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RESEARCH ARTICLE

Evaluation of the parasitoid *Copidosoma koehleri* for biological control of the potato tuber moth, *Phthorimaea operculella*, in Israeli potato fields

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The potato tuber moth *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) is a major agricultural pest of solaneceous crops in warm countries worldwide. The encyrtid polyembryonic parasitoid *Copidosoma koehleri* (Blanchard) has been successfully introduced for biological control of the moth in potato fields in South Africa and Australia; however, augmentative releases of the parasitoid in trial plots and in commercial potato fields in Israel did not reduce pest populations or infestation levels more than chemical treatment. *C. koehleri* accounted for 4–5% of parasitism on tuber moth caterpillars, while most parasitism was due to local species of larval parasitoids. The abundance and composition of local parasitoids did not differ between *C. koehleri* release plots and conventionally treated control plots. These findings can be interpreted as failure of the introduced parasitoids to survive and locate their hosts, or as mortality of *C. koehleri* within hosts in the field. Sentinel hosts, placed in trial plots and collected after 24 h, were rarely parasitized by *C. koehleri*, supporting the first interpretation. To test the second hypothesis, hosts parasitized by *C. koehleri* were placed in field plots for a week, collected, and reared out in the laboratory. The emergence rates of *C. koehleri* from these hosts resembled those of lab-reared controls, suggesting that mortality within hosts in the field is not a major cause of *C. koehleri*'s poor biocontrol performance.

Keywords: augmentative biocontrol; *Copidosoma koehleri* (Hymenoptera: Encyrtidae); local natural enemies; *Phthorimaea opeculella* (Lepidoptera: Gelechiidae); potato field

Introduction

The potato tuber moth (PTM, *Phthorimaea operculella* Zeller, Lepidoptera: Gelechiidae) is a major economic pest of solanaceous crops in warm countries around the globe (Kfir 2003). The larvae infest mainly the leaves and fruit of potatoes, tomatoes, eggplant and tobacco (Das and Raman 1994). The potato tuber moth can complete several generations within a 4-month potato spring growing

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season, and can thus build up its populations (Kroschel and Koch 1986). Adult PTM may migrate into potato fields from nearby volunteer plants and tuber dumps. Larvae feed on the leaves of the young plants at the beginning of the season (Coll and Yuval 2004). The leaves wilt when the tubers reach their final size, and the plants are subsequently defoliated by the growers. Tubers are left in the soil for three additional weeks before harvest to let their skin stabilize. When the foliage starts to wilt, PTM larvae migrate to the tubers through cracks in the soil. The larvae damage leaves and tubers directly by boring characteristic tunnels. These tunnels allow secondary infestation by fungi and bacteria.

Control methods of PTM include chemical and cultural treatment. Currently used products include the organo-phosphate methamidophos, the carbamate Cartap and the growth regulator Valnuron, applied to the foliage (Coll, Gavish, and Dori 2000). Cultural methods aim to reduce the access of PTM larvae to tubers through deep planting, ridging of soil to cover the tubers, and sealing soil cracks through pressing and appropriate irrigation protocols (Ali 1993; Coll et al. 2000). Exposure to PTM is also reduced through selection of PTM-resistant potato varieties, and early harvesting (Fenemore 1980; Hanafi 1999). Additional approaches to the control of PTM include mass-trapping using pheromone traps, treatment with *Bacillus thuringiensis* (Bt) products, application of granulosis viruses, and genetic modification of crop plants to express the Bt-toxin (Hanafi 1999; Mohammed et al. 2000).

PTM infestation of tubers was shown to increase with increased numbers of insecticide applications, possibly because of negative impacts on its natural enemies (Coll et al. 2000). This finding draws attention to the possible role of predators and parasites in controlling populations of this pest. Numerous predators and parasitoids of PTM were recorded in surveys in the USA, India, Ethiopia, Australia and Israel (Flanders and Oatman 1987; Trivedi and Rajagopal 1992; Horne 1993; Coll et al. 2000; Mulatu, Applebaum, and Coll 2004).

In the present study we focus on the evaluation of one PTM parasitoid, the encyrtid *C. koehleri*, for augmentative biological control in potato fields. This in an egg-larval polyembryonic parasitoid of South American origin. Female wasps lay their eggs inside PTM eggs, and each egg cleaves repeatedly to form a clone of 30–50 genetically identical embryos, while the host goes through four larval instars. The wasp embryos feed on the host's tissues, eventually killing it just before pupation. *C. koehleri* exhibits several traits that are considered advantageous for biological control agents, such as an exotic origin, high host specificity (van Lenteren et al. 2003), high reproductive rate (Lane, Mills, and Getz 1999) and targeting of an early stage in the host's life cycle (Murdoch and Briggs 1996). The successful introductions of *C. koehleri* into South African potato fields for PTM classical biocontrol provided an additional incentive for evaluating this natural enemy in Israel (Horne 1990; Kfir 2003). On the other hand, previous introductions of the parasitoid into fields in Israel and Italy did not lead to its widespread establishment (Berlinger and Lebiush-Mordechi 1997; Pucci, Spanedda, and Minutoli 2003). The reasons for the parasitoid's failure to establish were not documented. We therefore tested *C. koehleri*'s efficiency as an augmentative biocontrol agent, i.e., we mass-reared the parasitoid and released it repeatedly in potato fields, during two spring growing seasons. We evaluated survival of *C. koehleri* adults and their rates of parasitism in potato fields, and quantified

