

Control of *Liriomyza langei* on chrysanthemum by *Diglyphus isaea* produced with a standard or modified parasitoid rearing technique

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Abstract: Overproduction of male parasitoids during mass rearing will increase costs for biological control because wasp shipments contain fewer females and only females kill hosts directly. We have developed a rearing technique capable of significantly reducing male-biased sex ratios in *Diglyphus isaea* (Walker) (Hym., Eulophidae), a commercially reared parasitoid of agromyzid leafminers. In this study, we examined the effect of rearing technique on the efficacy of *D. isaea* for biological control of *Liriomyza langei* Frick (Dip., Agromyzidae) on chrysanthemum, *Dendranthema grandiflora* Tzvelev var. 'Miramar'. We produced *D. isaea* on mixtures of small and large hosts (our modified technique) or on only large hosts (simulating commercial mass-rearing) and compared: (1) control of *L. langei* with *D. isaea* produced by the two rearing techniques, and (2) damage and yield of unprotected and protected plants. The two rearing techniques produced similar numbers of wasps per rearing cohort, but the 'modified' technique reduced the proportion of males by approximately 13%. The two techniques also produced females of similar size, but the 'modified' technique produced smaller males. In greenhouse trials simulating leafminer infestations of potted chrysanthemums during commercial production, we found no significant differences between the levels of control obtained by releasing identical numbers and sex ratios of adult wasps produced by either rearing technique. Mine counts on plants protected by wasps of either rearing history were similar and around 30–70% less than unprotected plants during most of the 11-week crop cycle. At crop harvest, more than half of the foliage on protected plants was undamaged compared with < 10% on unprotected crops. Damage to the flower stems of protected plants was relatively light in the top half of the canopy compared with the bottom half. Protected plants were around 10–15% taller and produced twice as many flower buds compared with unprotected plants. Our 'modified' rearing technique can reduce overproduction of males in *D. isaea* with no compromise in biological control efficacy. Adoption of our rearing technique by commercial insectaries could reduce implementation costs for not only *D. isaea* but also other parasitoids that show host-size-dependent sex allocation.

Key words: Agromyzidae, augmentative biological control, Eulophidae, leafminer, mass rearing, sex ratio

1 Introduction

Augmentative biological control has two essential elements: (1) the mass production of a control agent, and (2) the agent's release and satisfactory suppression of the pest population. Aspects of quality control during mass production include management of genetic diversity, physiological state and behavioural variation among natural enemies under rearing conditions (reviewed in van Lenteren 2003; Lewis et al. 2003). A frequent problem with augmentative releases is the cost for the large numbers of agents needed to achieve acceptable levels of control. This problem is accentuated when only a certain stage or sex of the control agent attacks the pest. Overproduction of male parasitoids during mass rearing increases implementation costs for biological control. While males are needed for sexual

reproduction, only female wasps directly kill hosts by oviposition or host feeding.

Diglyphus isaea (Walker) (Hymenoptera: Eulophidae) is a commercially reared parasitoid of agromyzid leafminers. This wasp is marketed for control of *Liriomyza* species on vegetables (van der Linden 2004) and floricultural crops (reviewed in Chow and Heinz 2004) grown in protected culture. *Diglyphus isaea* sex ratios from commercial insectaries may be extremely male-biased (up to 0.77 proportion male) according to Heimpel and Lundgren (2000), which makes biological control expensive. Based on current prices, clientele pay up to \$95 US for 200–250 adult *D. isaea*. In cut-chrysanthemum production, augmentative biological control of *Liriomyza trifolii* (Burgess) with *D. isaea* may require weekly releases of up to 400 wasps (60–70% male) per 1000 plants during the entire

11-week crop cycle (Parrella et al. 1992; Heinz et al. 1993). The high cost and release rates for *D. isaea* make augmentative biological control of leafminers prohibitively expensive, no matter the effectiveness of the programme.

Manipulation of parasitoid behaviour to produce more females can potentially reduce the cost of leafminer biological control. Females of *D. isaea* are solitary ectoparasitoids that exhibit host-size-dependent sex allocation (Ode and Heinz 2002). Female wasps oviposit more daughters on large hosts than small hosts and females will assess a host to be large or small depending upon the distribution of host sizes encountered by the wasp.

Based upon our knowledge of *D. isaea* sex allocation behaviour, we have developed a technique for reducing male-biased sex ratios in *D. isaea* by altering the size distribution of hosts (Chow and Heinz 2005a,b). Using only large hosts produces sex ratios similar to those obtained for commercial shipments of *D. isaea*. Using mixtures of small hosts and large hosts reduces the proportion of males by an average of 10%, with no loss in overall reproductive output. While our rearing technique can reduce overproduction of males in *D. isaea*, it is important to demonstrate that wasp quality is not compromised by this manipulation of offspring sex ratio.

Our previous studies show using mixtures of small hosts and large hosts produces females similar in size to those produced on only large hosts, although males are significantly smaller in size (Chow and Heinz 2005b). In this study, our objectives were to determine if rearing technique affects the ability of *D. isaea* to suppress leafminer infestations and prevent crop damage. Our experimental system consisted of chrysanthemum, *Dendranthema grandiflora* Tzvelev var. 'Miramar', and the leafminer, *Liriomyza langei* Frick (Dip., Agromyzidae). Chrysanthemums are produced as cut flowers or potted flowering plants in greenhouses throughout North America and Europe. *Liriomyza langei* is an important agricultural pest in the US and often infests chrysanthemum (reviewed in CHOW and HEINZ 2004). We simulated mass rearing of *D. isaea* on mixtures of small hosts and large hosts or on only large hosts and compared: (1) control of *L. langei* on greenhouse chrysanthemum with releases of identical numbers and sex ratios of *D. isaea* produced by the two rearing techniques, and (2) damage and yield of unprotected plants and protected plants.

