

# Assessment of interspecific interactions among parasitoids on the outcome of inoculative biological control of leafminers attacking chrysanthemum

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## Abstract

Indigenous natural enemies occur within field grown crops at varying densities dependent upon a variety of other biotic and abiotic parameters. This natural control often does not provide adequate suppression, which results in the application of other pest management solutions including augmentative biological control. When releasing mass-reared natural enemies into a backdrop of existing natural enemy populations, competitive interactions are likely to occur. To assess the influence of these interspecific interactions on the outcome of such biological control practices studies were conducted in a simulated, field cage grown, cut chrysanthemum production system. Competitive interactions of two commercially available parasitoids were studied both in terms of parasitoid-host population dynamics and the impact of interspecific interactions on crop quality at harvest. The parasitoids *Diglyphus isaea* and *Dacnusa sibirica* attacking the leafminer *Liriomyza langei* were used as the model insect system. Both parasitoids are cosmopolitan and are known to occur in many ornamental production areas. Treatment comparisons included single species releases with complimentary releases of both species either simultaneously or with 2-week time lags, as well as a no release control to measure the background effects of natural mortality. Conclusions drawn from results of population-level studies replicated within and among years were that levels of interspecific competition among parasitoid species were undetectable at leafminer densities typical of field-grown ornamental crops (low densities), and thus, the efficacy of one species released into a backdrop of potentially competing parasitoids did not negatively or positively affect the outcome of the augmentative biological control, nor was there a positive outcome; however, crop quality at harvest was influenced.

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*Keywords:* *Liriomyza langei*; *Dacnusa sibirica*; *Diglyphus isaea*; Competition; Host suppression

## 1. Introduction

The practice of biological control of arthropod pests infesting ornamental crops has long resided within research laboratories, but only in a few cases has it become a regular pest management practice (Heinz, 2004). In many cases, implementation of biological control in ornamentals remains too costly versus grower expenditures for chemical control (Del Bene et al., 1994). Further, the high aesthetic

value of ornamental crops makes biological control in ornamentals difficult due to the near-zero tolerance to damage and arthropod presence exhibited by the commercial sector (Jones et al., 1986; Parrella and Jones, 1987; van Lenteren and Woets, 1988; Parrella, 1990; Osborne and Oetting, 1989; Parrella et al., 1992; Del Bene et al., 1994). Yet, biological control in protected culture remains a pest management technique desired by growers when practical, and it is gaining interest in North and South America, Europe and Japan. There are several reasons for the increasing interest in the use of biological control that include minimal legislative restrictions, worker safety, the regular occurrence of pest resistance to insecticides, and the

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absence of insecticide residues associated with its practice (Jones et al., 1986; Heinz, 1990).

The high aesthetic value of cut chrysanthemums originates from their attractive bouquet of flowers against a backdrop of dark green foliage (Carvalho and Heuvelink, 2003). Prior to the generation of the high value, harvested portion of the crop occurs in the later portions of the crop production cycle, there exists a window of time during which moderate damage is tolerated (Jones et al., 1986; Parrella, 1990; Chow and Heinz, 2004). This window is about 40 days after planting, after which time, successful control of foliar herbivores must be obtained as no pest damage to the harvestable foliage is tolerated (Jones et al., 1986; Heinz et al., 1990, 1993; Sher et al., 2000). As only the approximate top 81 cm of the plant is harvested with the bottom 41 cm of the harvested stems stripped of foliage, a vertically stratified damage threshold exists that is compatible with the use of biological control (Jones et al., 1986). The cut chrysanthemum system also offers potential for successful biological control because they are grown as a continuously overlapping rotation of annual crops, which allow for a long-term interaction between the pest and natural enemies (Parrella, 1990).

Cut chrysanthemums are one of the world's most popular floral crops (Murphy and Broadbent, 2004). In 2004 cut chrysanthemum production totaled over \$20 million and it ranked sixth in cut flower production in the United States. Approximately 60% of the United States floriculture production is grown in open fields or permeable to semi permeable protective structures (Jerardo, 2005). Continual pest and natural enemy invasions throughout the growing season often characterize these types of production systems. One group of common and devastating pests of cut chrysanthemums, agromyzid leafminers in the genus *Liriomyza*, are known to have communities of natural enemies occurring in both their native and invaded ranges (Murphy and LaSalle, 1999; Rauf et al., 2000). These natural enemies include the parasitoid genera *Chrysocharis*, *Diglyphus*, *Neochrysocharis*, *Halticoptera*, *Opius*, and *Dacnusa* (Murphy and LaSalle, 1999). Indigenous natural enemies may appear at any time to attack either an established or an incipient leafminer population. When natural control by these parasitoids does not provide adequate leafminer suppression, augmentative biological control may be used as a potential solution (Minkenberg and van Lenteren, 1987). However, as this augmentation process releases mass-reared parasitoids into indigenous parasitoid populations, this management technique introduces the possibility of interspecific competitive interactions. Understanding these interactions is important as they may affect the outcome of the biological control program (Smith, 1929; Ehler, 1979; Briggs, 1993; Godfray, 1994).

The importance of interspecific competition in regulating population dynamics has long been discussed within population ecology (Connell, 1983; MacNally, 1983; Briggs, 1993; Walter and Paterson, 1995), and its importance pest management has been discussed in the biological con-

trol literature since the early 1900's (Smith, 1929). Yet, there are still little data addressing the impact of parasitoid interspecific competition on parasitoid-host dynamics and biological control from field experiments (Godfray, 1994). Most experimental studies on parasitism and/or predation have examined effects of only one natural enemy attacking the host and do not address the questions of interspecific competition (Bográn, 2000). The purpose of this study was to examine interspecific competition on parasitoid and host population dynamics when two biological control agents are released together and when a one agent is released into a population of established natural enemies.

We report findings of manipulative field cage experiments that assessed competitive interactions between two species of parasitoids of an agromyzid leafminer *Liriomyza langei* Frick (Diptera: Agromyzidae). This study focused on the potential for control of *L. langei*, attacking cut chrysanthemums (Asteraceae: *Dendranthema grandiflorum* va. *Polaris*) by the commercially available parasitoids *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) and *Dacnusa sibirica* Telenga (Hymenoptera: Braconidae). The specific objectives of this study were to: (1) determine if interspecific competition occurs between these two parasitoids using different inoculative release strategies, (2) determine the impact of interspecific parasitoid interactions on leafminer population dynamics, and (3) determine how that impact influences crop conditions at harvest. These objectives were broken down into two questions. The first was to determine what impact releasing the two parasitoids simultaneously into a field would have on parasitoid and host population dynamics. The second was to determine the impact on parasitoid and host population dynamics by releasing a biological control agent into a field where an established natural enemy population already occurs.

