

**IMPACT OF INTERSPECIFIC INTERACTIONS AMONG PARASITIDS ON
INOCULATIVE BIOLOGICAL CONTROL OF LEAFMINERS ATTACKING
CHRYSANTHEMUM**

A Thesis

by

AMY ELAINE BADER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

May 2006

Major Subject: Entomology

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Approved by:

Co-Chairs of Committee,	Kevin M. Heinz Robert A. Wharton
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ABSTRACT

Impact of Interspecific Interactions Among Parasitoids on Inoculative Biological Control
of Leafminers Attacking Chrysanthemum. (May 2006)

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Co-Chairs of Advisory Committee: Dr. Kevin Heinz
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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 remedial pest management solutions including augmentative biological control. When releasing mass-reared natural enemies into a backdrop of indigenous 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 both in a laboratory and in a simulated, field grown, cut chrysanthemum (Asteraceae: *Dendranthema grandiflorum*) 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 in this type of system. 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. Conclusions drawn from laboratory experiments were that *D. sibirica* produces

more offspring than *D. isaea* over approximately the same number of days. Treatment comparisons in the field included single species releases with complimentary releases of both species either simultaneously or with two-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; thus, the efficacy of one species released into a backdrop of potentially competing parasitoids did not negatively affect the outcome of the augmentative biological control. The two species were able to coexist inside field cages for the duration of the crop. Most of the release treatments suppressed host densities lower than the control cages where no parasitoids were released, and there were no treatment effects on host suppression. Even though parasitoid release combination did affect the amount of damage visible at harvest, there was no influence on the number of flowers produced (yield).

DEDICATION

to my grandmother

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First of all, I would like to thank my co-chairs for sticking with me through this experience. I am sure it wasn't easy. Their guidance and patience was greatly appreciated. I would also like to thank my other committee members, Drs. Kirk Winemiller and Carlos Bográn, for their time and input on my research project. In addition, I would also like to thank the Texas A&M University Hispanic Leadership Program In Agriculture and Natural Resources for their financial support.

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CHAPTER I

INTRODUCTION

Introduction

When crops are grown outside in fields there is a chance for indigenous natural enemies to be present. Because this natural control often does not provide adequate suppression, augmentative biological control is a potential solution. Even though biological control in ornamentals is difficult and not widely practiced, cut flowers provide a unique opportunity for augmentative biological control to be successful. When releasing mass-reared natural enemies into this backdrop of an indigenous population, competitive interactions are likely to occur. To explore this possibility the parasitoids *Diglyphus isaea* and *Dacnusa sibirica* attacking the leafminer *Liriomyza langei* on cut chrysanthemums was used as the model insect system. Both parasitoids are commercially available, considered cosmopolitan and likely to be occurring in major ornamental production areas.

Floriculture industry

According to the 2004 Floriculture and Nursery Crops Situation and Outlook Yearbook greenhouse and nursery crop proceeds from direct sales totaled \$15.7 billion and floriculture proceeds totaled \$5.2 billion in the United States in 2004 alone (Jerardo 2005). The United States imports over \$1.3 billion in floriculture and nursery products

This thesis follows the style and format of Biological Control.

and over half of that is in cut flowers (Jerardo 2005). The majority of U.S. floriculture and nursery imports originated from Canada and the Netherlands. Worldwide, Columbia, Ecuador, and the Netherlands constitute the vast majority of exporters of cut flowers.

Domestic cut flower producers averaged \$786,000 per grower, in 2004, which is the highest income of floriculture crops. Bedding and garden plants follow at an average of \$591,300 per grower and then foliage plants with an average of \$424,600 per grower in 2004. Cut chrysanthemum production sales for 2004 were over \$20 million in the United States totaling over 15 million stems sold. There are 61 cut chrysanthemum producers in the United States averaging \$328,361 per grower in 2004. The United States imported \$67 million worth of cut chrysanthemum constituting 30% of the annual sale.