2 Materials and Methods

2.1 Insect colonies

We established colonies of *L. langei* from pupae collected near Salinas, CA, and *D. isaea* from adults obtained from Koppert Biological Systems Inc. (Romulus, MI). Protocol described by Ode and Heinz (2002) was used to rear *L. langei* on chrysanthemum and *D. isaea* on *L. langei*. To obtain the uniform cohorts of *L. langei* larvae necessary to illustrate clear differences between mass rearing systems, we exposed potted chrysanthemums to adult leafminers for approxi-

mately 4 h in cages. Potted chrysanthemums (three plants per pot) were grown from cuttings under greenhouse conditions for at least 1 month before exposure to leafminers.

We used 8-day-old *L. langei* larvae as large hosts and 6-day-old larvae as small hosts. Projected cross-sectional area of larvae (following Heinz and Parrella 1990) was used as a determinant of host size. Large hosts (2.04–3.51 mm²) were approximately three to four times the size of small hosts (0.45–1.10 mm²). All colonies were laboratory reared for at least 2 months at 21–25°C, 40–70% r.h., and under a L14 : D10 photoperiod before being used for our study.

2.2 Effect of rearing technique on sex ratio and wasp size

To produce wasps for our greenhouse trials, we simulated mass rearing of *D. isaea* using two techniques: rearing on mixtures of both large hosts and small hosts (Lg + Sm) or rearing on only large hosts (Lg). We caged nine plants for 3–4 h with approximately 200 adult *L. langei* to obtain plants with only large hosts. To obtain plants with both small hosts and large hosts, we caged nine plants for 1.5–2 h with adult *L. langei* and repeated the procedure 2 days later. When *L. langei* larvae were of the required age, we chose plants of uniform height (13–20 cm), leaf number (8–14 mature leaves) and host density (20–40 *L. langei* larvae).

To standardize wasps for the two rearing techniques, we selected wasps of similar size and age for each sex from cohorts that developed on 8- to 9-day-old *L. langei*. Groups of 20 adult males and 20 adult females (1- to 2-days old) were confined together in paper cups (9.5 cm diameter × 5.5 cm height) with honey solution for 48–72 h to ensure mating. To initiate rearing, we collected two groups of 80 female wasps and 80 male wasps (4- to 5-days old) and confined each group in a different plexiglass cage (38 cm width × 51 cm height × 46 cm length).

For three consecutive days, we exposed each group of wasps to nine plants infested with *L. langei* larvae. One group was exposed to plants infested with similar numbers of both large hosts and small hosts (Lg + Sm) and the second group to plants infested with only large hosts (Lg). We exchanged the plants daily with another set of identically infested plants and added 20 female wasps and 20 male wasps to maintain 160–200 wasps in each cage. We repeated this protocol for eight consecutive weeks. Treatment plants were cut when immature wasps were approximately 13 days old.

Cut plants containing wasp cohorts produced by a single technique during the same week were held together. We sexed and counted all adult wasps that eclosed from each weekly harvest. Sex ratio was estimated as the proportion of males among all offspring counted. The Wilcoxon matched-pairs signed-rank test was used to detect differences among the sex ratio and total offspring produced by the two rearing techniques. To compare the size of wasps produced by the two techniques, we used the methodology described by HEINZ (1991) and measured the hind tibia length of 40 female wasps and 40 male wasps from the second, fourth, sixth and eighth harvests of each technique. The effect of rearing technique on wasp size was tested with a three-way ANOVA using rearing technique, harvest and sex as factors.

2.3 Control of *L. langei* by *D. isaea*

We compared control of *L. langei* on potted chrysanthemums by *D. isaea*, produced by our two rearing techniques, under conditions simulating greenhouse production in Texas. We made no prediction on the degree of biological control success, but predicted that rearing history would not significantly affect the ability of wasps to suppress *L. langei* on

chrysanthemum. To test our prediction, we exposed chrysanthemum to only *L. langei*, *L. langei* and *D. isaea* produced by the 'Lg' technique, or *L. langei* and *D. isaea* produced by the 'Lg + Sm' technique. The number of replications was two per treatment and the three treatments were equally distributed within a randomized block design, using position within the greenhouse as the blocking factor.

Rooted chrysanthemum cuttings were transplanted four per pot (15.5 cm diameter, 15.5 cm depth) in soil-less potting mix (Sunshine Mix no. 1; Sun Gro Horticulture Canada Ltd., Bellevue, WA) and given liquid fertilizer (375 ppm N, Peters Professional Pot Mum Special, 15-10-30; Scotts-Sierra Horticultural Products Company, Marysville, OH) three times a week. Each replicate consisted of 84 potted plants (total = 21 pots) on a greenhouse bench enclosed by a PVC frame (305 cm long × 152 cm wide × 122 cm high) sheathed with Econet S[®] insect screen (Gintec Shade Technologies Inc., Windham Centre, Ont., Canada). The pots were placed within clear plastic pot holders (40.5 cm diameter) and spaced 45 cm apart in a 7 × 3 grid. We grew potted chrysanthemums following commercial US guidelines (Yoder Brothers Inc. 2001). All plants were pinched back to six to eight laterals after they had grown to 2.0 cm in height. Two weeks after pinching, we applied a plant growth regulator, daminozide (3750 ppm, B-Nine WSG; Uniroyal Chemical Company Inc., Middlebury, CT), to restrict plant height.

