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Combination of Mating Disruption and parasitoid Habrobracon hebetor against Plodia interpunctella in a chocolate factory

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Abstract

A field experiment of 4 years' duration was carried out to evaluate the efficacy of combining the mating disruption (MD) formulation Dismate ZETA (9Z,12E-tetradecadienyl acetate), with the parasitoid *Habrobracon hebetor* against the Indianmeal moth *Plodia interpunctella* in a chocolate factory. The experimental period began early in 2011 and ended in late 2014. Begane Dismate dispensers were placed in the facility from 2011 to 2014 and *H. hebetor* was released in 2014. Pheromone-baited traps were used to monitor the flight activity of the male moths and oviposition Petri dish cups were placed to assess the progeny production of *P. interpunctella* females. Following the start of MD, a decrease in the number of *P. interpunctella* males caught in monitoring traps was observed from 2011 to 2013. A further decline in the moth population was noted in 2014, when MD was combined with the release of parasitoids. The presence of larvae in the oviposition cups was occasionally observed throughout the monitoring period, from 2011 to 2014. This study demonstrates that the combined system of MD and parasitoids is an effective and reliable technique that can be used to successfully control *P. interpunctella*.

Keywords: food processing, Integrated Pest Management, *Plodia interpunctella*, mating-disruption, *Habrobracon hebetor*

Introduction

The use of pheromones for suppressing pest populations through mating-disruption (MD) has been widely studied for stored-product Lepidoptera. For stored-product moths such as *Ephestia* spp. and *Plodia interpunctella* (Hübner), a single pheromone compound, known as TDA [(9Z,12E)-tetradecadienyl-acetate] can act as a male attractant (Trematerra, 2012; Trematerra et al., 2013). The enclosed environment of storage facilities provides an ideal area for the application of MD, given that the sources of external infestation in this environment are limited (e.g., the introduction of infested raw materials or the immigration of mated females from untreated areas) (Trematerra and Fleurat-Lessard, 2015). For instance, in a recent study, Trematerra et al. (2011) illustrated the reduction in the population of various Pyralidae following the use of MD dispensers in several areas and facilities in Europe, which clearly indicated that this technique can be of widespread use.

MD can only lead to a reduction in chemical treatments, which however, remain necessary, because males of moths such as *P. interpunctella* can inseminate on average 6 females in their lifetime. Moreover, females need to be mated only once per lifetime to produce their full number of viable eggs. Consequently, a few successful copulations in an environment under MD treatment will guarantee the survival of the population. Therefore, the application of pheromone-based IPM tools should be accompanied by additional nonchemical measures, such as cleaning and sanitation, especially in critical areas, such as the inside of the machinery and around raw materials and packaging areas. More recently, it has been suggested that combining and integrating different management tools and the careful selection and timing of different approaches, together with an understanding of pest behaviour and ecology can result in greater effectiveness (Trematerra, 2013). Another approach to overcome the challenges faced by the stored-product industry due to stored-product insects is the integration of pheromone-based IPM tools with biological control, like the parasitoid wasp *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) (Ghimire and Phillips, 2010). *Habrobracon hebetor* can parasitize all larval instars of *P. interpunctella*, but significantly fewer early instars are paralyzed and reproduction requires last-instar larvae (Akinkurolere et al., 2009). In the past, various applications of *H. hebetor* in stored products have been investigated, for example, in grain spillage (Press et al., 1982), packaged products (Cline et al., 1984; Adarkwah et al., 2014), bulk peanuts (Brower, 1990) and bulk grain (Adarkwah and Schöller, 2012).

MD targets *P. interpunctella* adults, whereas the parasitoid *H. hebetor* should target *P. interpunctella* larvae. The purpose of this 4-year study (2011–2014), conducted in a chocolate factory, was to analyze the effectiveness of MD for the first time and to evaluate the combination of MD and the release as parasitoid *H. hebetor*.

Materials and Methods

The chocolate factory is located in Southern Italy, in the area of Ospedaletto d'Alpinolo (Campania region). In the factory, dried fruits were stored and processed and chocolate food was produced. The factory was constructed from concrete, and contained 3 floors with the first floor being dedicated to food production and having an area of about 2000 m2 and a height of 5 m (about 10000 m3 in total).