Biological control in ornamental crops

While frequently suggested as a component of integrated pest management (Heinz 2004), biological control has rarely become a common pest management practice in ornamentals in the U. S. In the 1990s biological control was considered to be too costly to use in ornamental crops and was thus unable to compete with chemical control methods (Del Bene et al. 1994). Also, it is difficult to use biological control in many ornamental crops because of the high aesthetic value placed on these crops, which results in a near-zero tolerance to damage and arthropod presence (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 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, increased resistance to pesticides, and absence of pesticide residues (Jones et al. 1986; Heinz 1990).

Cut chrysanthemums are grown for their bouquet of flowers that possess a high aesthetic value (Carvalho and Heuvelink 2003). Cut chrysanthemums are one of the world's most popular floral crops (Murphy and Broadbent 2004). In 2004 cut chrysanthemum proceeds totaled over \$20 million and was 6th in cut flower production in the United States. Chrysanthemum crops attract a large array of pests including thrips, aphids, leafminers, two-spotted spider mites, and several Lepidoptera pests (Jones et al. 1986; Del Bene et al. 1994). This large pest complex adds to the difficulty in achieving successful biological control. The failure to control one pest using biological control may result in pesticide use, disrupting the biological control of the rest of the pests (Jones et al. 1986; Murphy and Broadbent 2004). There are several other limitations of biological control in cut chrysanthemum, some of which include start up costs, no commercial natural enemies available for secondary pests, and new and continual pest emergence (Murphy and Broadbent 2004).

There is a window in cut chrysanthemum production, before the harvestable foliage begins to appear, when moderate damage is tolerated (Jones et al. 1986; Parrella 1990; Chow and Heinz 2004). This window is about forty days after planting for a typical variety, after which time; no pest damage is tolerated (Jones et al. 1986; Heinz et

al. 1990; 1993; Sher et al. 2000). Thus, full leafminer control in cut chrysanthemum is required 40 days after planting. Harvesting of only the top 32 inches of the plant, and leaving the bottom portions of the plant in the ground, provides a cropping system capable of sustaining some damage and thus lending itself to biological control (Jones et al. 1986). The cut chrysanthemum system also offers potential for successful biological control because the plants are grown sequentially and year round in most commercial production operations, which allows for a long-term period of interaction between the pest and natural enemies (Parrella 1990). Yet, to achieve acceptable levels of damage control, large-scale inundative releases of natural enemies are required (Heinz 1990, Parrella 1990).

Continual pest and natural enemy invasions throughout the growing season often characterize field production systems. Agromyzid leafminers attacking chrysanthemum 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 several genera of parasitoids (Murphy and LaSalle 1999). Indigenous natural enemies may appear at any time to attack either an established or an incipient leafminer population. Because this natural control often does not provide adequate suppression, augmentative biological control is a potential solution (Minkenberg and van Lenteren 1987). Currently, there are two parasitoid species commercially available for control against leafminer pests. When releasing mass-reared natural enemies into this backdrop of an indigenous population, competitive interactions are likely to occur.

The pest

The Agromyzidae (Diptera) are primarily known as leafminers, but only 75% of the known 1800 species actually mine leaves. The genus *Liriomyza* contains some of the most economically important species of leafmining agromyzids (Spencer 1973, Lanzoni et al. 2002). *Liriomyza* contains more than 300 species, of which 20 are considered economically important (Spencer 1973, Lanzoni et al. 2002). Species in the genus *Liriomyza* attack a wide range of plants (Spencer 1973). In areas of recent introduction a concern of *Liriomyza* species in general is their potential to exploit new hosts that may occur in an enemy free environment (Chow and Heinz 2004).

Liriomyza huidobrensis is a highly polyphagous species found primarily on peas. It is also found attacking ornamentals including chrysanthemum, gerbera, and gypsophila (Chow and Heinz 2004), and vegetables in the families Asteraceae, Cucurbitaceae, Fabaceae, and Solanaceae (Spencer 1973). *Liriomyza huidobrensis* was originally found in Argentina in 1926, but is also known from Israel, Brazil, Chile, Peru, Columbia, Venezuela, Europe, and California (Spencer 1973, Parrella 1987, van der Linden 1990, Lanzoni et al. 2002). In the Netherlands it is one of the most economically important pests in greenhouses (Spencer 1973, Parrella 1987, van der Linden 1990, Lanzoni et al. 2002).