mortality of parasitized hosts and parasitoid development within hosts under field conditions. Additionally, we collected data on the abundance and composition of local parasitoids of PTM, with and without augmentation by *C. koehleri*.

Materials and methods

General study conditions

The study was carried out during March–July of 2003–2006 in experimental plots and in commercial potato fields in the Western Negev, Israel's main potato growing region. Mean daily temperatures during this season ranged from 8.8 to 25.7°C (early March), and from 19.1 to 33.8°C (early July). Measurements of temperature and humidity under the potato canopy in mid-June show a daily relative humidity range of 40–100%, and a daily temperature range of 17–41°C. PTM infests 5–12% of potato tubers in commercial fields in the area, depending on growing season, potato cultivar, and agro-technical practice (unpublished data by YAHAM, the local potato growers' association). *C. koehleri* were mass-reared at Bio-Bee Ltd., Israel, and released as adults in 100-mL plastic cups containing 1000–10,000 individuals. The cups were supplied with honey-soaked filter paper strips to allow wasps to feed before dispersing, because feeding markedly improves adult longevity (Baggen and Gurr 1998).

Trial plot experiment

This experiment was aimed at comparing the efficiency of *C. koehleri* with the conventional chemical control of PTM. A second aim was to characterize the assemblage of PTM's local parasitoids in the presence and absence of *C. koehleri*. Sixteen 10 × 10-m potato trial plots (cv. Desiree) were established near Habessor Experimental Farm in early March, 2004. Adjacent plots were separated by 50 m of bare soil. The soil was sprayed with herbicide at the beginning of parasitoid release, and 6 weeks later, to reduce insect migration among plots through the vegetation. Aldicarb (Temik) was applied at planting to all plots to control the leafminer *Liriomyza huidobrensis* (Blanchard) on young seedlings. This systemic carbamate pesticide is absorbed by plant roots and is translocated throughout the plants, killing insects and mites that feed on them. Carbamate does not directly interfere with *C. koehleri*, which does not feed on potato vegetative tissue. However, indirect effects on *C. koehleri* through feeding on poisoned PTM larvae cannot be ruled out. We allocated plots into four experimental treatments arranged in a Latin square design. In three of the treatments, we released *C. koehleri* parasitoids at *high* (altogether 400,000 adult parasitoids/hectare), *medium* (200,000 adults/hectare) or *low* (40,000 adults/hectare) dosages, divided into four releases during the first 6 weeks of the growing season. In the fourth *conventional control* treatment, we used standard chemical treatment to control PTM (a single application of valnuron, 3 weeks before defoliation), without release of *C. koehleri*. Each treatment was replicated four times. We walked each plot for 15 min in search of leaves with fresh PTM tunnels (these leaves are likely to contain larvae). We conducted the first sampling 1 week before the first *C. koehleri* release, and continued sampling at weekly intervals until defoliation, 11 weeks after the first *C. koehleri* release. Thus, each plot was sampled for 12 weeks. We kept the leaves under PTM rearing conditions in the laboratory (Berlinger and

Lebiush-Mordechi 1997), and recorded the number of PTM, *C. koehleri* and other parasitoids that emerged from the samples. We also sampled tubers for PTM infestation at harvest. For each sample we harvested all tubers in three randomly determined strips of 2×1.4 m in each plot (ca. 500 tubers), and weighed them. We determined the number of PTM-infested tubers per sample, and weighed them as well. We estimated the fraction of the crop lost to PTM infestation by dividing the mass of the infested tubers by total sample mass.