## 2. Methods

### 2.1. Parasitoid-host system

The validity of the names used for the species referred to as *Liriomyza huidobrensis* have recently been clarified (Scheffer, 2000; Scheffer et al., 2001). Scheffer (2000) and Scheffer et al. (2001) have shown that *L. huidobrensis* consists of two monophyletic clades, one containing all the California and Hawaii populations, which Scheffer called *L. langei*, and all other populations, including those from South and Central America, which represent the true of *L. huidobrensis*. The name *L. langei*, originally proposed for a pest of peas and other crops in California (Frick, 1951), is available and should be applied to US populations. The *Liriomyza* colony used in these experiments was established from approximately 2000 pupae collected from Salinas, California in September 1998 from lettuce and will therefore be referred to as *L. langei*.

*Liriomyza langei* is a highly polyphagous species found attacking ornamentals including chrysanthemum, gerbera, and gypsophila (Chow and Heinz, 2004), and vegetables

including Asteraceae, Cucurbitaceae, Fabaceae, and Solanaceae (Spencer, 1973). *Liriomyza* damage is caused by larval feeding in the spongy mesophyll layer of the leaf and by the feeding and oviposition punctures of the females (Johnson et al., 1980). The feeding punctures can decrease photosynthesis (Johnson et al., 1980) and create entry sites for plant pathogens (Chow and Heinz, 2004). Larval mining can also decrease photosynthesis rates (Johnson et al., 1980) and can reduce tissue conductance (Chow and Heinz, 2004). The female flies lay their eggs on the adaxial side of the leaf and the larvae mine down to the abaxial side usually staying close to the midrib and lateral veins (Spencer, 1973; Johnson et al., 1980).

Two commercially available parasitoids, *D. sibirica* and *D. isaea* were used in this study because of their common use in augmentation biological control programs targeted at *Liriomyza* leafminers (van der Linden, 1994). *D. sibirica* is a synovigenic endoparasitoid without a preoviposition period (Minkenberg, 1990; Croft and Copland, 1994), and it is endemic to the Palaearctic region (Griffiths, 1966). Females oviposit in all host instars of *Liriomyza* species, regardless of size (Minkenberg and van Lenteren, 1987; Dicke and Minkenberg, 1991). The reproductive rate of *D. sibirica* decreases from 15 to 25 °C (Minkenberg, 1990) and is most commonly released at the beginning of the season against increasing host infestations (van der Linden, 2004). *D. isaea* is a synovigenic ectoparasitoid with a preoviposition period of 1–3 days also endemic to the Palaearctic region (Minkenberg, 1989). Females lay eggs on late second and third instar larvae (Minkenberg, 1989), but will host feed on all instars dependent up on availability (Minkenberg and van Lenteren, 1987). Cooler temperatures will decrease the development and oviposition rates of *D. isaea*; therefore *D. isaea* is commonly released in warmer climates and/or months when host populations are larger (Cheah, 1987; Minkenberg, 1989; van der Linden, 2004).

Both parasitoids can be purchased from commercial insectaries and are used around the world for biological control (van der Linden, 2004). The parasitoids used in these studies were purchased as units of 125 adults of each species from Koppert Biological Systems Inc. (Berkel En Rodenrijs, Zuid-Holland NL), which rears and distributes the product under the name Diminex™. Voucher specimens of parasitoids and leafminer have been deposited in the Texas A&M University Insect Collection.

## 2.2. Field site and insect material

Experiments were conducted at the Rangeland Field Site on the Texas A&M University campus in College Station, Texas, USA in fall of 2003 and fall of 2004, thus taking advantage of environmental conditions sufficient for flowering. Plots simulating cut chrysanthemum production were established as described in Kofranek (1980). Twenty-four field cages were used as experimental units where *L. langei* populations were established. Each cage measured

3.3 m (length) by 3.3 m (width) by 2.0 m (height) and was covered with 20 × 20 holes/cm mesh, Lumite™ fabric (Synthetic industries, Gainesville, Georgia, USA). The cages allowed for the replication of parasitoid releases and the exclusion of native pests and natural enemies.

In each cage, two 1 m by 2 m beds were planted with rooted chrysanthemum cuttings provided by a commercial propagator (Yoder Bros. Inc., Barberton, Ohio, USA) with 20 × 20 cm spacing of plants. Each bed contained 3 rows of plants totaling 30 plants per bed, and 1440 as the experiment total. Following commercial production practices, plant terminals were pinched 2 weeks after planting to promote rapid shoot development. Within each year, four cages were randomly selected for grouping into each of the six treatments. The six treatments included *D. sibirica* only, *D. isaea* only, *D. sibirica* and *D. isaea* released together, *D. sibirica* released first followed by *D. isaea*, *D. isaea* released first followed by *D. sibirica*, and no parasitoid release control. *L. langei* were reared at Texas A&M University on chrysanthemum (var. Mirimar) following the methods outlined in Ode and Heinz (2002). Relative humidity and temperature was measured and recorded inside and outside one cage using a HOBO® H8 Pro Series data loggers (Onset Computer Corporation, Bourne, MA), and two sets of recordings subsequently compared using a paired *t* test.

## 2.3. Inoculation rates of flies and parasitoids in field cages

Cages were inoculated with adult leafminers, of an unknown age, 4 days a week until crop harvest at a daily rate of one female and two males. Twelve hours before making releases into the field cages, the groups of three adult flies were placed in plastic tubes without a food source to help ensure mating. In each treatment in which wasps were released, two females and one males of an unknown age were released once a week beginning 2 weeks after the initial inoculation of flies for 7 weeks in 2003 and 2004. This was the same for each parasitoid species. Table 1 describes parasitoid release treatment combinations for the first weekly release and all subsequent releases. Koppert Biological Systems (<http://www.koppert.com/>) release recommendations are very low, 0.25 adults per square meter when releasing *D. isaea* and *D. sibirica* together. Based on this information we used the smallest release rate, which also allowed us to examine interspecific competition. This complementary release rate was used to insure that intraspecific interactions between the parasitoids were uniform across all treatments, and for ease of statistical analyses and interpretation.

Censusing of *L. langei*, *D. isaea*, and *D. sibirica* populations began immediately prior to the release of any parasitoids, and weekly thereafter. The sampling protocol involved the removal of 30 randomly selected mainstem leaves per cage, one per randomly selected plant. Each leaf was examined under a dissecting microscope (at 140× power) to determine the number of immature leafminers and immature parasitoids by species.