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, van der Linden 2004). The feeding punctures, referred to as stippling, can decrease

photosynthesis (Johnson et al. 1980, Rauf et al. 2002) 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 females lay their eggs on the upper side of the leaf and the larvae mine down to the underside usually staying close to the midrib and lateral veins (Spencer 1973, Johnson et al. 1980).

The validity of the names used for the species referred to as *L. huidobrensis* have recently been clarified (Scheffer 2000; and Scheffer et al. 2001). Frick (1951) described *L. langei* found primarily on peas and later described *Liriomyza dianthi* (Frick 1958) found on carnations. Frick (1964) synonymized *L. dianthi* with *L. langei*, and Spencer (1973) synonymized the North American *L. langei* with the South American *L. huidobrensis* (Scheffer 2000). In different areas of the world *L. huidobrensis* appears to have different preferred hosts and tolerances to insecticides, giving rise to the possibility of the existence of two or more cryptic species (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 represent the true *L. huidobrensis*. Currently there are no morphological differences to distinguish the two clades. As of 2001, *L. langei* has not been recorded anywhere outside of North America (Scheffer et al. 2001). Populations from South and Central America, as well as those from the Old World, belong to *L. huidobrensis* sensu stricto. 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 U.S. 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*.

Pesticide resistance

Early documentation of *Liriomyza* resistance to insecticides in several countries was summarized by Spencer (1973). The first known case of resistance resulting in management problems was from the Lea Valley in Hertfordshire, England in 1948, with *Liriomyza bryoniae* Kaltenbach after the use of dichloro-diphenyl-trichloroethane (DDT) and Lindane (BHC). This has also been the case in the Lower Rio Grande Valley of Texas where *Liriomyza subpusilla* Frost severely damaged peppers after DDT, tetrachlorodiphenylethane (TDE), and methoxychlor were applied in 1951. This is also true in Venezuela where an increase in leaf-mining pests coincided with the widespread use of insecticides beginning in the 1950s. In 1968 at Holtville, Imperial County, California a large leafminer infestation occurred on alfalfa due to development of resistance to diazinon. In Arizona, repeated use of DDT had no detrimental effect on the leafminer populations, but reduced the parasitoid populations dramatically.

Most recently Weintraub and Horowitz (1995) and Weintraub (1999) reported that *L. huidobrensis* arriving in Europe and Israel was already resistant to many locally used insecticides. Agromyzids repeatedly have become resistant to popular insecticides, resulting in increased difficulties in controlling population outbreaks (Schuster et al.

1991; Murphy and LaSalle 1999). In many of the cases leafminer resistance is an unintentional outcome induced by insecticide applications targeted at other pests such as external foliage feeding caterpillars (Jones et al. 1986).

Long-term use of insecticides to control pest populations may also disrupt natural control exerted by parasitoids. There is clear cut evidence that leafminer natural enemies are not resistant to pesticides, that their populations are reduced when growers over-spray for fly control, and that this results in exploding leafminer populations. The broad-spectrum insecticide methomyl, in California for example, increased *L. sativae* populations and decreased their parasitoid populations and when used against *Heliothis zea* (Boddie) decreased populations of *Diglyphus begini* (Ashmead) (Hymenoptera: Eulophidae) and *Chrysonotomyia punctiventris* (Crawford) (Hymenoptera: Eulophidae) (Johnson et al. 1980). In a study done by Schuster (1994) methomyl and permethrin were highly toxic to all stages of *D. intermedius* (Girault) and *Neochrysocharis punctiventris* (Crawford) (Hymenoptera: Eulophidae).

Natural enemies of Liriomyza pests

In natural conditions agromyzid pests are usually kept under control by their own parasitoid complexes (Johnson et al. 1980; Schuster 1994; Murphy and LaSalle 1999). Families of parasitoids reared from *Liriomyza* pests include Braconidae, Figitidae (Eucoilinae), and 23 species of Chalcidoidea primarily in the families Pteromalidae and Eulophidae (Spencer 1973; Minkenberg and van Lenteren 1990). The composition of field-collected samples may vary dramatically.