MD treatments

From 2011 to 2014, the pheromone component TDA [(9Z,12E)-tetradecadienyl- acetate] (Dismate, Russell IPM, Deeside, UK), was used for MD. Each cellulose acetate MD dispenser was baited with 100 mg TDA. In 2011, MD dispensers were placed in a small area of the chocolate factory (of about 320 m^2), but covered the entire chocolate factory (2000 m^2) from 2012 to 2014.

In 2011, MD dispensers were placed from May to December; in 2012 and 2013, MD dispensers were installed all-year round. In 2014, MD dispensers were put in place between July and December, during P. interpunctella adult flight activity. In 2011, 6 MD dispensers were installed. The MD dispensers were replaced with new ones every 2 months; in 2012, the number of MD dispensers was increased to 25, resulting in one dispenser per 50 m2 throughout the entire chocolate factory area.

Parasitoid release

During 2014, adults or pupae of *H. hebetor* (from Biologische Beratung Ltd., Berlin, Germany) were released within the chocolate factory. Starting from April 2014, 40 units containing 30 pupae or 30 adults each were released monthly (April 7, May 9, June 25, July 21, August 20, September 22, and October 19). Consequently, about 1200 pupae or adults were released monthly, representing a total of ca. 8400 individuals of *H. hebetor*.

Monitoring of *P. interpunctella* adult males

P. interpunctella adult males were monitored (as a control) by 7 pheromone-baited sticky traps (X-lure R.T.U. Combo 4, Russell IPM), each baited with 1 mg TDA. The traps were checked every 7 d. The TDA lures were replaced at 2 monthly intervals. The traps were placed at a height of 2.0–2.5 m above the ground. Monitoring started on March 31, 2011 and ended in December 2014. The following monitoring periods were analyzed: from April 11 to December 3, 2011; from May 14 to October 14, 2012; from June 10 to December 15, 2013; and from February 17 to November 9, 2014.

Monitoring of *P. interpunctella* oviposition

A Petri dish cup containing 15 g crushed peanuts was placed near each pheromone monitoring trap, which was used as a control oviposition trap. Seven oviposition Petri dish cups were placed throughout the entire facility (Cups: Cu1–Cu7). The cups were replaced on each trap-check date (i.e., at 7-d intervals), and the old cups were brought to the laboratory, where they were placed in incubators at 27.5 °C and 70% relative humidity after 45–60 d, the Petri dish cups were examined for emerging individuals in most cases, larval stages and usually last-instar larvae.

Data analysis

he number of *P. interpunctella* males caught in the traps during 2011–2014 was analyzed using the Duncan test and compared using Kruskal–Wallis one-way analysis of variance on ranks, and the means were separated using the Tukey's Test ($P < 0.05$) (using SigmaStat software, San Jose, CA, USA).

Results

The number of *P. interpunctella* males that were trapped in different areas of the chocolate factory between 2011 and 2014 is reported in Figures 1 and 2. Figure 2 and Tables 1 and 2 depict the capture trends from 2011 to 2014 in the pheromone monitoring traps. The catches obtained during the 4 years differed, and a progressive decrease in the number of *P. interpunctella* caught from the second to the fourth year of monitoring was observed. In all years of the experiment, an increase in the number of individuals caught during the hottest months (late spring and summer) was observed. During the cold months, low temperatures negatively affected the population of *P. interpunctella*, resulting in fewer trapped adults. From 2011 to 2014, the monitoring traps caught 2127 moths, and during 2011, 2012, 2013, and 2014, pheromone traps caught 706, 784, 416, and 221 moths, respectively.

The mean catches of individual traps from 2011 to 2014 are presented in Table 3. There was a significant effect of trap location on the number of moths caught (Kruskal– Wallis one-way analysis of variance on ranks, H = 23.568, df = 6, P < 0.001). However, only traps 2 and 3 caught significantly more moths than trap 7. Trap 2 caught the highest number of *P. interpunctella* individuals (mean ± SD 127.50 ± 41.66), whereas trap 7 caught the fewest individuals (20.25 ± 1.66). This might be due to their location in the chocolate factory; trap 2 was located in the packaging area, which was characterized by windows and a door that can facilitate the migration of moths inside, which are attracted by odors of the sweet products. Traps 7 and 4 were placed in the production area of nougat, a seasonal sweet; when the machinery is operational, the temperature is high enough to impede colonization of the area by moths.