Table 1  
Summary of parasitoid (*D. isaea* and *D. sibirica*) release combinations used in 2003 and 2004 field tests of the influence of interspecific interactions between parasitoid species on parasitoid and host population dynamics and crop yield and quality

Treatment	Release 1	Release 2–7
<i>D. isaea</i> only	2 females and 1 male of <i>D. isaea</i>	2 females and 1 male of <i>D. isaea</i>
<i>D. sibirica</i> only	2 females and 1 male of <i>D. sibirica</i>	2 females and 1 male of <i>D. sibirica</i>
Both	2 females and 1 male of <i>D. isaea</i> and <i>D. sibirica</i>	2 females and 1 male of <i>D. isaea</i> and <i>D. sibirica</i>
<i>D. isaea</i> first, <i>D. sibirica</i> second	2 females and 1 male of <i>D. isaea</i>	2 females and 1 male of <i>D. isaea</i> and <i>D. sibirica</i>
<i>D. sibirica</i> first, <i>D. isaea</i> second	2 females and 1 male of <i>D. sibirica</i>	2 females and 1 male of <i>D. sibirica</i> and <i>D. isaea</i>

In both 2003 and 2004, 7 weekly releases were made.

#### 2.4. Occurrence of interspecific competition

In making the leafminer dissections, all leafminer larvae within each leaf were examined under a dissecting microscope for the occurrence of *D. isaea* eggs and larvae discovered adjacent to or near (less than 5 host lengths away) hosts. Each leafminer larva was then extracted from the leaf, dissected, and examined for *D. sibirica* eggs and larvae within it. These tallies were used to calculate parasitoid population densities by dividing the total numbers of parasitoids per cage by the numbers of susceptible host larvae per cage. The average number of parasitoids collected was pooled over the weeks due to the low populations of parasitoids and a mean seasonal abundance for each parasitoid was generated for each treatment. This standardized parasitoid population density estimate of immature parasitoids was compared using a two-way ANOVA with year and treatment as variables. If competition was occurring at an undetectable level, then the mean seasonal parasitoid abundances should not be significantly different among treatment comparisons.

To determine how each species influenced the population density of the other, the mean seasonal abundance for each species in the simultaneous release treatment was compared to each parasitoid's mean population density in the treatment where they were released individually. To determine the effect of releasing a parasitoid into an area where an existing natural enemy population is present, each parasitoid's mean seasonal abundance in the simultaneous release treatment was compared to the treatment in which that species was released first. This was done for both parasitoids to mimic a system where one species was naturally pre-existing. The null hypothesis relative to this analysis was for the mean seasonal abundance to not differ significantly among the treatment comparisons.

#### 2.5. Impact of interspecific competition on host suppression

The leafminer suppression estimates were calculated using all three larval instars from counts made from the leafmine dissections. The numbers of leafminer larvae in each parasitoid release treatment relative to the number in the control treatment were used to calculate the within-treatment suppression levels using the following equation:

$$S_t = \frac{N_c - N_t}{N_c} \quad (1)$$

where  $S_t$  is the average host suppression in a release treatment,  $N_c$  is the average number of leafminers in the control treatment, and  $N_t$  is the average number of leafminers in a parasitoid release treatment. These averages were obtained from the weekly sample counts of live leafminer larvae.

To assess the impact of interspecific interaction on host population dynamics the observed levels of leafminer suppression relative to the no release control, data obtained from the simultaneous release treatment were compared to the expected levels of leafminer suppression calculated using the single release treatments for each parasitoid. The expected level of suppression was determined using the following equation (Bográn, 2000, 2002):

$$\hat{S}_{a+b} = (S_a + S_b) - (S_a \times S_b) \quad (2)$$

where  $\hat{S}_{a+b}$  is the expected suppression by parasitoids a and b together,  $S_a$  is the observed host suppression by parasitoids species a when released singly and  $S_b$  is the observed host suppression by parasitoid b when released singly. To test for a significant effect of interspecific interactions on host suppression when both parasitoid species were released simultaneously, a replicated goodness of fit test (*G*-statistic) (Sokal and Rohlf, 1995) was performed on the observed suppression in the simultaneous release treatments and the expected levels of leafminer suppression based on the two single releases for each week in both 2003 and 2004. The replicated *G*-test examined weekly cage homogeneity and was used to test for a significant departure of observed from the expected levels of suppression. If interspecific competitive interactions occur at undetectable levels, then levels of suppression observed from the simultaneous release treatment should not differ significantly from the additive levels of suppression calculated from the single release treatments. Significant deviations from this expectation would suggest that releasing both parasitoids together would disrupt host suppression.

To examine the impact of interspecific interactions on host suppression, due to the release of one parasitoid when another is already present, the observed level of suppression in the simultaneous release (treated as the expected level of suppression) and both staggered releases were compared for each week in both years using a replicated good-

ness of fits test ( $G$ -statistic). Eqs. (1) and (2) were again used to calculate the expected levels of suppression. If the occurrence of the pre-existing population had no negative effect on the second released species, then the level of suppression observed from the simultaneous treatment should not be significantly different from the suppression levels observed from the staggered release treatments. Significant deviations from this expectation after week 3 would suggest that releasing either parasitoid into a population of the other would disrupt host suppression.

## 2.6. Crop quality

Crop quality at harvest was assessed using the number of days until harvest, number of flowers, and proportion of leaves damaged. Plants were harvested on a per plant basis when in 50% bloom. Bloom was defined as a flower that was completely open, meaning the bottom petals were flat. In each cage, 30 plants were harvested, where the top 33 cm of the plant was cut and placed into a black plastic bag. For each day a plant was harvested, the date, number of fully open flowers, total number of leaves, and damaged (both adult puncturing and larval mining) leaves were recorded.

The number of days to harvest, number of open flowers, and the proportion of leaves damaged at harvest were assessed. To assess the effect on crop cycle length of releasing the two parasitoids simultaneously, the average number of days until harvest for each cage in the control, *D. isaea* only, *D. sibirica* only, and the simultaneous release treatments were compared using a two-way ANOVA with year and treatments as variables. To determine the effect of releasing a parasitoid into an area where an indigenous natural enemy population already exists, the average number of days until harvest for each cage in the control, simultaneous, *D. isaea* first, and *D. sibirica* first treatments were compared also using a two-way ANOVA with year and treatment as variables. The same analyses were conducted to detect differences in the proportion of leaves damaged and the number of flowers at harvest among treatments. The null hypotheses were the absences of significant treatment effects for the average number of days to harvest, open flowers, and proportion of damage leaves. A square root transformation to the proportion of leaves damaged was applied to ensure homogeneity of data.

## 3. Results

Within-cage air temperatures closely tracked temperature recorded outside of the cages (Fig. 1), and considering the one-to-one correspondence between the recorded values from the different localities, mean temperatures were not significantly different ( $t = -2.362$ ,  $df = 5$ ,  $P = 0.065$ ). However, the within-cage relative humidity (Fig. 1) was consistently and significantly 1–15% higher than the values recorded outside of the cages ( $t = -5.963$ ,  $df = 5$ ,  $P = 0.002$ ). This difference in relative humidity did not have an observable effect on the numbers of immature leafminers present in the cages prior to parasitoid releases, as the starting numbers of leafminer larvae were not statistically different between years ( $F_{1,35} = 1.84$ ,  $P = 0.667$ ) or treatments ( $F_{5,35} = 1.17$ ,  $P = 0.343$ ). Thus, any observed differences among treatments were most likely due to treatment effects and not differences in temperature or starting conditions.