Beginning 12 days after the cuttings were transplanted, we released one adult male and one adult female *L. langei* into each replicate three times per week for eight consecutive weeks and twice during the ninth week (total = 52 flies over 26 releases per replicate). The flies were 96 ± 24 h old when released on the Monday, Wednesday, and Friday of each week. Prior to release, flies were caged in groups of 50 males and 50 females for 2–3 days with honey solution and chrysanthemum plants to ensure mating and egg maturation.

We expected the 'Lg + Sm' technique to produce the same number of adult wasps but proportionately fewer males than the 'Lg' technique. Comparisons between the efficacy of *D. isaea* from the two rearing techniques would have been complicated if wasp releases varied in sex ratio. Thus, all releases of *D. isaea* were identical in both wasp number and sex ratio.

Beginning 3 days after leaf mining was observed in any of the replicates, three adult female *D. isaea* and five adult male *D. isaea* were released once a week for eight consecutive weeks into all replicates assigned a treatment with wasps (total = 64 wasps over eight releases per replicate). We based our release rates on recommendations by Koppert Biological Systems Inc. (John Wolf, personal communication, Biological Sales Specialist, Koppert USA, Romulus, MI) for the number of wasps needed to control *Liriomyza* leafminers on greenhouse crops. Adult wasps were obtained from the weekly harvests for the two rearing techniques (see Section 2.2). We randomly selected wasps of each sex from cohorts that eclosed over 72 h. To simulate commercial shipping, we confined three female wasps and five male wasps in paper cups (9.5 cm diameter × 5.5 cm height) with honey solution for 24 h in Styrofoam boxes with ice packs. The wasps were released on the Friday of each week.

The experiment was conducted in a greenhouse from 17 September to 6 December 2004. Daily temperature was monitored at 4-h intervals inside the enclosed benches with HOBO[®] H8 Pro Series data loggers (Onset Computer Corporation, Bourne, MA). On the day that the first mine was found in any replicate, we counted every mine on the plants of all replicates. Subsequent mine counts were taken every 7 days for seven consecutive weeks. We harvested the entire crop when approximately one-fourth to one-third of

the flowers were fully open. After harvest, all mined leaves were examined to estimate both wasp number and leafminer mortality for each replicate. To estimate wasp number, we counted wasp exit holes and immature or adult wasps within mined leaves. To estimate leafminer mortality, we counted dead leafminers within mined leaves. We also selected 10 pot holders from each cage after the harvest and counted all leafminer puparium in the pot holders.

For ANOVA, we transformed mine counts, wasp counts, dead leafminer counts and pupae counts to their square root values to meet assumptions of normality and homogeneity of variance. For presentation of results, back-transformed means were reported with 95% confidence intervals. We analysed weekly mine counts with repeated-measures one-way ANOVA tests (SPSS Inc. 2000) using treatment as the main effect and the Greenhouse–Geisser adjustment to correct for sphericity (Hand and Crowder 1996). We used one-way ANOVA to compare between treatments: wasp number, puparium number and leafminer mortality counted only at the end of the study.

2.4 Plant yield and damage

We compared two parameters among replicates: yield (numbers of flower stems and flower buds) and damage distribution. For each plant, we measured its height and counted all leaves, flower stems and flower buds (open or unopened). To examine damage distribution, we divided all the plants of each replicate into two sections: floral canopy (leaves of flower stems) and main canopy (leaves of the main plant stem). A vertical stratification scheme was used to further subdivide each section. We assigned each leaf on a flower or main stem to a stratum. Leaves closest to the flower or pinch point at the top of a stem were assigned to stratum I. We then assigned sequentially higher stratum numbers to each leaf down the stem. Few flower or main stems had more than 13 leaves; therefore, we standardized main and floral canopies into 13 strata by pooling all leaves below the 12th leaf into the 13th stratum. To estimate damage distribution, we counted all undamaged leaves and damaged leaves in each stratum. We considered a leaf damaged if it was mined or had no mines but five or more stipules.

For ANOVA, we transformed flower stem, flower bud and leaf counts to their square root values and proportions of leaves damaged to their arcsine values to meet assumptions of normality and homogeneity of variance. For presentation of results, back-transformed means were reported with 95% confidence intervals. We used one-way ANOVA to compare treatments: plant height, total leaves, total flower stems, total flower buds and proportion of leaves damaged. The effect of canopy on leaf damage was tested with two-way ANOVA using canopy and treatment as factors. The effect of treatment on damage distribution in floral or main canopies was tested with two-way ANOVA using stratum and treatment as factors.

3 Results

3.1 Effect of rearing technique on sex ratio and wasp size

We found no significant differences between the numbers of wasps produced by either technique over 8-weekly harvests (mean \pm SE = 435.6 ± 20.1 , $n = 16$) (Wilcoxon signed-rank test: $n = 8$, $Z = -0.280$; $P = 0.779$), but found significantly different sex ratios (Wilcoxon signed-rank test: $n = 8$, $Z = -2.100$;

$P = 0.036$). In six of eight cohorts, the 'Lg + Sm' technique yielded offspring with fewer males than the 'Lg' technique (fig. 1). Mean sex ratio for harvests produced by the 'Lg + Sm' technique (mean \pm SE = 0.53 ± 0.02 , $n = 8$) was approximately 13% less male-biased than by the 'Lg' technique (mean \pm SE = 0.61 ± 0.01 , $n = 8$).