Two parasitoids, the braconid *D. sibirica*, and the eulophid *D. isaea*, are commercially available for control of *Liriomyza* pests (van der Linden 1994). Species in the genus *Diglyphus* have been studied as control agents for inoculative release programs in field and indoor vegetables and for inundative programs in greenhouse ornamentals (Jones et al. 1986, van Lenteren and Woets 1988, Heinz and Parrella 1990a, b, c, Minkenberg and Parrella 1990, Minkenberg and van Lenteren 1990; Del Bene et al. 1994, Bazzocchi et al. 2003). *Dacnusa sibirica* Telenga (Hymenoptera: Braconidae) is known to attack many agromyzids throughout most of the old world (Spencer 1973). In greenhouses *Liriomyza* species are being controlled by both *D. sibirica* and *D. isaea*. In the UK and Netherlands *D. isaea* is recommended for release during the warm summer months (Cheah 1987, van der Linden 1994) and *D. sibirica* during the early spring and fall (van der Linden 1994). Successful control has been achieved in the UK (Buxton and Finlay 1993), the Netherlands (Ravensberg and Altena 1993) and Canada (Murphy and Broadbent 2004).

Dacnusa sibirica is a synovigenic endoparasitoid without a preoviposition period (Minkenberg 1990; Croft and Copland 1994) endemic to the Palaearctic region (Griffiths 1966). Females oviposit in all host instars of *Liriomyza* species, regardless of size and do not host feed (Minkenberg and van Lenteren 1987; Dicki and Minkenberg 1991). The reproductive rate of *D. sibirica* decreases with increasing temperature (Mickenberg 1990) and is most commonly released at the beginning of the season against increasing host infestations (van der Linden 2004).

Diglyphus isaea is a synovigenic ectoparasitoid with a preoviposition period of 1 to 3 days also endemic to the Palaearctic region (Minkenberg 1989). Females lay eggs on late second and third instar larvae, but will host feed on these and late first instar larvae (Minkenberg 1989). Cooler temperatures will decrease the development and oviposition rates of *D. isaea*; therefore *D. isaea* is commonly released later in the season when host populations are larger (Cheah 1987, van der Linden 2004). Both parasitoids can be purchased from Koppert Biological Systems Inc. (Berkel En Rodenrijs, Zuid-Holland NL) and are used around the world for biological control (van der Linden 2004).

Competition

Competition can be defined as any interaction that is mutually detrimental between species sharing the same limited resource (Smith and Smith 2001). Competition that occurs between different species is called interspecific competition (Pianka 2000). Interactions involving resource depression or depletion are called exploitative competition (Pianka 2000). Competitive exclusion is defined as a system, which does not exhibit a deterministic tendency towards coexistence (Armstrong and McGehee 1980) so one species eliminates another entirely when they come into contact (Pianka 2000).

The role of interspecific competition in population dynamics has been a frequently studied phenomenon (Connel 1983; MacNally 1983; Briggs 1993; Walter and Paterson 1995), and its influence on the outcome of biological control has been discussed in the literature since the early 1900s (Smith 1929). It is possible that

interspecific interactions can lead to lower levels of parasitization, reducing pest regulation (Ehler 1979). There are still little data addressing the impact of parasitoid interspecific competition on parasitoid-host dynamics from field experiments (Godfray 1994). Laboratory studies can provide important data on competition mechanisms, but have limited applicability to nature without field data (Godfray and Waage 1991; Bográn 2000). Many 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 et al. 2002). For this project both lab and field experiments were conducted to increase the applicability to biological control practitioners.

There have been cases where combining insect natural enemies proved to be more efficient (Kuhne 1998) than releases of a single species. This issue, single versus multiple species release, has been a heated topic since Pemberton and Willard (1918) claimed that careful study of parasitoid interactions need to take place before multiple species releases are made. Before this it was general practice to release all available species of primary parasitoids, with little consideration given to the possibility of interference with one another (Pemberton and Willard 1918).