### 3.1. Occurrence of interspecific competition

As desired for this study conducted on an aesthetically important crop, the leafminer and parasitoid populations remained within the range of values reported for this crop in the greenhouse and field studies (see Chow and Heinz, 2004; Parrella, 1987 for reviews). Establishment of *D. isaea* could not be verified by the recovery of parasitoid eggs or larvae from the samples removed from the cages where both parasitoid species were released simultaneously in 2003, and in the cages where it was released when *D. sibirica* was already present in 2004. Establishment by *D. sibirica* was verified in all its released cages for both years.

A two-way analysis of variance, on mean seasonal abundance of *D. isaea* in the simultaneous release treatment and the treatment in which *D. isaea* was released alone showed there were no significant differences between the years ( $F_{1,11} = 0$ ,  $P = 1$ ), treatments ( $F_{1,11} = 1.472$ ,  $P = 0.251$ ), or in the year-by-treatment interaction ( $F_{1,11} = 1.16$ ,  $P = 0.304$ ) (Fig. 2A). When comparing the mean seasonal abundance of *D. sibirica* in the simultaneous release treatment to the single release treatment, there were no significant year ( $F_{1,10} = 0.92$ ,  $P = 0.360$ ), treatment ( $F_{1,10} = 0.28$ ,  $P = 0.606$ ), or year-by-treatment interaction effects ( $F_{1,10} = 0.10$ ,  $P = 0.756$ ) (Fig. 2B). The population

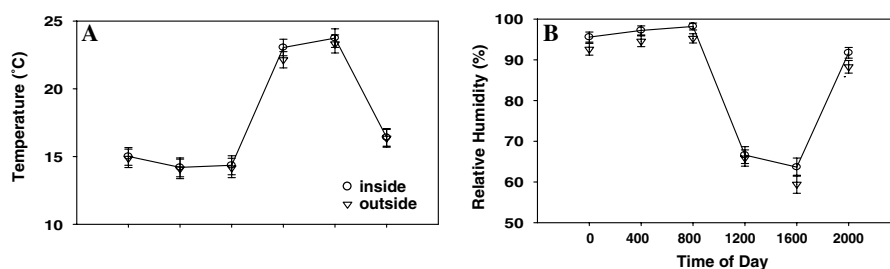


Fig. 1. Mean air temperature (°C) and relative humidity (%) inside and outside the cages during 2003. Comparisons were conducted using paired  $t$  tests (A)  $t_5 = -2.362$ ,  $P = 0.065$  and (B)  $t_5 = -5.963$ ,  $P = 0.002$ . Values represent means ( $\pm$ SE) of recordings every 4 h.

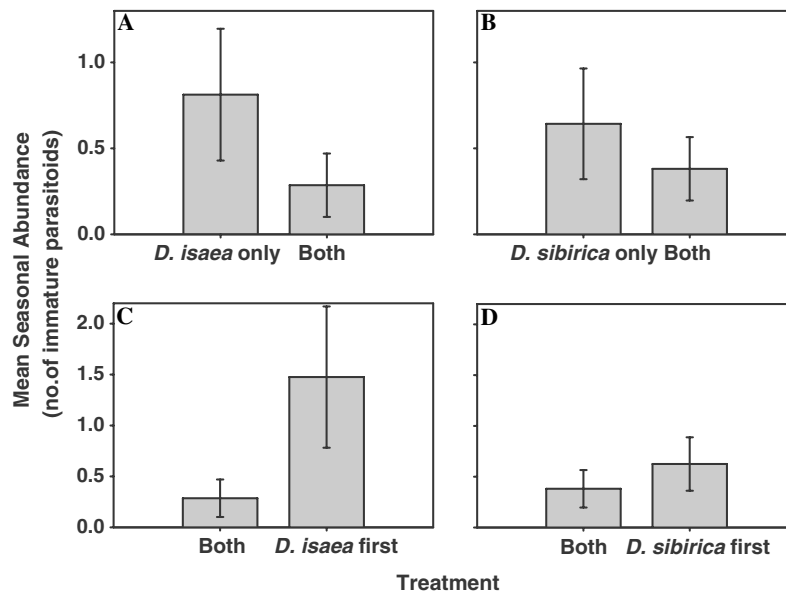


Fig. 2. Mean population density of (A) *D. isaea* in the *D. isaea* only and the simultaneous release treatments and of (B) *D. sibirica* in the *D. sibirica* only and the simultaneous release treatments. No significant differences among the treatments were found for (A)  $F_{1,11} = 1.472$ ,  $P = 0.251$  or (B)  $F_{1,10} = 0.283$ ,  $P = 0.606$ . Mean population density of (C) *D. isaea* in the simultaneous release and *D. isaea* first treatments and of (D) *D. sibirica* in the simultaneous release and the *D. sibirica* first treatments. No significant differences among the treatments were found for (C)  $F_{1,10} = 2.877$ ,  $P = 0.121$  or (D)  $F_{1,11} = 0.371$ ,  $P = 0.555$ . Plotted values represent means ( $\pm$ SE) for each treatment in both 2003 and 2004 combined.

densities of each parasitoid when they were released together were not significantly different from their population densities when released alone.

When comparing the mean population density of *D. isaea* in the simultaneous release treatment to the treatment where *D. isaea* was released first and *D. sibirica* second no significant differences were detected between the years ( $F_{1,10} = 1.54$ ,  $P = 0.243$ ), treatments ( $F_{1,10} = 2.88$ ,  $P = 0.121$ ), or year-by-treatment interaction ( $F_{1,10} = 4.12$ ,  $P = 0.070$ ) (Fig. 2C). Similarly, when comparing the mean population density of *D. sibirica* in the simultaneous release treatment to the treatment where *D. sibirica* was released first and *D. isaea* second, no significant differences were detected between the years ( $F_{1,11} = 1.20$ ,  $P = 0.296$ ), treatments ( $F_{1,11} = 0.37$ ,  $P = 0.555$ ), or year and treatment interaction ( $F_{1,11} = 0.13$ ,  $P = 0.722$ ) (Fig. 2D).

### 3.2. Host suppression

In 2003, each parasitoid release treatment was successful in reducing the number of leafminer larvae relative to the no parasitoid release control after week 5 (see Fig. 3A, C, and E for comparisons). By the end of week 4 in 2004 the parasitoid release treatments rarely reduced the number of leafminer larvae below the number in the control (see Fig. 3B, D, and F for comparisons). This result in 2004 may be due to the substantially lower numbers of leafminer larvae in 2004 control cages compared to 2003.