Commercial field experiment

This experiment aimed to study the combined effect of *C. koehleri* with chemical control on PTM infestation, as compared to chemical treatment only. An additional aim was to test *C. koehleri*'s biocontrol potential under realistic cultivation conditions. Fifteen commercial fields of late-harvested PTM-susceptible potato varieties were used for the experiment in the spring of 2005. Rectangular fields that were at least 10 ha in area, and separated from each other by more than 500 m, were selected. The fields were separated from one another by wild vegetation and roads. Soil type, potato variety and agro-technical practice varied among fields. We marked four 20×20 m areas in each field. Three of the areas were located at the corners of the field, and the fourth area was marked 50 m diagonally from a corner, toward the field's center. We released *C. koehleri* in two dosages in two areas at diagonal field corners, and used the remaining areas as untreated controls. The *high-dosage* treatment consisted of 200,000 wasps/ha, released in equal amounts over the first 3 weeks of the experiment. In the *low-dosage* treatment we released 100,000 wasps/ha, using the same schedule. The third corner area was monitored as a *margin control* area, in the absence of *C. koehleri*, because PTM is known to primarily infest field margins. We also sampled the untreated *center control* areas, located 50 m away from the high-dosage release areas towards the center of the field, as estimates to PTM infestation levels away from field edges. An additional aim of the center control areas was to detect possible migration of the parasitoids released in the high-dosage areas. We sampled PTM infestation in leaves using the same protocol as in the trial plot experiment. The first sampling (pre-release) was conducted immediately before the release of the parasitoids. The first post-release sampling was conducted 2 weeks after parasitoid release, and was followed by five additional sampling sessions at weekly intervals. Most fields were defoliated during the last week of leaf sampling. We sampled all tubers from three 2×2 m quadrates in each plot at weekly intervals between defoliation and harvest. Depending on harvest date, this protocol yielded 2–3 sampling days per plot. We recorded infestation rates in the tuber samples (number of infested tubers/total number of tubers sampled). We reared out the infested tubers in the laboratory and determined parasitism levels on the infesting PTM larvae.

Host release-recovery experiments

Sentinel host experiment

The aim of this experiment was to evaluate the ability of *C. koehleri* adults to survive and parasitize their hosts in the field. A total of 10×10 m trial plots, planted with potatoes (cv. Desiree) and 25 m apart, were established near Habessor Experimental Farm in 2003. The strips between the plots were hoed repeatedly during the growing

season to prevent weed growth. In treatment plots, adult *C. koehleri* were released *once*, *twice* or *four* times during the first 6 weeks of the growing season. The total release dosage was 2,500,000 wasps/ha in all three treatments. Control plots received no biological or chemical control. Each release frequency was replicated in five plots, using a Latin square design. Parasitoids were released on fixed dates, regardless of the moth population densities. We set up sentinel hosts by attaching 20 PTM eggs to a potato tuber, using chicken egg-white as an adhesive. We placed three such tubers under the foliage of one plant in a margin row of each plot at weekly intervals for 9 weeks, and collected the tubers 24 h later. Thus, *C. koehleri* (but not local parasitoids, which attack larval stages) had an opportunity to parasitize the hosts while they were in the field. We reared out the tubers in the laboratory, and recorded the number of emerging PTM and *C. koehleri* adults.

Parasitized host experiment

The aim of this experiment was to evaluate the ability of *C. koehleri* juveniles, developing inside hosts, to complete their development under field conditions. Parasitoids may die as juveniles due to mortality of parasitized hosts (i.e., hosts die before the parasitoids have completed their development), or due to mortality of juvenile parasitoid stages within the host's body. One possible cause for mortality (of hosts and/or juvenile *C. koehleri*) could be superparasitism by local larval parasitoids. To evaluate mortality of parasitized hosts, we compared the survival of parasitized versus non-parasitized hosts in the field and in the laboratory. To evaluate the mortality of *C. koehleri* juveniles inside hosts, we assessed their emergence rates from field-reared versus lab-reared parasitized hosts.

Thirty to 100 mated PTM females oviposited on tissue paper for 24 h. One half of the papers were exposed to parasitism by *C. koehleri* for an additional 24 h in a standard insectary cage. The remaining oviposition papers were left unparasitized. We cut the papers into small (ca. 2×1 cm) fragments (114 parasitized, 110 non-parasitized), and counted the number of hosts on each fragment. We stapled each fragment to the foliage of a potato plant, along the margin of a single potato field, alternating between parasitized and non-parasitized fragments. We collected the branches that carried the hosts 8 days later, and searched them for PTM larvae. We reared out these larvae in the laboratory, and determined the composition of emerging insects. As controls, we reared parasitized ($n = 10$ paper fragments carrying hosts) and non-parasitized hosts ($n = 10$ paper fragments) under laboratory conditions, without previous field exposure. We recorded host survival and *C. koehleri* emergence rates in all treatments.

Data analysis

The trial plot and the commercial field experiment were sampled repeatedly for the effects of *C. koehleri* on the number of infested leaves and the number of sampled PTM. The sentinel host experiment was sampled repeatedly for the effects of *C. koehleri* on sentinel parasitism levels. We used repeated-measures ANOVAs to analyze these effects. We used week number as the within-subject variable, and experimental treatment as the between-subject variable. In the trial plot and the sentinel host experiments we treated each plot as an independent data point. In the

commercial field experiment, we controlled for differences among the fields by treating the field variable as a covariate.