Currently it is common practice to release *D. sibirica* and *D. isaea* in combination. *D. isaea* and *D. sibirica* exhibit some niche and resource (host instars) overlap (Minkenbergh 1989; Minkenbergh and van Lenteren 1987; Dicki and Minkenbergh 1991), which might lead to interspecific competition between the two affecting biological control of the host (Kakehashi et al. 1984; Mills and Getz 1996; Briggs and

Latto 2001; Collier and Hunter 2001). With the presence of more than one natural enemy, competitive exclusion, due to resource depletion, and coexistence are possible outcomes (Ciros-Perez et al. 2001; Collier and Hunter 2001). *D. sibirica* and *D. isaea* have equal access to their host and resource depletion by one species could affect the survival of the other. With the occurrence of exploitative competition individuals are indirectly affected by the abundance of the food source and the difficulty in finding this food (Kawata 1997, Pianka 2000). Interaction between species will alter individual survival and reproduction because as the two populations increase, so will the effects of exploitative competition (Kawata 1997). Competitive exclusion due to resource depletion occurs when one species reduces the resource below that required for population growth of the other species (Ciros-Perez et al. 2001).

There are four possible outcomes of interspecific competition between *D. sibirica* and *D. isaea* based on the Lotka and Volterra model describing interactions between two species sharing the same resource (Pianka 2000). These are: *D. sibirica* always wins, *D. isaea* always wins, either species can win, and neither species can contain the other, meaning there is a stable coexistence. Coexistence or a stable equilibrium occurs when neither species can inhibit the other, both species inhibit their own population growth more than that of the other species.

Both parasitoids are cosmopolitan and likely to be occurring in major ornamental production areas. Each species may be purchased individually with the possibility of being released into a population of the other. When species occur simultaneously, whether naturally or by release, it is important to examine their competitive interactions

(Ehler 1979). Several studies have shown that *D. sibirica* naturally enters greenhouses in the late fall and winter in Northern Europe and growers then release *D. isaea* into the same greenhouse in late spring as leafminer populations increase (Cheah 1987, van der Linden 2004).

According to 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. Unless the two species aggregate independently of each other the host density is lower with a second parasitoid than when either is acting alone (May and Hassell 1981, Briggs 1993). A second parasitoid can invade a system when both of the parasitoids limit their own abundance more than that of the competitors (Mills and Getz 1996). Coexistence is possible as long as the second parasitoid is more efficient and can distinguish between previously parasitized and non-parasitized hosts (Briggs 1993).

Mitsunaga and Yano (2004) studied the effect of multiple parasitism by these two species when attacking *L. trifolii* in a laboratory setting. They conclude that *D. sibirica* is the competitively inferior wasp and shows competitive avoidance of *D. isaea*.

This is due to *D. isaea* being an idiobiont ectoparasitoid and killing both the host larvae and the endoparasitoid *D. sibirica* (Mitsunaga and Yano 2004). There have been no field experiments examining the interaction or efficacy of these two parasitoids when attacking *L. langei*.

Project focus

This project focuses on the potential interspecific competition between the two commercially available parasitoids, *D. sibirica* and *D. isaea*, used against *Liriomyza langei*, attacking chrysanthemums (Asteraceae: *Dendranthema grandiflorum* va. *Polaris*). Although these parasitoids are currently available to be released alone or in tandem, their competitive interactions have not been closely examined. The objectives of this study were four-fold: 1) to assess the life history traits (longevity, total offspring production and host kill) of both parasitoids, 2) to assess interspecific competition, (3) to assess what impact parasitoid interactions have on host population dynamics, and (4) assess how that impact influences crop condition at harvest.

The objectives were achieved by conducting a lab and a field experiment. In the lab experiment the longevity and total offspring production of *D. isaea* and *D. sibirica* when attacking *L. langei* alone were assessed. The effects of host feeding on host mortality was also assessed because *D. isaea* host feeds and *D. sibirica* does not (Minkenbergh and van Lenteren 1987; Dicki and Minkenbergh 1991). In the field experiment, using conditions simulating cut chrysanthemum production; parasitoid interactions and suppressive capabilities were assessed.