For each week the overall  $G$ -statistics ( $G_T$ ) were partitioned into terms representing pooled goodness of fit ( $G_P$ ) and heterogeneity among the cages ( $G_H$ ). The heterogeneity

of the cages was tested first and found to be non-significant ( $P > 0.05$ ), indicating that cage densities did not significantly differ from each other among weeks. Deviations from the null hypothesis represented by  $G_P$ , are non-significant ( $P > 0.05$ ) in every week. The total  $G$  suggests that the data as a whole fits the expected levels of suppression.

In summary, in both 2003 and 2004 the levels of suppression observed from the simultaneous release treatment did not differ significantly from the additive levels of suppression calculated from the single release treatments (expected suppression) (Fig. 4) In both 2003 and 2004 the cages in which *D. isaea* was released first (Fig. 5A and B) and the cages in which *D. sibirica* were released first (Fig. 5C and D) had similar levels of suppression as did the simultaneous releases.

### 3.3. Production of a harvestable crop

The full model analysis of variance on the average number of days until harvest showed there was a significant year effect when comparing the control, *D. isaea* only, *D. sibirica* only, and the simultaneous release treatments ( $F_{1,24} = 140.72$ ,  $P < 0.001$ ) and when comparing the control, simultaneous, *D. isaea* first, and *D. sibirica* first treatments ( $F_{1,24} = 138.18$ ,  $P \ll 0.01$ ). Therefore, subsequent analyses of variance on days until harvest were performed separately for the 2 years using a one-way ANOVA. There were no significant among-treatment differences in 2003 ( $F_{3,12} = 0.95$ ,  $P = 0.447$ ) or in 2004 ( $F_{3,12} = 0.48$ ,  $P = 0.701$ ) when comparing the control, *D. isaea* only, *D. sibirica* only, and the simultaneous release treatments. The same was true when comparing the control, simulta-

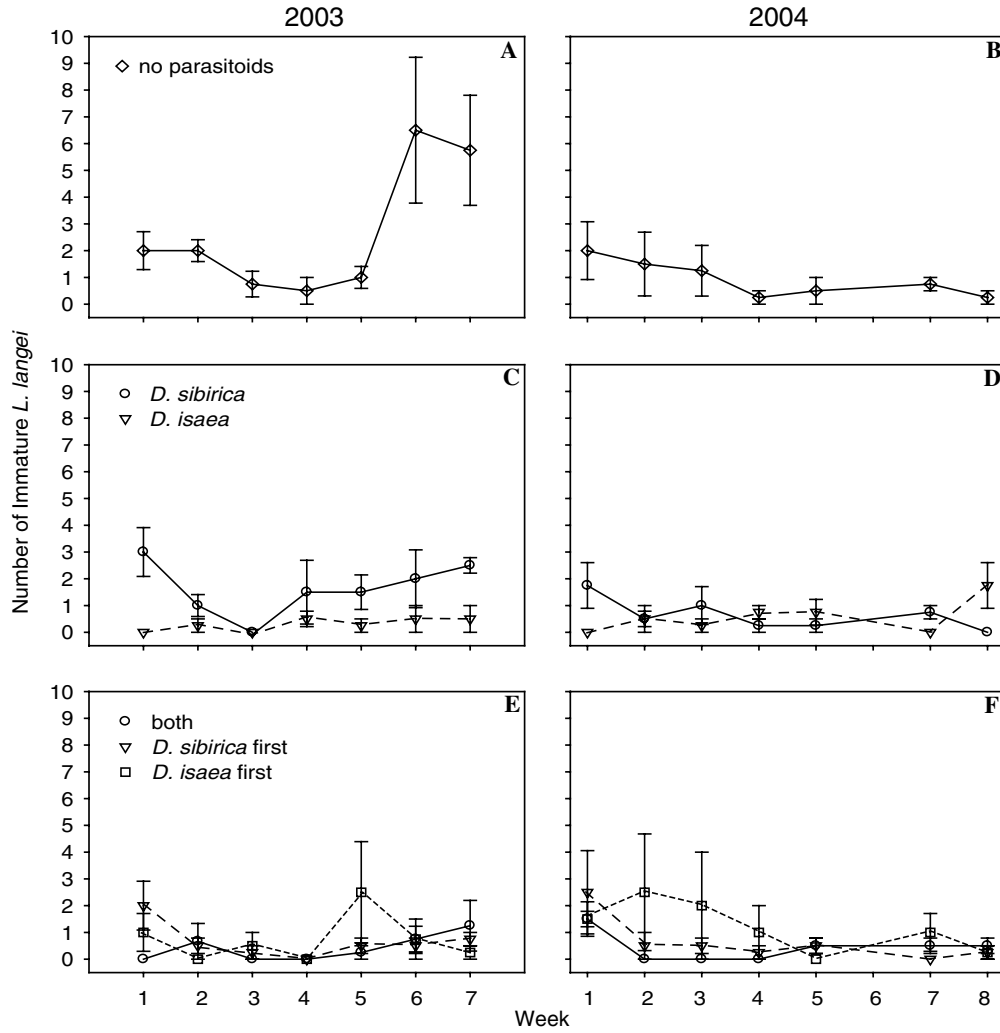


Fig. 3. Leafminer population density over time in the no parasitoid release control (A and B), the individual releases (C and D), and the combination releases (E and F) in 2003 and 2004, respectively.

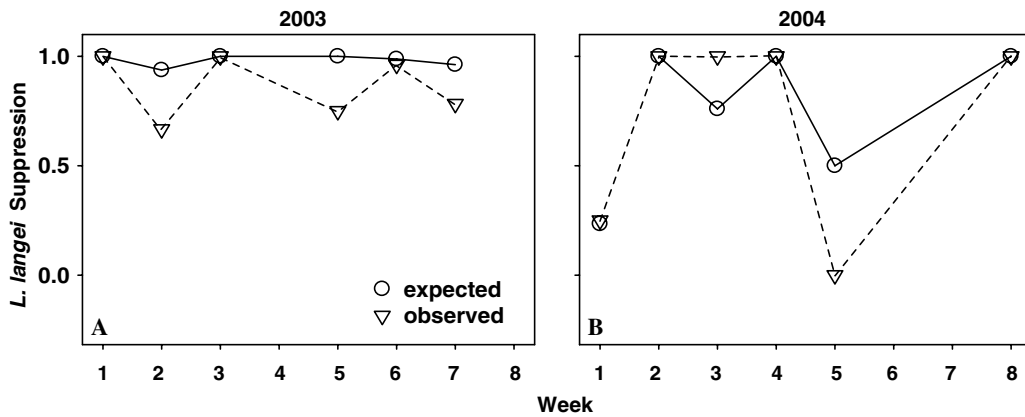


Fig. 4. Observed and expected levels of leafminer suppression in the treatment where *D. isaea* and *D. sibirica* were released simultaneously in (A) 2003 and (B) 2004. Expected mortality for the simultaneous release was calculated using the mortality caused by individual species in the absence of competitors and observed values are leafminer means for all replicates. Values represent cage means.

neous, *D. isaea* first, and *D. sibirica* first treatments, no significant treatment effects in 2003 ( $F_{3,12} = 0.67$ ,  $P = 0.587$ ) or in 2004 ( $F_{3,12} = 2.11$ ,  $P = 0.153$ ). The growing season

in 2004-averaged  $87.4 \pm 0.27$  days (mean  $\pm$  MSE,  $n = 24$ ), 11 days longer than in 2003, which averaged  $76.5 \pm 0.68$  (mean  $\pm$  MSE,  $n = 24$ ).