Several plots in the field experiments did not contain any PTM in all sampling sessions, yielding missing values for parasitism rates. We were therefore unable to use repeated-measures procedures to test for treatment effects on parasitism rates, and used mixed linear models instead. In these analyses, we treated parasitism rates as the dependent variable, and treatment as a fixed factor. We defined week as a repeated effect. Parasitism proportions were arcsine-transformed prior to analysis.

In the field experiments, we tested for pre-release differences between plots using one-way ANOVA, with field number defined as a random effect. We used SPSS 14.0 for all statistical analyses.

Results

Trial plot experiment

The pre-release sampling yielded only six PTM-infested leaves, and a single PTM individual, from all experimental plots combined. The number of infested leaves increased during the first half of the growing season in all treatments. Later in the season the number of infested leaves stabilized, and eventually decreased as the foliage wilted (Figure 1). The effect of week on the number of infested leaves was statistically significant, but the effect of parasitoid dosage was not (repeated-measures ANOVA with Greenhouse–Geisser correction: $F_{4.7,57.3}=34.588$, $P<0.001$ for week of experiment; $F_{3,\ 12}=0.329$, $P=0.804$ for dosage). Similarly, week of experiment, but not parasitoid dosage, significantly affected the number of PTM individuals sampled per plot ($F_{5.9,61.2}=18.321$, $P<0.001$ for week of experiment; $F_{3,12}=1.216$, $P=0.346$ for dosage). The proportions of hosts parasitized by any parasitoid increased with the growing season in all treatments (Figure 1), but did not

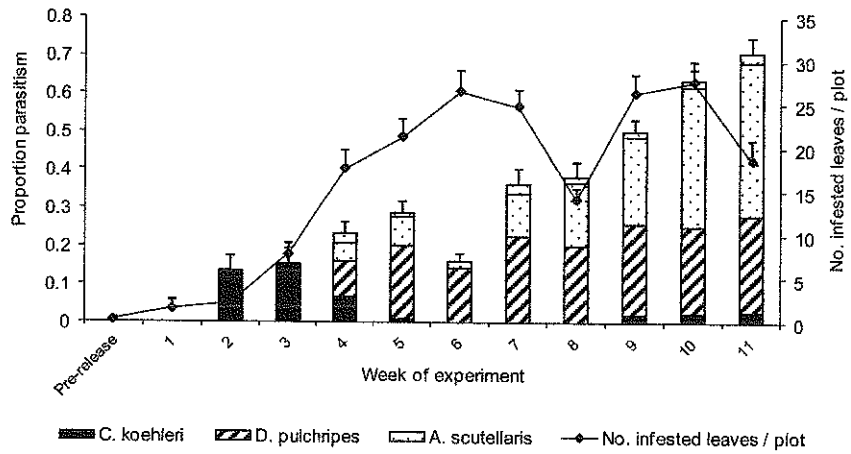

Figure 1. Left *y*-axis: Mean (+SE) proportion of parasitized hosts (by all parasitoid species together) per plot in the trial plot experiment, all treatments combined. The relative frequency of *C. koehleri* versus the two dominant local species is indicated within each bar. Right *y*-axis: Mean (+SE) number of infested leaves per plot, all treatments combined.

vary significantly among release dosages (mixed linear model, $F_{4,106.6}=1.892$, $P=0.117$). The main parasitoids recovered were *Diadegma pulchripes* Kokujev (Ichneumonidae) (47.9% of 1149 recovered parasitoid individuals) and *Apanteles scutellaris* Muesebeck (Braconidae) (44.0% of recovered parasitoids). Sixty-two larvae parasitized by *C. koehleri* were collected, mainly on weeks 5 and 10–11 of the experiment, and accounted for 4.4% of the total parasitism. Other parasitoids included *Bracon gelechiae*, *Temelucha decorata*, and five additional unidentified braconids. The number of species, and individuals, of local parasitoids sampled per plot was significantly affected by week of experiment, but not by *C. koehleri* dosage (No. of species: repeated-measures ANOVA: $F_{3.35,\ 55.28}=6.931$, $P<0.001$ for week of experiment, $F_{3,\ 12}=0.623$, $P=0.614$ for dosage; No. of Individuals: repeated-measures ANOVA: $F_{2.89,34.69}=10.017$, $P<0.001$ for week of experiment, $F_{3,12}=0.866$, $P=0.485$ for dosage, Table 1). The mean ($\pm$SE) proportion of PTM-infested tubers at harvest, combined over all plots, was 0.072 ± 0.045. Tuber infestation rates did not significantly differ among release dosages (ANOVA: $F_{3,12}=0.143$, $P=0.93$). Parasitism on PTM in tubers was not determined.