The number of *P. interpunctella* individuals trapped during the whole period of MD and MD + parasitoid experimentation in 2011–2014 decreased in all traps. The overall trend of the weekly catches from 2011 to 2014 is shown in Figure 2. Following the start of MD, a decrease in the number of *P. interpunctella* moths caught in monitoring traps was observed and the number ranged from 706 in 2011 to 416 in 2013. A further decrease in the population was observed during 2014, when MD was combined with the release of *H. hebetor* parasitoids, with 221 moths being caught in the monitoring traps.

In 2011 and 2013, the number of *P. interpunctella* individuals captured was extremely high before the beginning of the MD programme, with a rapid decrease in adults observed soon after the placement of new MD dispensers. That situation was less evident during 2014 when *H. hebetor* was released, in addition to MD activity (Figure 2).

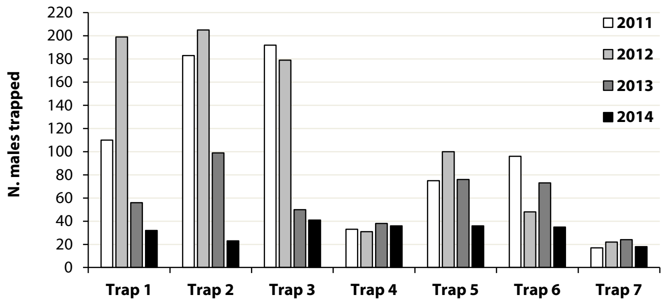

Fig. 1 *Plodia interpunctella* males caught by different pheromone traps 1-7 from 2011 to 2014.

The percentage reduction in moth captures between 2012 and 2013 reflected the increase in the number of dispensers (from 6 MD dispensers to 25 MD dispensers) and was 46.94% (Table 3). During the MD period in 2012, captures ranged from 22 (trap 7) to 205 (trap 2); in 2013, captures per trap ranged from 24 (trap 7) to 99 adults (trap 2) (Table 1). During 2014, when control with MD was only performed from July to November, and was associated with H. hebetor parasitoids, the number of trapped moths was lower, with a 46.87% reduction compared to that in 2013 (Table 4). In 2014, the moth captures per trap varied from 18 (trap 7) to 41 (trap 3) (Table 1). In 2014, the mean number of *P. interpunctella* individuals caught by each trap was significantly lower than at the beginning of the experiment (2011–2012) (Table 2).

The presence of larvae in the oviposition cups was observed occasionally during the monitoring period from 2011 to 2014. Except for positions Cu1, Cu3 and Cu5, all positions were infested at least once (Table 4).

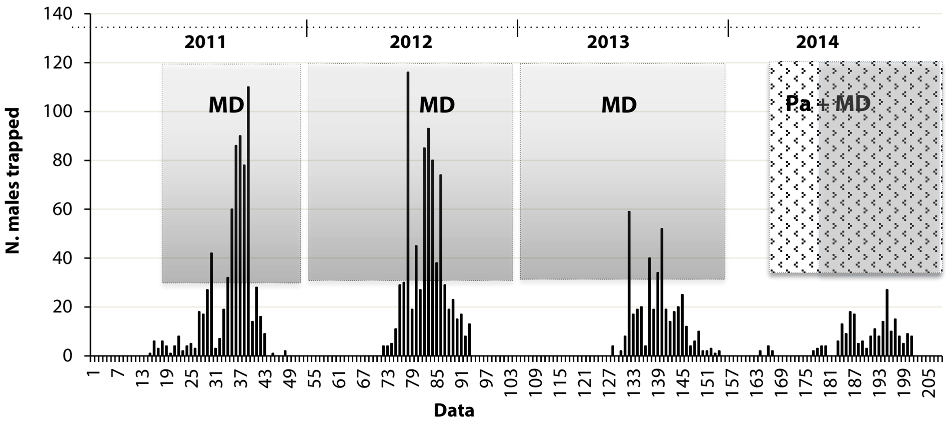

Fig. 2 Number of *Plodia interpunctella* males caught during 2011-2014 with indication of mating disruption treatments (MD) and parasitoids release (Pa).