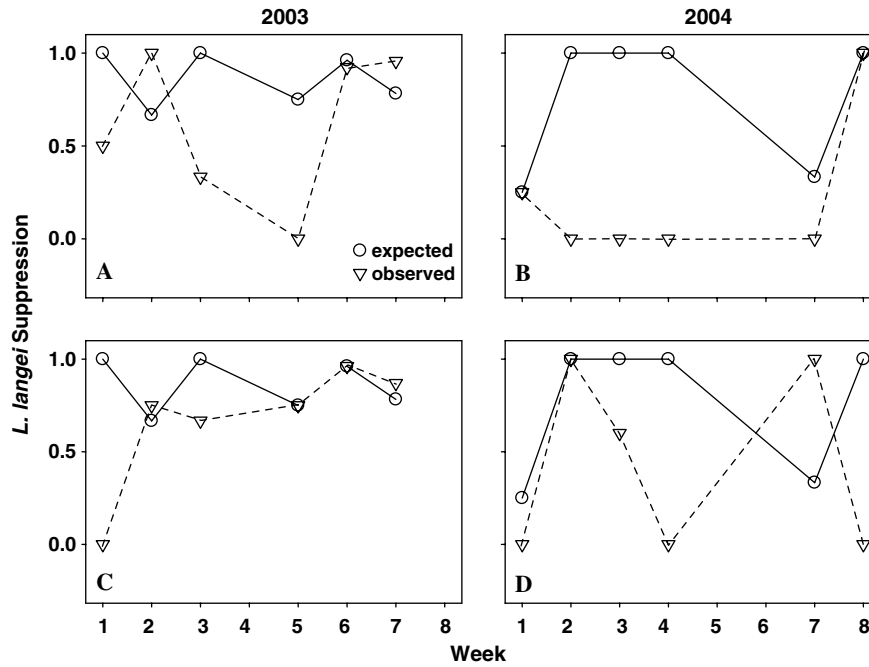


Fig. 5. Observed and expected levels of leafminer suppression in the treatment where *D. isaea* was released first in (A) 2003 and (B) 2004 and where *D. sibirica* was released first in (C) 2003 and (D). Expected mortality was calculated using the mortality caused when both species were released simultaneously and observed values are leafminer means for all replicates. Values represent cage means.

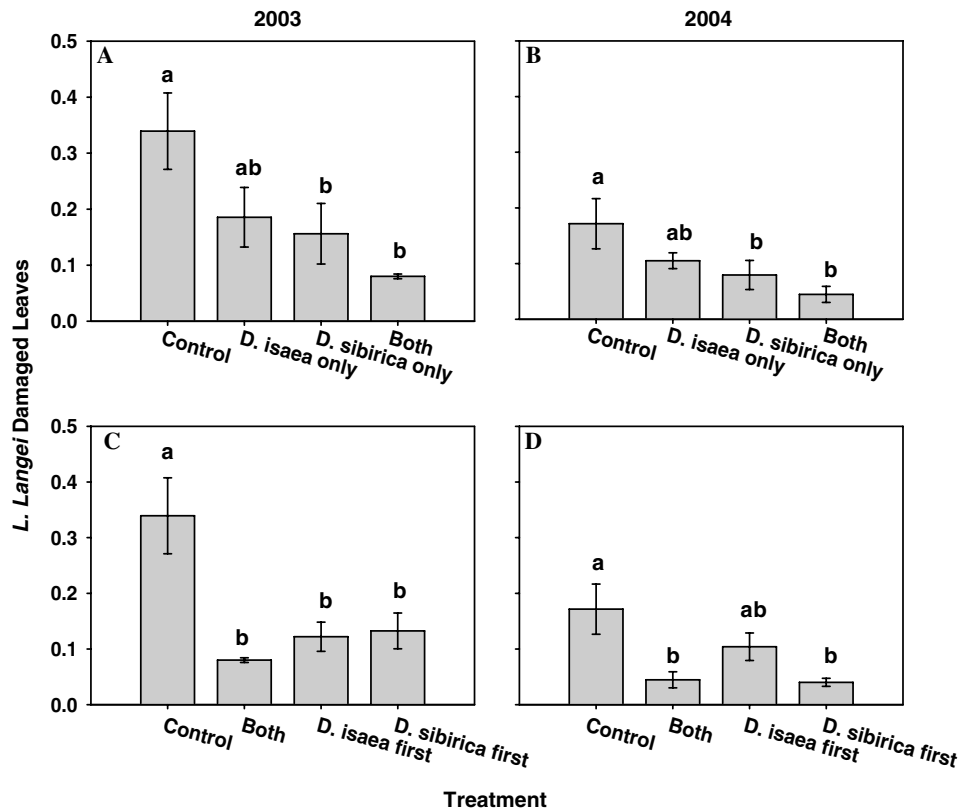


Fig. 6. Mean proportion of damaged leaves in the control, *D. isaea* only, *D. sibirica* only, and the simultaneous release treatments in (A) 2003 and (B) 2004. Mean proportion of damaged leaves in the control, simultaneous, *D. isaea* first, and *D. sibirica* first treatments in (C) 2003 and (D) 2004. Significant differences among the treatments were found for (A)  $F_{3,12} = 4.808$ ,  $P = 0.020$ , (B)  $F_{3,12} = 3.746$ ,  $P = 0.041$ , (C)  $F_{3,12} = 9.079$ ,  $P = 0.002$  (D)  $F_{3,12} = 5.996$ ,  $P = 0.010$ . Plotted values represent means ( $\pm$ SE) for each treatment in 2003 and 2004.