Commercial field experiment

The number of infested leaves and of PTM individuals did not vary significantly among areas allocated to different treatments in the pre-release sampling ($F_{3,\ 56}=2.51$, $P=0.07$ for infested leaves, $F_{3,\ 42}=1.14$, $P=0.34$ for PTM numbers). Leaf infestation levels were much lower than in the trial plot experiment, and did not show a clear temporal trend (Figure 2). Leaf infestation differed significantly among sampling weeks, but not among *C. koehleri* release dosages (repeated-measures ANOVA with Greenhouse–Geisser correction: $F_{1.9,\ 39.7}=4.283$, $P=0.02$ for week of experiment; $F_{3,\ 21}=1.70$, $P=0.20$ for dosage). The numbers of sampled PTM in foliage were not significantly affected by the week of experiment or by release dosage ($F_{2.3,\ 39.4}=1.38$, $P=0.27$ for week of experiment; $F_{3,\ 17}=2.22$, $P=0.12$ for dosage).

Local larval parasitoids emerged from two out of six PTM larvae collected in the pre-release sampling (one from a high-dosage area, the second from a low-dosage area). No parasitized hosts were collected in the center control areas throughout the experiment. PTM larvae in the leaves were parasitized mainly by *Diadegma pulchripes* (in 40.1% of 74 parasitized hosts) and *Bracon gelechiae* (31.1%). *C. koehleri* emerged from 5.4% of the parasitized hosts (Figure 2).

Table 1. Local parasitoids in the trial plot experiment.

Treatment	Mean ($\pm$SE) species/plot/week	Mean ($\pm$SE) individuals/plot/week
High dosage	1.63 ± 0.15	7.19 ± 1.62
Medium dosage	1.81 ± 0.18	7.47 ± 1.20
Low dosage	1.63 ± 0.15	8.19 ± 1.60
Conventional control	1.72 ± 0.16	5.34 ± 0.83

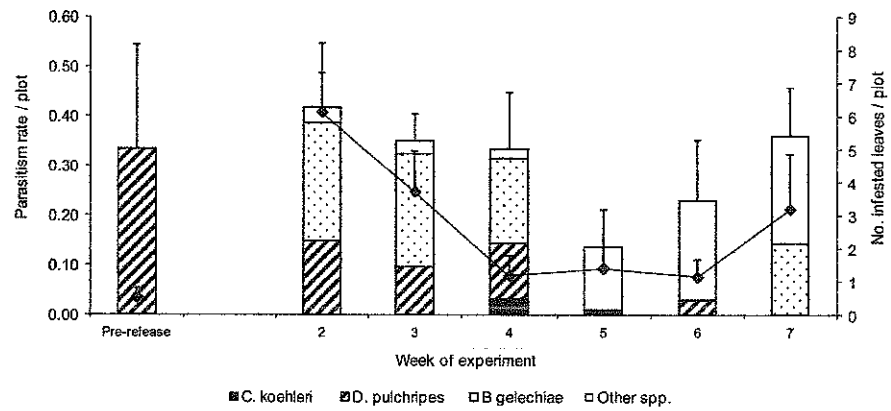

Figure 2. Left *y*-axis: Mean (+SE) proportion of parasitized hosts (by all parasitoid species together) per plot in the commercial field experiment, all treatments combined. The relative frequency of *C. koehleri* versus the two dominant local species is indicated within each bar. Right *y*-axis: Mean (+SE) number of infested leaves per plot, all treatments combined.

The mean ($\pm$SE) proportion of PTM-infested tubers, combined over all sampled areas, was 0.069 ± 0.009 ($n = 33$ samples) at defoliation. Ten days later, this proportion increased to 0.077 ± 0.007 ($n = 39$), and reached 0.097 ± 0.011 ($n = 26$) at harvest. The proportions of infestation at harvest were not significantly affected by *C. koehleri* release dosage ($F_{3,\ 14} = 0.84$, $P = 0.49$). Similarly, dosage did not affect the number of PTM that emerged from the sampled tubers at harvest ($F_{3,\ 14} = 0.96$, $P = 0.44$). Parasitoids emerged from 16 tuber samples, i.e., 0.9% of the samples. Two of these samples yielded *C. koehleri*.

Low parasitism rates by *C. koehleri* were recorded in the trial plot experiment, and in the commercial field experiment. We investigated possible reasons for the parasitoids' low performance through the two host release-and-recovery experiments.