Wasp size varied considerably in this study (three-way ANOVA: $F_{1,624} = 86.366$; $P < 0.001$) (fig. 2). Females were always larger than males (three-way ANOVA: $F_{3,624} = 1096.760$; $P < 0.001$) and this difference was independent of rearing technique or harvest (three-way ANOVA: $F_{3,624} = 1.477$; $P = 0.220$). However, there was a significant interaction between rearing technique and sex (three-way ANOVA: $F_{3,624} = 58.361$; $P < 0.001$).

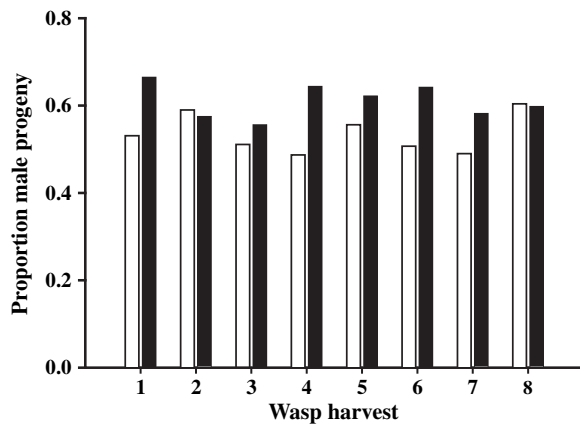


Fig. 1. Offspring sex ratios for weekly harvests of *Diglyphus isaea* cohorts produced over 8 weeks by two rearing techniques. Wasps reared from 8-day-old *Liriomyza langei* (Lg) (■). Wasps reared from mixtures of 8-day and 6-day-old *L. langei* (Lg + Sm) (□).

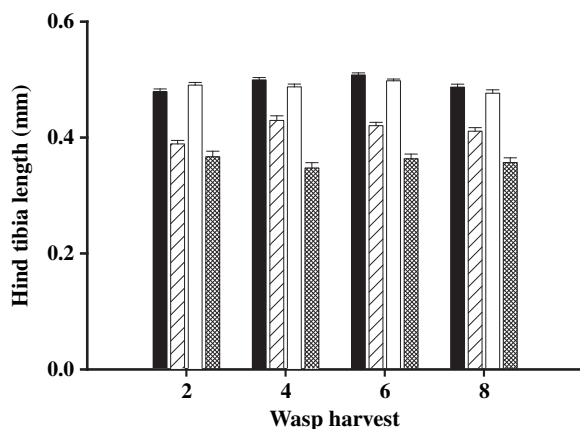


Fig. 2. Hind tibia lengths (mm) of male and female *Diglyphus isaea* from four of eight groups of wasps produced weekly over 8 weeks by two rearing techniques. Wasps reared from 8-day-old *Liriomyza langei* (Lg females = ■; Lg males = ▨). Wasps reared from mixtures of 8-day and 6-day-old *L. langei* (Lg + Sm females = □; Lg + Sm males = ▩). Each column = mean (+SE), $n = 40$ wasps

The two techniques produced females of similar size, but the 'Lg + Sm' technique produced smaller males than the 'Lg' technique (fig. 2).

3.2 Control of *L. langei* by *D. isaea*

Daily temperatures inside the enclosed benches ranged from 18 to 36°C during September and gradually decreased to 13–26°C during December. The plants were pinched 13 days after transplanting and required approximately 11 weeks of growth before they could be harvested. Weekly releases of *D. isaea* substantially decreased but did not prevent the build-up of mines on chrysanthemum. We first observed mines and began wasp releases during the third week after transplanting, but mine counts increased for all replicates with each successive week (repeated-measures one-way ANOVA: $F_{7,21} = 122.768$, Greenhouse–Geisser-adjusted $P < 0.001$) (fig. 3).

Although there were significant differences between mine counts on plants exposed to only leafminers (unprotected) or both leafminers and wasps (protected) (repeated measures two-way ANOVA: $F_{2,3} = 15.164$; $P = 0.027$) (fig. 3), there was no significant interaction between week and treatment (repeated-measures one-way ANOVA: $F_{14,21} = 1.656$, Greenhouse–Geisser-adjusted $P = 0.273$). Unprotected plants always had the most mines, but mine counts on protected plants did not vary significantly with wasps released from either rearing technique. Mine counts were around 30–70% greater for unprotected plants than protected plants from the second to the eighth week of mine counts.

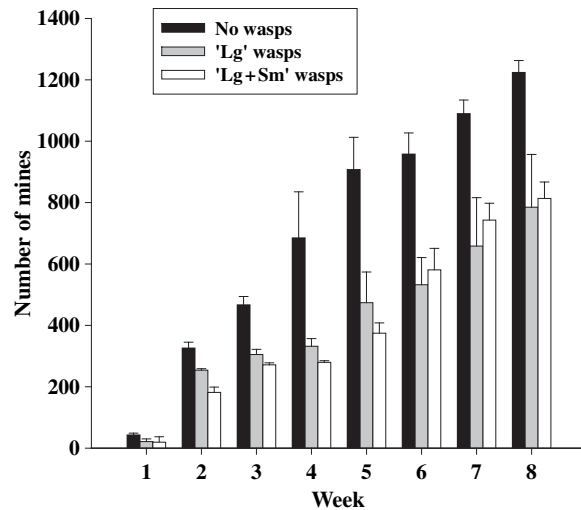


Fig. 3. Weekly counts of *Liriomyza langei* mines (mean + SE) on potted chrysanthemums exposed to 'no wasps' (no wasps = ■) or *Diglyphus isaea* reared from 8-day-old *L. langei* (Lg wasps = ▨) or *D. isaea* reared from mixtures of 8-day and 6-day-old *L. langei* (Lg + Sm wasps = □), $n = 2$ per treatment, 84 plants per replicate. Releases of *L. langei* began on the 12th and *D. isaea* on the 19th day after the crop was planted. Mines were counted once every week for 8 weeks after the first mine was found