CHAPTER II
REPRODUCTIVE POTENTIAL AND HOST KILL ASSESSMENT OF
DIGLYPHUS ISAEA* AND *DACNUSA SIBIRICA* ON *LIRIOMYZA LANGEI
ATTACKING CHRYSANTHEMUM

Introduction

Liriomyza langei Frick (Diptera: Agromyzidae) is a highly polyphagous species attacking ornamental plants including *Dendranthema grandiflorum*, *Gerbera jamesonii*, and *Gypsophila paniculata* (Chow and Heinz 2004), as well as several vegetable species in the families Asteraceae, Cucurbitaceae, Fabaceae, and Solanaceae (Spencer 1973). Female flies lay their eggs adaxially and upon eclosion the larvae mine down to the abaxial side usually staying close to the midrib and lateral veins (Spencer 1973; Johnson et al. 1980). Damage to crops is a result of larval feeding in the spongy mesophyll layer of the leaf and feeding and oviposition punctures created by female adults (Johnson et al. 1980). Larval mining can also decrease photosynthetic rates (Johnson et al. 1980), reduce tissue conductance, and create entry points for plant pathogens (Chow and Heinz 2004).

Augmentation biological control, which relies on the purchase and release of mass-reared natural enemies, is frequently proposed as a means of managing agromyzid pests within agriculture (Heinz and Parrella 1989, 1990a, 1990b; Johnson and Hara 1987; Minkenberg and van Lenteren 1987, 1990; Heinz et al. 1993; Petitt and Wietlisbach 1993). While many parasitoids attack agromyzid leafminers (see Schuster

and Wharton 1993; Murphy and La Salle 1999 and van der Linden 2004 for reviews on this topic), only *Dacnusa sibirica* Telenga (Hymenoptera: Braconidae) and *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) are available from commercial insectaries (van der Linden 2004). *Dacnusa sibirica* is a proovigenic koinobiont endoparasitoid (Minkenberg 1990; Croft and Copland 1994), which oviposits in all host instars of *Liriomyza* species but apparently does not host-feed (Minkenberg and van Lenteren 1987; Dicke and Minkenberg 1991). *Diglyphus isaea* is a synovigenic idiobiont ectoparasitoid (Minkenberg 1989), which oviposits on late second and third host instars but may puncture and host-feed upon all immature stages (Minkenberg and van Lenteren 1987). As an ectoparasitoid, *D. isaea* will oviposit into a host already parasitized by *D. sibirica*, thus killing the braconid (Mitsunaga and Yano 2004). Being that *D. sibirica* is a koinobiont endoparasitoid that requires an actively feeding host to complete development it is unlikely that it will oviposit into a host previously parasitized by *D. isaea* (Mitsunaga and Yano 2004).

As a result of worldwide introductions for biological control, both parasitoids are cosmopolitan and likely to be occurring in major ornamental production areas (Minkenberg 1989; Bazzocchi et al. 2003). Each species may be purchased in combination or individually with the possibility of being released into a population of the other. In order to assess potential interspecific interactions and the potential impact of competitive interactions on performance in a biological control context, it is useful to have baseline information on individual performance in the absence of competition. The purpose of this study is to compare data on biological traits that might assist in

predicting the outcome of competitive interactions between *D. isaea* and *D. sibirica* when attacking *L. langei* on chrysanthemum.

Pre-introduction criteria for parasitoids have been studied in depth (see van Lenteren 1986 for a review of selection criteria). Criteria proposed for selecting which parasitoids to be used in an augmentative biological control program include type of development on or in the host, non target effects, synchronization with the host, reproductive capacity or population growth, host kill rate (rate of killing host through oviposition, host feeding, and/or mutilation), searching efficiency, and density responsiveness (van Lenteren 1986; Minkenberg and van Lenteren 1987, 1990). Although intrinsic rate of increase and net reproductive rate are the usual parameters measured when examining population growth rates, longevity and fecundity have often been used as a more readily comparable measure of reproductive capacity (Minkenberg 1989; Patel and Schuster 1991; Haile et al 2002). The amount of host mortality derived from each parasitoid under consideration is also valuable when comparing potential biological control agents (Scholler and Hassan 2001; Haile et al. 2002). In combining parameters, the parasitoid with a higher reproductive potential and ability to cause greater leafminer mortality should be the more effective parasitoid (Minkenberg and van Lenteren 1987; van Lenteren 1986; Scholler and Hassan 2001). Regardless of the parameters assessed, predicting outcomes based solely on individual or arrays of populational parameters between potentially competing parasitoids is difficult because of multiple interactions among life history traits (Minkenberg and van Lenteren 1990).