Host release-recovery experiments

Sentinel host experiment

A mean ($\pm$SE) of 2.36 ± 0.15 PTM and 0.16 ± 0.39 mummies parasitized by *C. koehleri* developed from each of the tubers that carried 20 sentinel hosts ($n = 540$ tubers, combined over all dates and plots). Week of experiment, but not parasitoid release frequency, significantly affected the number of PTM that developed per tuber (repeated-measures ANOVA with Greenhouse–Geisser correction: $F_{4.4,\ 244.5} = 20.481$, $P < 0.0001$ for week of experiment; $F_{3,\ 55} = 8.314$, $P = 0.374$ for release frequency). Similarly, the number of parasitized sentinel hosts per tuber was significantly influenced by week, but not by *C. koehleri*'s release frequency (repeated-measures ANOVA: $F_{8,\ 440} = 4.988$, $P < 0.0001$ for week of experiment; $F_{3,\ 55} = 0.174$, $P = 0.873$ for release frequency). Notably, no *C. koehleri* emerged from any sentinel host in some weeks of the experiment (Figure 3).

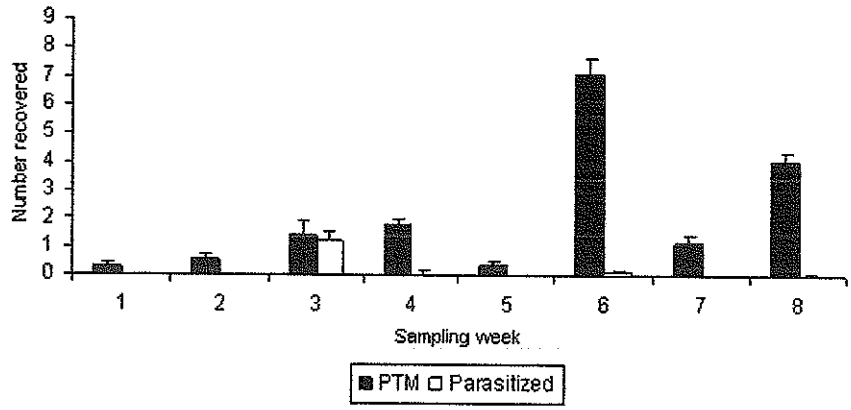

Figure 3. Mean (+SE) number of emerging PTM and *C. koehleri* broods, per replicate, in the sentinel host experiment. Each replicate consisted of 20 hosts, glued to a tuber and placed in a potato lot for 24 h.

Parasitized host experiment

The proportions of hosts that survived to emergence of an adult insect (PTM, *C. koehleri* or a local parasitoid) varied significantly among treatments ($F_{3,\ 237} = 55.54$, $P < 0.001$), and were much lower for hosts placed in the field for 8 days than for the lab-reared controls (Figure 4). Local parasitoids emerged from some of the surviving field-reared hosts (both parasitized and non-parasitized), but not from lab-reared hosts. This suggests that local larval parasitoids successfully oviposited into some field-reared hosts. The emergence frequency of *C. koehleri* from hosts that had been exposed to the parasitoids for 24 h hosts did not differ significantly between the field-reared and the lab-reared treatments ($t_7 = 1.41$, $P = 0.20$).

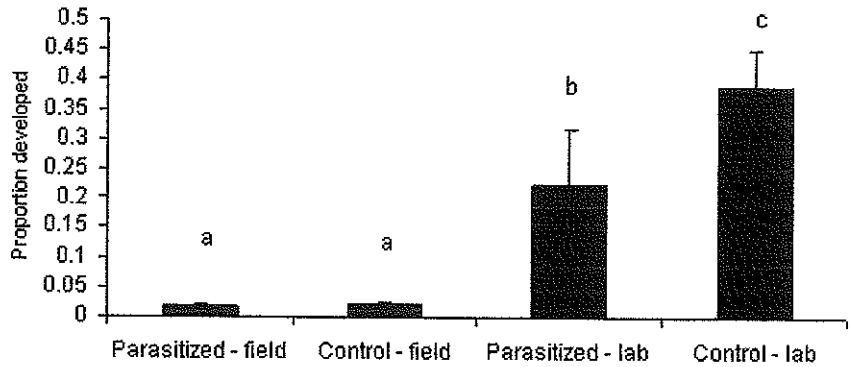

Figure 4. Proportion of parasitized and non-parasitized hosts that completed development after 8 days in the field, or after rearing under laboratory conditions. Different letters denote different grouping by Tukey's *post-hoc* test.

Discussion

Augmentative releases of *C. koehleri* in potato trial plots in Israel were as effective as a pesticide-based control treatment in reducing PTM populations and their damage to foliage and tubers. The application of aldicarb at planting may have caused some PTM mortality in the first weeks of the trial plot experiment. This could possibly complicate the interpretation of the results, because aldicarb is not part of the conventional treatment against PTM in Israel. In the commercial field experiment, *C. koehleri* release combined with insecticide application provided equal control as chemical treatment only. No aldicarb was applied in this experiment, yet *C. koehleri* did not improve PTM control above the level attained by conventional treatment. These findings suggest that *C. koehleri* release, alone or in addition to the current chemical control practices, does not suffice to reduce PTM below economic threshold.