The greatest difference in mine counts was during the fifth week with, on average, 400–500 more mines on unprotected plants than protected plants (fig. 3).

Leafminer mortality differed greatly between unprotected and protected plants (one-way ANOVA: $F_{2,5} = 39.031$, $P = 0.007$). We counted around 10 times as many dead leafminers on protected plants than unprotected plants, but wasp rearing history did not significantly affect leafminer mortality (fig. 4). The ratio of dead leafminers to wasp progeny ranged from 3 : 1 to 2 : 1; thus, most leafminers were killed but not parasitized. We also recovered approximately 25 times as many leafminer puparium from the pot holders of unprotected plants than protected plants (one-way ANOVA: $F_{2,5} = 39.031$, $P = 0.007$) (fig. 5). The numbers of wasps recovered from protected plants did not differ significantly with wasp rearing history (mean = 384.4, 95% CI = 140.2–749.1, one-way ANOVA: $F_{2,4} = 0.041$, $P = 0.858$).

3.3 Plant yield and damage

Releases of *D. isaea* on chrysanthemum resulted in a substantial gain in plant yield. The heights of plants protected by 'Lg' wasps (mean = 23.7 cm, 95% CI = 23.2–24.3, $n = 168$) and 'Lg + Sm' wasps (mean = 22.1 cm, 95% CI = 21.6–22.6, $n = 168$) were similar and, on average, 10–15% taller than unprotected plants (mean = 20.4 cm, 95% CI = 20.0–20.9, $n = 168$). Protected and unprotected plants produced similar numbers of leaves (mean = 4145.97, 95% CI = 3336.91–5042.78, one-way ANOVA: $F_{2,5} = 0.175$, $P = 0.848$) and flower stems (mean = 317.6, 95% CI = 273.7–364.8, one-way ANOVA: $F_{2,5} = 2.477$, $P = 0.232$), but different numbers of flower buds (one-way ANOVA: $F_{2,5} = 17.734$, $P = 0.022$). We counted similar numbers of flower buds on plants protected

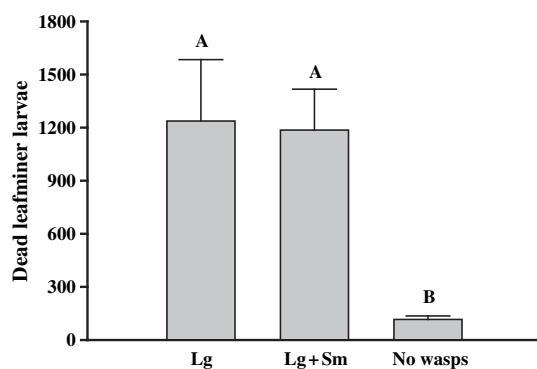


Fig. 4. Numbers of dead *Liriomyza langei* larvae (mean + SE) recovered after harvest of potted chrysanthemums exposed to 'no wasps' or *Diglyphus isaea* reared from 8-day-old *L. langei* (Lg) or *D. isaea* reared from mixtures of 8-day and 6-day-old *L. langei* (Lg + Sm), $n = 2$ per treatment, 84 plants per replicate. Different letter(s) above bars indicate significant differences ($P \leq 0.05$) determined by one-way ANOVA for square-root-transformed data followed by the Bonferroni method. Untransformed mean and SE are presented here

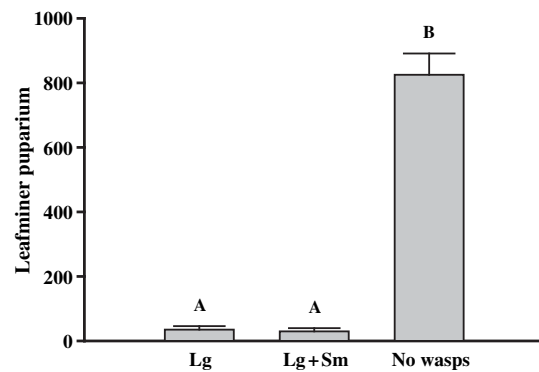


Fig. 5. Numbers of *Liriomyza langei* puparium (mean + SE) recovered from pot holders after harvest of potted chrysanthemums exposed to 'no wasps' or *Diglyphus isaea* reared from 8-day-old *L. langei* (Lg) or *D. isaea* reared from mixtures of 8-day and 6-day-old *L. langei* (Lg + Sm), $n = 2$ per treatment, 84 plants per replicate. Different letter(s) above bars indicate significant differences ($P \leq 0.05$) determined by one-way ANOVA for square-root-transformed data followed by the Bonferroni method. Untransformed mean and SE are presented here

by wasps of either rearing history, but protected plants produced twice as many flower buds as unprotected plants (fig. 6).