The full model analysis of variance on the proportion of damaged leaves at harvest showed significant year effects when comparing the control, *D. isaea*, *D. sibirica*, and simultaneous release treatments ( $F_{1,24} = 10.42$ ,  $P = 0.004$ ) and when comparing the control, simultaneous release, *D. isaea* first, and *D. sibirica* first treatments ( $F_{1,24} = 14.05$ ,  $P = 0.001$ ). Therefore, subsequent analyses of variance on proportion of damaged leaves at harvest were performed separately for the 2 years using a one-way ANOVA. When comparing the control, *D. isaea*, *D. sibirica*, and simultaneous release treatments, there was a significant treatment effect in both 2003 ( $F_{3,12} = 4.81$ ,  $P = 0.020$ ) and 2004 ( $F_{3,12} = 3.75$ ,  $P = 0.041$ ) (Fig. 6A and B). In both 2003 and 2004 (Fig. 6A and B), the no parasitoid control had a significantly higher proportion of damaged leaves than the cages in which *D. sibirica* was released alone and the cages in which both species were released simultaneously. However, the cages in which *D. isaea* was (Fig. 6A and B) released alone had the same proportion of damaged leaves as the control cages.

When comparing the control, simultaneous release, *D. isaea* first, and *D. sibirica* first treatments, there was a significant treatment effect in both 2003 ( $F_{3,12} = 9.08$ ,  $P = 0.002$ ) and 2004 ( $F_{3,12} = 5.99$ ,  $P = 0.010$ ) (Fig. 6C and D). In 2003, the no parasitoid release control had significantly more damage than the other treatments (Fig. 6C). In 2004, the no parasitoid control had significantly more damage than the simultaneous release treatment and the treatment in which *D. sibirica* was released first, but was the same as the treatment where *D. isaea* was released first (Fig. 6D).

The number of open flowers in the control, *D. isaea* only, *D. sibirica* only, and the simultaneous release treatments did not differ between the years ( $F_{1,24} = 0.34$ ,  $P = 0.566$ ), the treatments ( $F_{3,24} = 2.95$ ,  $P = 0.053$ ), or the year-by-treatment interaction ( $F_{3,24} = 0.42$ ,  $P = 0.739$ ). The same was true when comparing the number of open flowers in the control, simultaneous, *D. isaea* first, *D. sibirica* first treatments, there was no year effect ( $F_{1,24} = 0.70$ ,  $P = 0.410$ ), treatment effect ( $F_{3,24} = 0.78$ ,  $P = 0.517$ ) or year-by-treatment interaction ( $F_{3,24} = 0.83$ ,  $P = 0.490$ ). Even though parasitoid release combination did affect the amount of damage at harvest, the amounts of flowers generated were not influenced by release treatment.

#### 4. Discussion

The field experiments conducted using cut chrysanthemums permitted an assessment of the occurrence competitive interactions among parasitoids at low host densities, the effect of these interactions on host suppression, and their influence on the quality and quantity of harvest for an aesthetically important crop. We were unable to detect the presence of interspecific interactions between the two commercially available parasitoids of *L. langei* attacking chrysanthemum in a field cage situation. Even though

parasitoid release combination did affect the amount of damage visible at harvest, there was no influence on yield as measured in the number of flowers harvested. Data suggest that having both parasitoids present in the field cage concurrently did not disrupt host suppression. When parasitoids were released, either alone or in tandem, the leaf-miner population was lower than when no parasitoids were released except in 2004. In this single case, releasing *D. sibirica* where there was a pre-existing *D. isaea* population resulted in a leaf-miner population density that exceeded the density in the no release control.

We are unaware of any field or greenhouse studies that have evaluated the competitive interactions of *D. isaea* and *D. sibirica* outside of a laboratory. *Diglyphus isaea* and *D. sibirica*, both Palearctic in origin, can now be found outside of their native range due to the numerous inoculative introductions made worldwide (Minkenberg, 1989; Bazzocchi et al., 2003). Because of *D. isaea* and *D. sibirica*'s cosmopolitan distribution it is likely that these or a closely related species will colonize hosts in or near a producer's field. Although limited to a two-parasitoid species system, the results from our studies showed that the presence of a potential competitor did not affect the seasonal abundance of either *D. sibirica* or *D. isaea*. When both parasitoids were present from the beginning of the season their population densities were similar to that of when they were released alone. Releasing either species in the presence of the other did not disrupt the existing species mean seasonal abundance.

The absence of competition in the field cage study may be due to the species-specific difference in preference of host stages exhibited by the two parasitoids. We were unable to corroborate models showing that a parasitoid that attacks later host stages is competitively inferior unless it can utilize previously parasitized-hosts (Briggs, 1993). Based on this model and the studies reported in Mitsunaga and Yano (2004), *D. isaea* should be at a competitive advantage even though *D. sibirica* attacks and completes development in earlier host stages (Minkenberg and van Lenteren, 1987; Dicke and Minkenberg, 1991). Mitsunaga and Yano (2004) showed that in the laboratory *D. isaea* will oviposit and host feed on a host previously parasitized by *D. sibirica*. In our field cage study there was no evidence of *D. isaea* ovipositing or host feeding on any host already parasitized by *D. sibirica*. It is unlikely that *D. sibirica*, being a koinobiont endoparasitoid, will attack hosts already parasitized by the idiobiont *D. isaea* since the female *D. isaea* paralyze their hosts and in cage studies *D. sibirica* shows competitive avoidance of *D. isaea* (Mitsunaga and Yano, 2004).

The seasonal abundance estimates from the five release treatments were based on a weekly sample size of 120 leaves per treatment. This small sample does not fully represent the average scale of production, measured as 4.5 ha (11 ac) and over 65,000 plants, planted by large US floriculture producers (Jerardo, 2005). Although no significant differences between mean seasonal abundance of parasitoids were detected among the treatments, trends were

observable. When comparing each parasitoid's mean seasonal abundance in the individual releases to the simultaneous release the seasonal abundance in the simultaneous release was lower for both parasitoids. A similar pattern is detectable when comparing each parasitoid's mean seasonal abundance in the treatment where it was released first to the simultaneous release treatment. For example, there is no significant difference in the mean seasonal abundance of *D. isaea* when it was released first compared to its seasonal abundance in the simultaneous release. This lack of a statistically significant difference may result from the large within-date variation associated with the logistical restrictions placed on the number of cages (replicates) used in each year of the study.

To test the effect of sample size on the conclusions drawn from the statistical analyses performed on data generated from the studies, post hoc sample size calculations (Sokal and Rohlf, 1995) were applied to the data from 2003 to 2004 combined using the equation:

$$N \geq 2 \left( \frac{\sigma}{\delta} \right)^2 \{ t_{\alpha[v]} + t_{2(1-P)[v]} \}^2 \quad (3)$$

where  $N$  is the number of replication,  $\sigma$  the true standard deviation,  $\delta$  the smallest true difference to be detected,  $v$  is the degrees of freedom of the sample standard deviation with a groups and  $n$  replications per group,  $\alpha$  is the significance level,  $P$  is the desired probability that a difference will be found to be significant (power of the test) and  $t_{\alpha[v]} + t_{2(1-P)[v]}$  represent values from a two tailed  $t$  table. The goal was to calculate the number of replicates necessary to achieve statistical significance and to assess whether this sample size estimate was consistent with commercial production levels common to the cut chrysanthemum industry.