Parasitism rates by *C. koehleri* were low in the trial-plot and the commercial field experiments. This is consistent with previous failures to establish *C. koehleri* as a classical biocontrol agent in Israel (Berlinger and Lebiush-Mordechi 1997). *C. koehleri* was collected, in small numbers, in release as well as in control plots of both experiments. This could indicate that the parasitoids dispersed from their release sites to control site, complicating the interpretation of the results (alternatively, small indigenous populations of *C. koehleri* may exist in the fields). Nevertheless, it is clear that *C. koehleri* did not become the dominant natural enemy in both field seasons, and did not increase in efficiency with increasing release dose.

Why does *C. koehleri* provide more effective control in South Africa than in Israel? One obvious possibility is that abiotic conditions are unfavorable to *C. koehleri* in Israel. Kfir (1981) demonstrated *C. koehleri*'s requirement for high relative humidity, and suggested that it explains the parasitoid's seasonal abundance pattern in South African potato fields. *A. subandinus* is the dominant PTM parasitoid at the beginning of the growing season, while *C. koehleri* dominates the second half of the season. It may be favored by the high-humidity microclimate under the canopy of fully grown potato plants towards the end of the season. In our experiments, however, *C. koehleri* did not increase towards the end of the growing season, reducing the likelihood of a humid microclimate as a limiting factor. High temperatures could have caused some parasitoid mortality, as parasitized hosts do not tolerate temperatures above 32°C (Horne and Horne 1991). The combination of high temperatures and low humidity for several hours a day likely reduced *C. koehleri*'s survival.

Several biotic factors may have limited *C. koehleri* in our experiments as well. Nectar sources for adult feeding improve the parasitoid's survival and parasitism efficiency (Baggen and Gurr 1998). Although we provided honey to the wasps before release, they did not have access to nectariferous plants in the field after they were released in any of the experiments. Additional possible biotic limiting factors include low efficiency of the parasitoid in searching for hosts; low host densities at the times of parasitoid release (perhaps in response to pesticide applications); high host mortality before the parasitoid has completed its development as compared to non-parasitized hosts; and/or low survival of *C. koehleri* juveniles inside hosts, possibly because of superparasitism.

The sentinel host experiment showed that only 6.3% of the recovered hosts were parasitized after a 24-h exposure to high parasitoid densities in the field. This can be due to high adult mortality, or to low searching efficiency in the field. The sentinel host experiment also suggests high mortality of PTM during development, as only 12.6% of the sentinel hosts completed their development to emergence of an adult (PTM or *C. koehleri*) after 24 h in the field.

In the parasitized host experiment, only 2% of parasitized and un-parasitized hosts completed their development after a week in the field. Emergence rates of *C. koehleri* from parasitized hosts were similar under field and lab conditions. The high mortality of hosts in the field concords with the findings of the sentinel host experiment, and suggests that premature mortality of parasitized hosts may prevent the development of the parasitoids, and can therefore further reduce *C. koehleri*'s reproduction under field conditions.

The hypothesis that *C. koehleri*'s failure results from low inter-specific competitive abilities predicts that (a) host individuals parasitized by *C. koehleri* are also attacked by other parasitoids and (b) such super-parasitism leads to lower emergence of *C. koehleri*, as compared to hosts parasitized by *C. koehleri* alone. While the parasitized-host experiment is compatible with the first prediction, the second prediction is not supported: emergence rates of *C. koehleri* from field-reared hosts (exposed to larval super-parasitism) and from lab-reared hosts (protected from larval super-parasitism) were similar. The similarity in composition and abundance of natural enemies among treatments in the trial plot experiment suggests no adverse effects in the opposite direction, namely reduction of local parasitoids by *C. koehleri.*

Our data point to the potential importance of local parasitoids of PTM in reducing the populations of this pest. Parasitism rates by these natural enemies reached 70% in the trial plot experiment. Conservation of food plants and overwintering habitat for these beneficial species may enable them to provide effective control, either with or without augmentation by exotic species.

Rational selection of natural enemies is a key factor that affects success of biological control programs. In this context, success stories are as important as failures in deciding which biocontrol agents are the most promising. Moreover, it is important to identify the factors that contribute to failure, so that they can be controlled in future attempts (Gurr and Wratten 2002). Our experiments contribute to both of these aims.

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AQ1 **References**

Ali, M.A. (1993), "Effects of Cultural Practices on Reducing Field Infestation of Potato Tuber Moth (*Phthorimaea operculella*) and Greening of Tubers in Sudan," *Journal of Agricultural Science*, 121, 283–287.

Baggen, L.R., and Gurr, G.M. (1998), "The Influence of Food on Copidosoma koehleri (Hymenoptera: Encyrtidae), and the Use of Flowering Plants as a Habitat Management

Tool to Enhance Biological Control of Potato Moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae)," *Biology Control*, 11, 9–17.