Releases of *D. isaea* also reduced leaf damage considerably. On average, <10% of the leaves on unprotected plants were free of damage (fig. 7). In comparison, more than half of the leaves on protected plants were free of damage. Canopy (two-way ANOVA: $F_{1,11} = 9.881$, $P = 0.020$) and treatment (two-way ANOVA: $F_{2,11} = 21.883$, $P = 0.002$) had significant effects on leaf damage, but there was no significant interaction between canopy and treatment (two-way

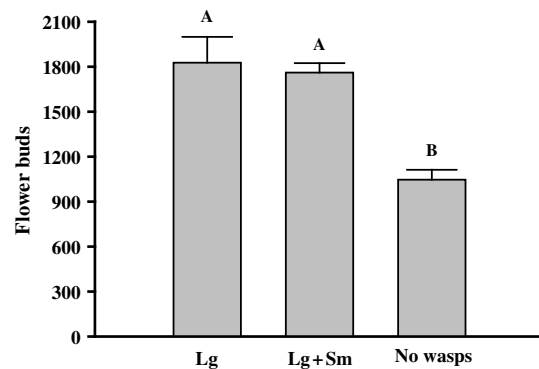


Fig. 6. Numbers of flower buds (mean + SE) produced by potted chrysanthemums infested by *Liriomyza langei* and exposed to 'no wasps' or *Diglyphus isaea* reared from 8-day-old *L. langei* (Lg) or *D. isaea* reared from mixtures of 8-day and 6-day-old *L. langei* (Lg + Sm), $n = 2$ per treatment, 84 plants per replicate. Different letter(s) above bars indicate significant differences ($P \leq 0.05$) determined by one-way ANOVA for square-root-transformed data followed by the Bonferroni method. Untransformed mean and SE are presented here

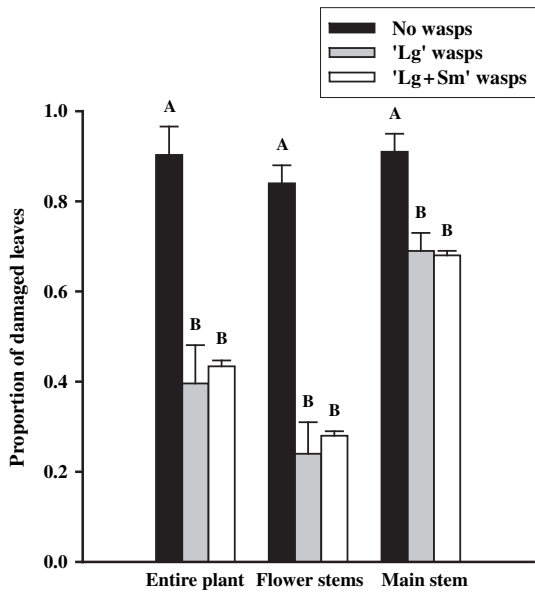


Fig. 7. Leaf damage (proportion of leaves mined and/or stippled) by *Liriomyza langei* for entire plants, flower stems and main stems after harvest of potted chrysanthemums exposed to 'no wasps' (no wasps = ■) or *Diglyphus isaea* reared from 8-day-old *L. langei* (Lg wasps = ■) or *D. isaea* reared from mixtures of 8-day and 6-day-old *L. langei* (Lg + Sm wasps = □), $n = 2$ per treatment, 84 plants per replicate. Different letter(s) above bars within groups representing entire plants, flower stems, or main stems indicate significant differences ($P \leq 0.05$) determined by one-way ANOVA for square-root-transformed data followed by the Bonferroni method. Untransformed mean and SE are presented here

ANOVA: $F_{2,11} = 2.697$, $P = 0.146$). Most damaged leaves were mined. On average, <3% of damaged leaves were only stippled and not mined. Main canopies always had more damaged leaves than floral canopies. Corresponding canopies were always more damaged on unprotected than protected plants (fig. 7). However, damage to main or floral canopies was similar for plants protected by wasps of either rearing history.

Damage to the floral canopy varied greatly with strata (two-way ANOVA: $F_{12,77} = 9.888$, $P < 0.001$) and treatment (two-way ANOVA: $F_{2,77} = 128.474$, $P < 0.001$), but treatment by stratum interaction was not significant (two-way ANOVA: $F_{24,77} = 0.574$, $P = 0.924$). Damage to the floral canopy was substantially less on protected than unprotected plants, but not significantly affected by wasp rearing history (fig. 8). For all plants, the proportion of leaves damaged increased with distance from the top of the flower stem. On protected plants, damage to the top three strata was <12% of all leaves (fig. 8). However, damage increased to 30% by the sixth stratum (half-way down the stem), 50% by the eighth stratum, and ranged from 60% to 80% for the ninth to the 13th stratum (fig. 8). On unprotected plants, damage to the top stratum was around 70% and gradually increased to 90% by the 13th stratum.