Studies on the life history characteristics of both *D. sibirica* and *D. isaea*, derived from studies conducted on several *Liriomyza* species and at different temperature ranges, suggest that both are candidates for biological control of leafminers (Minkenberg 1989, 1990; Minkenberg and van Lenteren 1987; van der Linden 1991). However, there are no published studies that examine the life history characteristics of these parasitoids with *L. langei* as host. *Liriomyza* parasitoids are effective in attacking several leafminer pests on several host plants (Christi and Parrella 1987; Heinz and Parrella 1990a; La Salle and Parrella 1991; Murphy and La Salle 1999), however each host and host plant may differentially influence the values obtained for life history parameters characterizing attack behavior (Christi and Parrella 1987; Heinz and Parrella 1989; van der Linden 1994; Bazzocchi et al. 2003). In addition, host-induced affects, such as encapsulation, are known to affect the success of *D. sibirica* when attacking *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) (Parrella 1987).

The objectives of this study were to (1) compare the reproductive potential defined in terms of longevity and total offspring production for these commercially available parasitoids using potted chrysanthemums infested with larvae of *L. langei* as the host plant and pest species and (2) compare host death by oviposition and host feeding for each parasitoid as *D. isaea* host feed (Minkenberg and van Lenteren 1987) and *D. sibirica* apparently does not (Minkenberg and van Lenteren 1987; Dicki and Minkenberg 1991). These comparisons were done with the goal of predicting which parasitoid species will have the competitive advantage when released against *L. langei*.

Methods

Insect host and host plants

The *Liriomyza* colony used in these experiments was established from approximately 2000 pupae collected from Salinas, California in September 1998. The protocol described by Ode and Heinz (2002) was used to rear *L. langei* on chrysanthemum. The following procedures were used in preparing *L. langei* hosts for exposure to parasitoids. Uninfested potted chrysanthemums (3 plants per 15.5cm diameter × 14.5cm depth pots, ITML Inc., Ontario, Canada) were exposed to a colony of adult *L. langei* (100-200 females), four pots at a time, for 1 hour during 5 consecutive days. This staggered exposure regime was used to provide parasitoids with plants containing all three host developmental stages. Potted plants were then isolated from leafminers in conditions similar to the rearing environment ($25 \pm 5^{\circ}\text{C}$ and $60 \pm 10\%$ R.H. mean \pm MSE) for two days to allow for larval development prior to exposure to the parasitoids.

Exposure to parasitoids

Measurements of parasitoid life history characteristics were made from four cohorts of three mated females of unknown age each of *D. sibirica* and *D. isaea*. Each cohort represented a different shipment of parasitoids from Koppert Biological Systems (Berkel En Rodenrijs, Zuid Holland, NL). The parasitoids were used within 24 hours of receipt. Although the parasitoids were of unknown age, they were known to have had no previous experience with hosts. Use of commercially reared parasitoids was favorable in making comparisons between the two species as it would best represent conditions experienced by biological control practitioners making releases in the field. One adult female and two adult males were released (three cages per parasitoid species) into each of six Plexiglas® cages (36 (length) × 36 (width) × 46 (height) cm), all cages were held at $25 \pm 10^\circ\text{C}$ and $60 \pm 10\%$ R.H (mean \pm MSE, n = 56 days). Each cage contained one pot with three chrysanthemum plants infested with a cumulative total of 11-100 first, second and third-instar *L. langei*. Males that died before completion of the experiment were replaced to insure that females were not sperm-limited. Adult longevity measured in days (d) was recorded for each female parasitoid used in the study.

After exposure to the wasps for 24 hours, the chrysanthemum pots were removed, and held in the same environmental conditions as above. Three days after removal, all *L. langei* larvae and pupae were removed from the leaf, counted and classified as unparasitized live, parasitized live (in the *D. sibirica* treatments), unparasitized dead or parasitized dead (in the *D. isaea* treatments). All larvae were then dissected with the aid of microscopy under (at 100 - 140× power) and the numbers of parasitoid eggs and larvae

of each species were censused. Any leafminer that was classified as unparasitized dead was assumed to have died naturally or by host feeding. It was not always possible to determine if a leafminer died of natural causes or from host feeding, thus a direct count of dead leafminers caused by host feeding was not attempted. Three other cages containing infested plants did not receive parasitoid releases and were used to assess natural leafminer mortality under these laboratory conditions. Similar to the other treatments, larvae within these plants were assessed as live or dead three days after the pots were removed from the cages.