Berlinger, M.J., and Lebiush-Mordechi, S. (1997), "The Potato Tubermoth in Israel: A Review of its Phenology, Behavior, Methodology, Parasites and Control," *Trends in Entomology*, 1, 137–155.

Coll, M., and Yuval, B. (2004), "Larval Food-Plants Affect Flight and Reproduction in an Oligophagous Insect Herbivore," *Environmental Entomology*, 33, 1471–1476.

Coll, M., Gavish, S., and Dori, I. (2000), "Population Biology of the Potato Tuber Moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae) in Two Potato Cropping Systems in Israel," *Bulletin of Entomology Research*, 90, 309–315.

Das, G.P., and Raman, K.V. (1994), "Alternate Hosts of the Potato Tuber Moth, *Phthorimaea operculella* (Zeller)," *Crop Protection*, 14, 631–636.

Fenemore, P.G. (1980), "Susceptibility of Potato Cultivars to Potato Tuber Moth, *Phthorimaea operculella* Zell. (Lepidoptera: Gelechiidae)," *New Zealand Journal of Agricultural Research*, 23, 539–546.

Flanders, R.V., and Oatman, E.R. (1987), "Competitive Interactions among Andophagous Parasitoids of Potato Tuberworm Larvae in Southern California," *Hilgardia*, 55, 1–34.

Gurr, G., and Wratten, S. (2002), *Biological Control: Measures of Success*, Dordrecht/Boston/London: Kluwer.

Hanafi, A. (1999), "Integrated Pest Management of Potato Tuber Moth in Field and Storage," *Potato Research*, 42, 373–380.

Horne, P.A. (1990), "The Influence of Introduced Parasitoids of the Potato Moth *Phthorimaea operculella* Lepidoptera Gleechiidae in Victoria, Australia," *Bulletin of Entomology Research*, 80, 159–164.

Horne, P.A. (1993), "Sampling for the Potato Moth (*Phthorimaea operculella*) and its Parasitoids," *Australian Journal of Experimental Agriculture*, 33, 31–96.

Horne, P.A., and Horne, J.A. (1991), "The Effects of Temperature and Host Density on the Development and Survival of *Copidosoma koehleri*," *Entomological Experiments & Applications*, 59, 289–292.

Kfir, R. (1981), "Fertility of the Polyembryonic Parasite *Copidosoma koehleri*, Effect of Humidities on Life Length and Relative Abundance as Compared with that of *Apanteles subandinus* in Potato Tuber Moth," *Annals of Applied Biology*, 99, 225–230.

Kfir, R. (2003), ""Biological Control of the Potato Tuber Moth *Phthorimaea operculella* in Africa," in *Biological Control in IPM Systems in Africa*, eds. P. Neuenschwander, C. Borgemeister and J. Langewald, Oxfordshire, UK: CABI International Publishing, pp. 77–85,.

Kroschel, J., and Koch, W. (1986), "Studies on the Population Dynamics of the Potato Tuber Moth *Pthtorimaea operculella* Zell (Lep., Gelechiidae) in the Republic of Yemen," *Journal of Applied Entomology*, 118, 327–341.

Lane, S.D., Mills, N.J., and Getz, W.M. (1999), "The Effects of Parasitoid Fecundity and Host Taxon on the Biological Control of Insect Pests: The Relationship between Theory and Data," *Ecology Entomology*, 24, 181–190.

van Lenteren, J.C., Babendreier, D., Bigler, F. *et al.* (2003), "Environmental Risk Assessment of Exotic Natural Enemies used in Inundative Biological Control," *Biocontrol*, 48, 3–38.

Mohammed, A., Douches, D.S., Pett, W. *et al.* (2000), "Evaluation of Potato Tuber Moth (Lepidoptera: Gelechiidae) Resistance in Tubers of Bt-cry5 Transgenic Potato Lines," *Journal of Economic Entomology*, 93, 472–476.

Mulatu, B., Applebaum, S.W., and Coll, M. (2004), "A Recently Acquired Host Plant Provides an Oligophagous Insect Herbivore with Enemy-Free Space," *Oikos*, 107, 231–238.

Murdoch, W.W., and Briggs, C.J. (1996), "Theory for Biological Control: Recent Developments," *Ecology*, 77, 2001–2013.

Pucci, C., Spanedda, A.F., and Minutoli, E. (2003), "Field Study of Parasitism Caused by Endemic Parasitoids and by the Exotic Parasitoid *Copidosoma koehleri* on *Phthorimaea operculella* in Central Italy," *Bulletin of Insectology*, 56, 221–224.

Trivedi, T.P., and Rajagopal, D. (1992), "Distribution, Biology, Ecology and Management of the Potato Tuber Moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae): A Review," *Tropical Pest Management*, 38, 279–285.

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