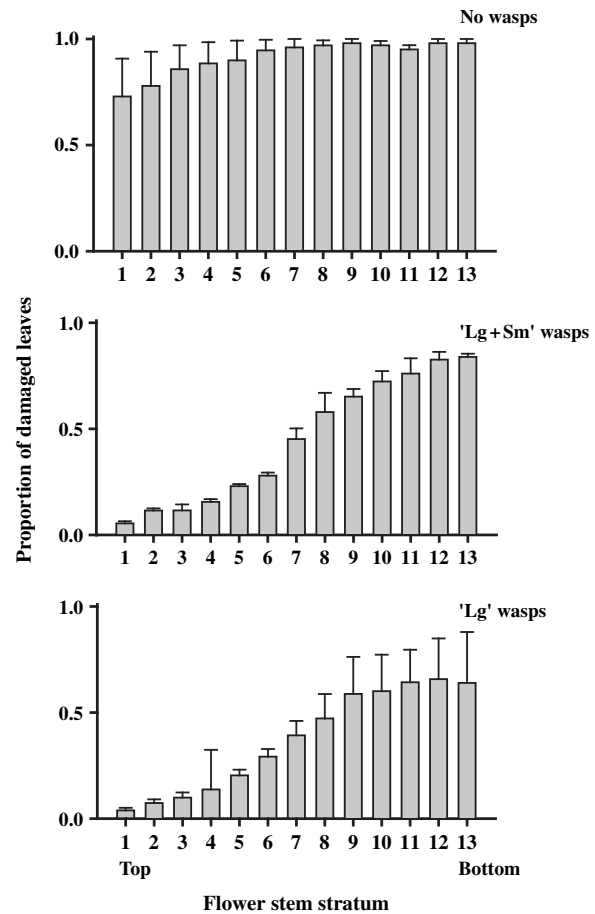


Fig. 8. Within-plant vertical distribution of leaf damage (proportion of leaves mined and/or stippled) by *Liriomyza langei* on flower stems after harvest of potted chrysanthemums exposed to 'no wasps' or *Diglyphus isaea* reared from 8-day-old *L. langei* (Lg) or *D. isaea* reared from mixtures of 8-day and 6-day-old *L. langei* (Lg + Sm), $n = 2$ per treatment, 84 plants per replicate. Leaves closest to the flower at the top of the stem are represented by stratum 1. Each column = mean (+SE). Untransformed mean and SE are presented here

Damage to the main canopy also varied considerably with strata (two-way ANOVA: $F_{12,77} = 24.154$, $P < 0.001$) and treatment (two-way ANOVA: $F_{2,77} = 62.598$, $P < 0.001$). Leaf damage within the main canopy was substantially less on protected than unprotected plants and not significantly affected by wasp rearing history (fig. 9). As with the floral canopy, treatment by stratum interaction was not significant (two-way ANOVA: $F_{24,77} = 0.912$, $P = 0.586$). However, damage to the main canopy decreased with stratum distance from the top (fig. 9). On protected plants, damage to the top three strata was over 90%, but gradually decreased to around 40% by the 13th stratum. On unprotected plants, damage ranged from 100% for the top stratum to 70% by the 13th stratum (fig. 9).

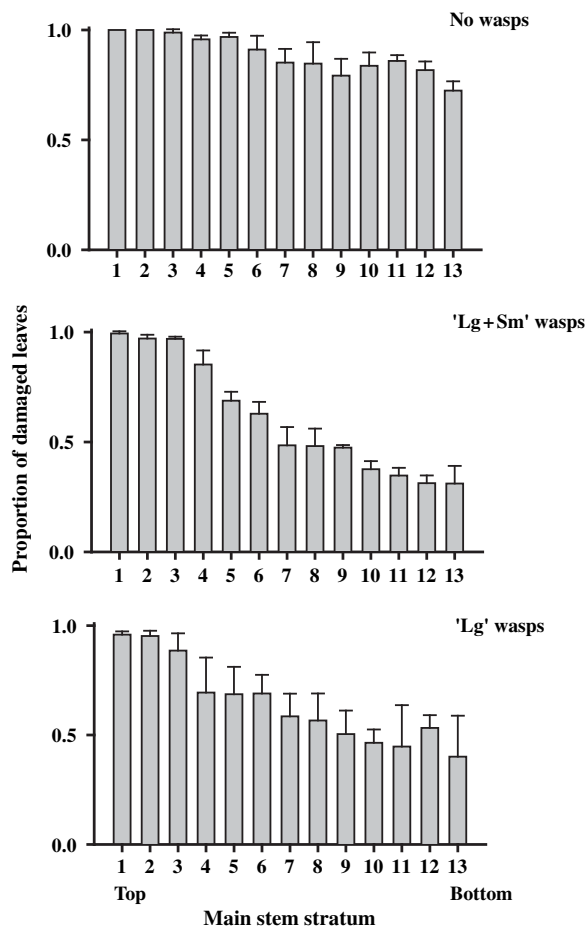


Fig. 9. Within-plant vertical distribution of leaf damage (proportion of leaves mined and/or stippled) by *Liriomyza langei* on main stems after harvest of potted chrysanthemums exposed to 'no wasps' or *Diglyphus isaea* reared from 8-day-old *L. langei* (*Lg*) or *D. isaea* reared from mixtures of 8-day and 6-day-old *L. langei* (*Lg* + *Sm*), $n = 2$ per treatment, 84 plants per replicate. Leaves closest to the 'pinch' (where the main stem was terminated to restrict plant height) at the top of the stem are represented by stratum 1. Each column = mean (+SE). Untransformed mean and SE are presented here

4 Discussion

Our study demonstrated that *D. isaea* could be reared on mixtures of large hosts and small hosts to reduce male-biased sex ratios with no detectable compromise in either wasp quality or quantity compared to rearing on only large hosts. Wasps produced from the two rearing systems and subsequently released in identical numbers and sex ratios in augmentation biological control trials showed no difference in their abilities to protect potted chrysanthemum from *L. langei*. Thus, modification of *D. isaea* mass-rearing systems to increase female wasp production may provide a plausible methodology for reducing the cost of augmentative biological control for agromyzid leafminers.