For our calculations, we set the power of the  $F$  test at 0.95 (as recommended by Sokal and Rohlf (1995) to increase the probability of rejecting the null hypothesis when it is false) for detection of a 0.5 difference in the mean seasonal abundances. To see an effect of releasing both parasitoids at the same time on the mean seasonal abundance of *D. isaea*, 80 (4800 plants) replicates would be needed; and on the mean seasonal abundance of *D. sibirica*, 49 (2940 plants) replicates would be needed. To see an effect of releasing *D. isaea* into a population of *D. sibirica*, 43 (2580 plants) replicates would be needed. To see an effect of releasing *D. sibirica* into a population of *D. isaea* 217 (13,020 plants) replicates would be needed. These calculations suggest that on a large-scale commercial production system (65,000 plants) statistically significant treatment effects on mean seasonal abundances would be distinguishable. Thus, on a large-scale commercial production system competition between the parasitoids may occur there by reducing the mean seasonal abundance of one or both of the parasitoids.

As results from our studies were unable to detect significant treatment effects on host suppression, the occurrence of a statistically significant interspecific interaction may not

be detrimental to biological control. This result may be due to the occurrence of the interspecific competition on the hosts shared by the competing parasitoids. When interactions occur through a shared host, there by killing the host, the interactions still generate high levels of host mortality (Heinz and Nelson, 1996) even when there is a reduction in one of the competing species population. Further, a compensatory host kill effect of one species may occur when the other species population is reduced. Under these conditions interspecific interactions are unlikely to negatively affect biological control of the host (Heinz and Nelson, 1996).

There have been cases where combining insect predators and or parasitoids proved to be more efficient (Heinz and Nelson, 1996; Kuhne, 1998) than releases of a single species. This issue, single versus multiple species release, has been discussed widely in the literature since Pemberton and Willard (1918) claimed that careful study of parasitoid interactions need to take place before multiple species releases are made. Before these studies, it was general practice to release all available species of primary parasitoids without considering potential negative consequences of antagonistic interactions between and among introduced species or with native species (Pemberton and Willard, 1918). More recently, it has been argued that the most effective parasitoid species would decrease the host population to a lower level when acting alone rather than when competing with another species (Turnbull, 1967). Conversely, May and Hassell (1981) had argued that it was not necessary to identify the best parasitoid species available, by providing cases where the use of more than one species has improved pest control (May and Hassell, 1981). This is important to this study because *D. isaea* and *D. sibirica* are recommended for release in combination at certain host densities (<http://www.koppert.com>). Our study showed that there was no difference in host suppression between releasing both species together than the additive suppression achieved by both species acting alone.

Based on a model described by May and Hassell (1981) the addition of a second parasitoid species into a population of an already established species can have one of four outcomes (Waage and Hassell, 1982). The most desired outcome is where the released parasitoid establishes and coexists with the first, increasing the level of suppression. Another satisfactory outcome is where the second parasitoid displaces the first, but suppresses the host at a lower level than with the first alone. An undesirable third outcome is when the released parasitoid does not establish, which is frequently seen in biological control. The least desirable outcome is where the interactions of the two parasitoids create periodic outbreaks of the pest. When both species established, our studies showed that *D. sibirica* and *D. isaea* coexisted for the entire growing season in both years. Releasing *D. isaea* into a population of *D. sibirica* did not affect host suppression. The suppression level was similar to what we expected based on the suppression achieved when there was no difference in release time.

The same was true for releasing *D. sibirica* into cages where a *D. isaea* population occurred.

Even though we were unable to show that interspecific competition occurred between *D. sibirica* and *D. isaea*, we did detect treatment-specific differences in the amount of damage at the end of the growing season. Based on laboratory-derived results, Mitsunaga and Yano (2004) claimed that the effect of simultaneous releases of *D. sibirica* and *D. isaea* was the same as making a single release of *D. isaea*. In both years of our study, the proportion of damaged leaves at the time of harvest was similar when the species were released individually and when released simultaneously. However, the amount of damage where only *D. isaea* was released was the same as if no parasitoids were present. This would indicate that having *D. sibirica* present enhances the biological control of the pest.

The differences between our study and Mitsunaga and Yano's (2004) may be due to differing environmental conditions. Mitsunaga and Yano (2004) suggest that environmental conditions during their study favored fecundity and development of *D. isaea*. In the UK and The Netherlands *D. isaea* is recommended for release during the warm summer months (Cheah, 1987; van der Linden, 1994) and *D. sibirica* during the cooler early spring and fall months (van der Linden, 2004). Many studies have shown that the optimal environmental conditions (mainly temperature) differ for these two species. The reproductive rate of *D. sibirica* decreases with increasing temperature (Minkenberg, 1990) and this species is therefore most commonly released at the beginning of the season against increasing leafminer infestations in Europe (van der Linden, 2004). Cooler temperatures will decrease the development and oviposition rates of *D. isaea*. Therefore, in Europe, *D. isaea* is commonly released later in the season when host populations are larger (van der Linden, 1994). The environmental conditions experienced during our study were substantially different than those described in the European studies. The early part of the Texas, fall growing season was the warmest with days cooling toward harvest. These conditions initially favored *D. isaea*, but as the season progressed, conditions shifted to favor *D. sibirica*. Even as the temperatures dropped there were only very few days that reached optimal temperatures for *D. sibirica* development.

These results have direct applicability to biological control practices in commercial ornamental production because we successfully mimicked grower conditions and practices, we utilized insectary-based recommended release rates, and our response variables are ones important to cut chrysanthemum production practices. We showed that the two commercially available parasitoids used for *Liriomyza* control were able to coexist throughout the chrysanthemum-growing season. However, the post hoc sample size calculations showed that on a larger scale, representative of large commercial producers, competitive interactions are likely to occur. To conduct a research experiment with the number of equivalent replicates indicated by the calculations would be impractical due to logistical constraints.

This restriction is likely to remain for studies conducted in ornamental crops due the low population densities required by the marketplace in these agricultural systems.

The results generated from our studies suggest that releases of either of the parasitoids tested should reduce the population of leafminers to below those in the absence of parasitoids. Results from this simple two-parasitoid system also suggest that if interspecific interactions between *D. isaea* and *D. sibirica* occur, they are not at levels adversely effecting biological control. However, as the complexity of the parasitoid community increases in terms of the number of parasitoid species, any introductions of natural enemies of *Liriomyza* species should take into account the indigenous natural enemy community and appropriate steps should be taken to conserve these native populations (Murphy and LaSalle, 1999).

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