it can also be used in larger stores. This study revealed that the host-location ability and the successful parasitism of *T. evanescens* has potential for biological control of *C. cephalonica* infesting bagged rice in both paper and jute bags.

Acknowledgements

Julius Kühn-Institut – Federal Research Centre for Cultivated Plants, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin, provided laboratory facilities and equipment for this work. The staff of the Institute, to whom we are most grateful, assisted in various ways. Parasitoids were provided by Biologische Beratung Ltd., Berlin, Germany.

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