Reducing overproduction of male *D. isaea* should increase mass-rearing efficiency and lower prices, but clientele will still benefit even if insectaries did not lower the price per unit for *D. isaea*. *Diglyphus isaea* is sold in units of 250 wasps that can cost US\$95.00 per unit (if shipped to the US), but only 58 wasps would be females assuming a sex ratio of 0.77 (as reported for commercial shipments in Heimpel and Lundgren 2000). However, units with a sex ratio of 0.53 (obtainable with our modified technique) would have 118 females and the actual cost per female wasp would decrease from US\$1.64 to US\$0.81. By providing approximately twice the number of female wasps per unit compared with typical shipments, our modified rearing technique could significantly reduce the number of *D. isaea* units required for control of agromyzid leafminers on some crops. Clientele would benefit from needing fewer units of wasps for pest suppression, and insectaries could expand their clientele base if costs for biological control and conventional chemical control of leafminers become more comparable.

van Lenteren and Nicoli (2004) argued that optimizing mass production of natural enemies requires approaches that address not only natural enemy number but also natural enemy quality. We found that presentation of both large hosts and small hosts to *D. isaea* produced similar numbers of wasps, similar sized females, but smaller males than presentation of only large hosts. Many studies support the premise that being small has negative fitness consequences for parasitoid wasps (van den Assem et al. 1989; Heinz, 1991; Ode and Strand 1995; Ueno 1999; Wang and Messing 2004), but some do not (Boivin and Lagace 1999; Ji et al. 2004). The conclusions of these laboratory studies were based on measuring specific life history parameters that included adult lifespan, fecundity and reproductive success for different sized adults. However, evaluation of natural enemy performance also requires tests that quantify either effective pest suppression or prevention of economic damage to the crop (van Lenteren and Manzaroli 1999). Our study showed that releases of *D. isaea* from cohorts, with small males, produced by our modified rearing technique did not compromise biological control of *L. langei* during production of greenhouse chrysanthemum.

Feeding by adult and larval stages of agromyzid leafminers can cause considerable physiological damage to ornamentals (Parrella et al. 1985; Parrella 1987). Adult female leafminers use their ovipositors to puncture leaf surfaces for feeding on sap or laying of eggs (stippling). Feeding by leafminer larvae in the leaf mesophyll may result in extensive mining of foliage. Stippling and mining by *Liriomyza* species can substantially reduce photosynthetic rates and both stomatal and mesophyll conductance in greenhouse chrysanthemum (Parrella et al. 1985). Leafminer damage also provides entry sites for infection of chrysanthemum by plant pathogens (Matteoni and Broadbent 1988). Heavy leafminer damage can severely stunt or kill young plants (Elmore and Ranney 1954). We found that reduction of feeding damage by *L. langei*

had a substantial effect on the growth and yield of chrysanthemum. In our study, more than half of the foliage on the protected plants was free of damage compared with <10% on unprotected plants. Chrysanthemums protected by wasp releases were taller and produced twice as many flower buds compared with unprotected plants.

Diglyphus species can be effective augmentative control agents for agromyzid leafminers, but their effectiveness depends on the type of crop. Augmentative tactics always result in some plant damage because of the delay between introductions of the natural enemies and reduction in pest populations. Biological control is not suitable when the entire plant is sold and leafminer damage to any part is unacceptable. In cut flowers, only the upper portion of the flower stem is harvested and leafminer damage to the lower portion is acceptable as it is neither harvested nor sold. Successful biological control in cut flowers is based not only on the overall reduction in the pest population but on the ability of the natural enemies to prevent damage from occurring to the aesthetically important portions of the harvested crop.

In the US, this approach to augmentative biological control has been successfully tested with *Diglyphus begini* (Ashmead) to control *L. trifolii* on chrysanthemums grown for cut flowers at scales characteristic of commercial production (Parrella et al. 1992; Heinz et al. 1993). We conducted our trials with a potted chrysanthemum variety, a much shorter plant than the chrysanthemum varieties used for cut flowers, but the results still validate this approach for controlling leafminers on certain ornamental crops. In our study, weekly releases of *D. isaea* did not prevent the build-up of substantial numbers of mines on chrysanthemum. However, leafminer damage to the flower stems of protected plants was relatively light in the top half of the canopy compared with the bottom half.

In conclusion, we have demonstrated the feasibility of a mass-rearing technique that can be used to reduce the overproduction of males in *D. isaea* without compromising biological control performance. Given the ongoing concerns over the development of insecticide resistance among several *Liriomyza* species, it is important to develop more cost-competitive control alternatives to pesticide-based programmes. Despite the feasibility of using augmentative releases of *Diglyphus* species to control agromyzid leafminers, their use in commercial settings has been restricted by high economic costs. Although *D. isaea* is commercially available, control of agromyzid leafminers with insecticides is presently more cost effective on most crops. For example, control of agromyzid leafminers on 1000 greenhouse chrysanthemums for an entire crop cycle with insecticides would cost around US\$600 (Mark Smith, personal communication, Color Star Growers, Giddings, TX) compared with almost US\$1700 with *D. isaea* (Chow and Heinz, 2005b). Adoption of our rearing technique by commercial insectaries could increase rearing efficiency and reduce implementation costs for not only *D. isaea* but also other parasitoids that show host-size-dependent sex allocation.

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