Discussion

A reduction in the number of *P. interpunctella* moths in pheromone-baited monitoring traps was observed after placing MD dispensers at the beginning of the annual flight period of *P. interpunctella* (in May) and also after increasing the density of MD dispensers from a localized application to a general application (Figure 2). After placing the MD dispensers according to the protocol of 1 dispenser per 50 m2, the number of moths caught in the monitoring traps was drastically reduced and this trend was evident throughout the entire experimental period, particularly from 2012 to 2014. All sites showed a decrease in the number of trap catches after the introduction of MD. At 2 sites with low initial moth catches (trap 4 and trap 7 areas), the trap catches after the introduction of MD remained constant. In contrast, in areas with a higher initial population density, the reduction in trap catches was initially very low, especially in 2011, when MD dispensers were placed only in a small area of the chocolate factory. In 2013 and 2014, there was a clear reduction in the number of individuals caught in traps 1, 2, 3, and 5, especially in 2014, when *H. hebetor* parasitoids were used against *P. interpunctella* larvae, in addition to MD treatment; it is known that MD is more effective at low population densities (Trematerra et al., 2011; Trematerra, 2012).

Tab. 1 Mean number (± SD) of *Plodia interpunctella* adults caught in pheromone traps 1–7 from 2011 to 2014, per trap.

Trap	Area	Mean number of trapped moths (± SD)
1	Almond brittle	99.25 ± 37.04 ab
2	Packaging	127.50 ± 41.66 a
3	Dried fruit	115.50 ± 40.55 ab
4	Nougat	34.50 ± 1.56 ab
5	Ovens	71.75 ± 13.25 ab
6	Cooling	63.00 ± 13.54 ab
7	Nougat	20.25 ± 1.66 b

Data followed by a different letter differ significantly (Tukey's test, $P < 0.05$).

Tab. 2 Mean number (± SD) of *Plodia interpunctella* male adults caught in pheromone traps 1–7 from 2011 to 2014.

Year	Mean number of trapped moths (± SD)

2011	100.86 ± 25.58 b
2012	112.00 ± 30.71 b
2013	59.43 ± 9.57 ab
2014	31.57 ± 3.08 a

Data followed by a different letter differ significantly (Duncan test, $P < 0.05$).

Tab. 3 Changes in mean adult males trapped from 2012 to 2014 after MD and parasitoids (Pa)+MD treatments.

Traps	**Adult males trapped**					
	Years			**MD vs. MD**		**MD vs. Pa+MD**
	2012	**2013**	**2014**	**2012 vs. 2013**	**2012 vs. 2014**	**2013 vs. 2014**
1	119	56	32	- 52.94	- 73.11	- 42.86
2	205	99	23	- 51.71	- 88.78	- 76.77
3	179	50	41	- 72.07	- 77.09	- 18.00
4	31	38	36	+ 22.58	+ 16.13	- 5.26
5	100	76	36	- 24.00	- 64.00	- 52.63
6	48	73	35	+ 52.08	- 27.08	- 52.05
7	22	24	18	+ 9.09	- 18.18	- 25.00
1-7	784	416	221	- 46.94	- 71.81	- 46.87

Tab. 4 Chocolate factory 2011–2014, control dates with *Plodia interpunctella* infestation in oviposition cups (Cu1-Cu7), all other dates without infestation.

Petri dishes	Data	Cu1	Cu2	Cu3	Cu4	Cu5	Cu6	Cu7
2011	4.XI	-	-	-	yes	-	-	Yes
25.XI		-	-	-	-	-	-	-
2012	13.I	-	-	-	yes	-	-	-
17.II		-	-	-	yes	-	-	-
4.V		-	-	-	-	-	-	Yes
8.VI		-	-	-	-	-	yes	-
2013	12.VII	-	yes	-	-	-	-	-
6.IX		-	-	-	yes	-	-	-
2014	1.VIII	-	-	-		-	yes	-

During the MD application period (2011–2014), the moth density, overall moth activity and degree of infestation was remarkably lower than in previous years without such treatment. In addition, the chocolate factory manager reported that fewer customer complaints were made in 2014 than in 2011.

In Europe, stored-product moths are among the most serious pests in stored grain, in the retail trade, mills, the food processing industry and private households. The use of pheromones to control populations of stored-product moths has been demonstrated (Trematerra, 2012) and MD has proven successful against stored-product moths in commercial field settings (Ryne et al., 2001, 2006, 2007; Burks et al., 2011; Trematerra et al., 2011; Burks and Kuenen, 2012; Trematerra and Savoldelli, 2013). In addition, other methods that involve pheromones in mass trapping (Phillips et al., 2000; Trematerra & Gentile, 2010), attract-and-kill methods (Trematerra and Capizzi, 1991; Phillips et al., 2000; Nansen and Phillips, 2004; Campos and Phillips, 2013, 2014) and the auto-confusion system (Trematerra et al., 2011) have been successfully used to manage stored-product moths. However, in these cases, complete elimination of the moth infestations was never achieved. The monitoring of pyralid moths using pheromone traps is likely to be affected by MD. One alternative to pheromone traps for monitoring is the use of water traps; however, these have also been shown to have a control effect (Trematerra and Savoldelli, 2013). In this study, oviposition cups were used as an alternative approach to pheromone traps; however, the number of infested cups was too low to analyze the population trends based on the data.

In the present experiment, the release of the parasitoid *H. hebetor* against *P. interpunctella* larvae helped to reduce the residual moth population in the chocolate factory in 2014 (Trematerra et al., 2017).

In Italy, the use of parasitoids in food facilities is rare, and our study represents one of the first applications. In Europe, and particularly in Germany, parasitoids have been evaluated in commercial food-processing facilities since 1995, and they have been commercially available since 1998. In the United States, insect natural enemies were technically designated as insecticides, in order to be regulated, and then they were exempted from a requirement for a tolerance level in food.

Recently, Trematerra (2013) suggested that combining and integrating different management tools and the careful selection and timing of different approaches, together with an understanding of pest behavior and ecology can be more effective in pest control.

According to our results, the combination of MD and biological control can be used to control *P. interpunctella* infestation in a chocolate factory. These techniques must be considered as part of an Integrated Pest Management programme and should not be considered in isolation. MD is effective against *P. interpunctella* adults, but cannot control larval and pupal stages; in contrast, *H. hebetor* can attack larval instars, and could also be used against overwintering larvae in warehouses or food facilities, when environmental conditions are suitable.

The use of insect natural enemies and MD in an integrated approach against stored-product moths is an opportunity for the biologically based management of storage pests. MD combined with natural enemies has been shown to be promising, and might be especially relevant in the organic food industry when *P. interpunctella* and other pyralid moths are the target pests.

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Host-age preference of *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae), a larval parasitoid of the lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) and the cowpea weevil, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae)

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Abstract

The pteromalids (Hymenoptera: Pteromalidae) *Anisopteromalus calandrae* (Howard), *Dinarmus basalis* (Rondani), *Lariophagus distinguendus* (Förster), *Pteromalus cerealellae* (Ashmead) and *Theocolax elegans* (Westwood) are solitary larval ectoparasitoids used to suppress several species of stored-product insects that infest storage grains. We investigated host-age preference of *T. elegans* using no-choice laboratory experiments. Lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) larvae (9, 11, 13, 15, 17, 19, 21 and 23 days-old) in wheat grain kernel and cowpea weevil, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) larvae (5–19 days-old) in cowpea beans were exposed to neonate *T. elegans* mated females to lay their eggs for two days. Our results showed that the highest number of parasitoids emerged from 23 days-old *R. dominica* larvae. The numbers of parasitoids emerged from 19, 21 and 23 days-old *R. dominica* larvae were statistically significantly different in experiments (F-test, 0.05). Progeny of *T. elegans* reared from *R. dominica* and *C. maculatus* larvae were either fully-winged (macropterous), short-winged (brachypterous) or wingless (apterous). Female *T. elegans* were rarely host-feeding on *C. maculatus* larvae. *Theocolax elegans* progeny were emerging from 14 days-old *C. maculatus* larvae only. We discussed insectary mass production of *T. elegans* for biological control.

Keywords: Biological control, *Callosobruchus maculatus*, host-age preference, *Rhyzopertha dominica*, stored-product insects