Data analysis

Data from the experiments were used to calculate longevity and total offspring production for each parasitoid species and the number of hosts each parasitoid killed by host feeding and parasitization. The occurrence of a significant between-parasitoid species difference in adult longevity was assessed using a two-way ANOVA with cohort and species as variables. Significant between-species differences in lifetime offspring productions were detected using a separate two-way ANOVA with cohort and species as variables.

To assess host kill by each parasitoid we examined the number of unparasitized dead hosts, parasitized hosts, and total mortality caused by each parasitoid. To detect the presence of a significant between-treatment difference in the numbers of hosts killed without oviposition by *D. sibirica* and *D. isaea*, a third two-way ANOVA was performed with adult longevity and treatment (including mortality data from the control

cages) as variables. This comparison was done to determine if there were more dead hosts in the cages containing parasitoids than should have occurred naturally. To detect the presence of a significant between-species difference in the numbers of hosts parasitized, a fourth two-way ANOVA was performed with adult longevity and treatment as variables. In addition, significant differences between the numbers of dead and or parasitized leafminer larvae resulting from attacks by *D. sibirica* and *D. isaea* were detected using a fifth two-way ANOVA with the adult longevity and treatment as variables.

Results

Longevity and total offspring production

Diglyphus isaea female wasps lived an average of 5.6 days whereas *D. sibirica* female wasps lived an average of 7.2 days (Table 1). Results from the two-way ANOVA detected no significant differences of longevity between the cohorts ($F_{3,16} = 2.143, P = 0.135$), species ($F_{1,16} = 2.242, P = 0.154$), or in the cohort-by-species interaction ($F_{3,16} = 0.702, P = 0.565$) (Table 1). There were no significant differences in total offspring production between the cohorts ($F_{3,16} = 0.088, P = 0.966$) or in the cohort-by-species interaction ($F_{3,16} = 0.121, P = 0.946$), but there was a significant difference between the species ($F_{1,16} = 6.787, P = 0.019$) (Table 1). *Dacnusa sibirica* on average produced 87.7 offspring over 7.2 days, whereas *D. isaea* on averaged produced 37.9 offspring over its average lifespan of 5.6 days.

Table 1. Summary of the reproductive capabilities of *D. isaea* and *D. sibirica*.

Values represent mean \pm SE and were compared using a two-way ANOVA, N = 12 female parasitoids.

	Longevity	Total Offspring Production
<i>D. isaea</i>	5.6 \pm 0.7 a	37.9 \pm 9.5 a
<i>D. sibirica</i>	7.2 \pm 0.9 a	87.7 \pm 13.6 b

Note: Means followed by the same letter with columns are not significantly different ($P > 0.05$).

Host death

A two-way ANOVA detected a significant difference ($F_{2, 187} = 27.23, P < 0.001$) between the average number of unparasitized dead leafminers in the *D. isaea* cages, *D. sibirica* cages, and the control cages (Figure 1), but no significant difference was detected among days ($F_{10, 187} = 1.61, P < 0.11$) or in the day-by-treatment interaction ($F_{17, 187} = 0.89, P = 0.59$). The numbers of dead leafminers in the *D. sibirica* cages were similar to the numbers of dead leafminers counted in plants from the control treatments, but both had significantly fewer dead leafminer larvae than the cages containing *D. isaea*. There was a significant difference in the number of hosts parasitized by each species ($F_{1, 112} = 14.43, P < 0.001$) (Figure 2) without a day ($F_{10, 112} = 1.87, P = 0.058$) or treatment-by-day effect ($F_{7, 112} = 0.92, P = 0.49$). However, total *L. langei* mortality (dead plus parasitized larvae) in the *D. isaea* cages and the *D. sibirica* cages were not significantly different ($F_{1, 113} = 1.46, P < 0.23$) (Figure 3) nor was there a day ($F_{10, 113} = 1.65, P < 0.10$) or a day-by-treatment effect ($F_{7, 113} = 1.26, P < 0.28$).

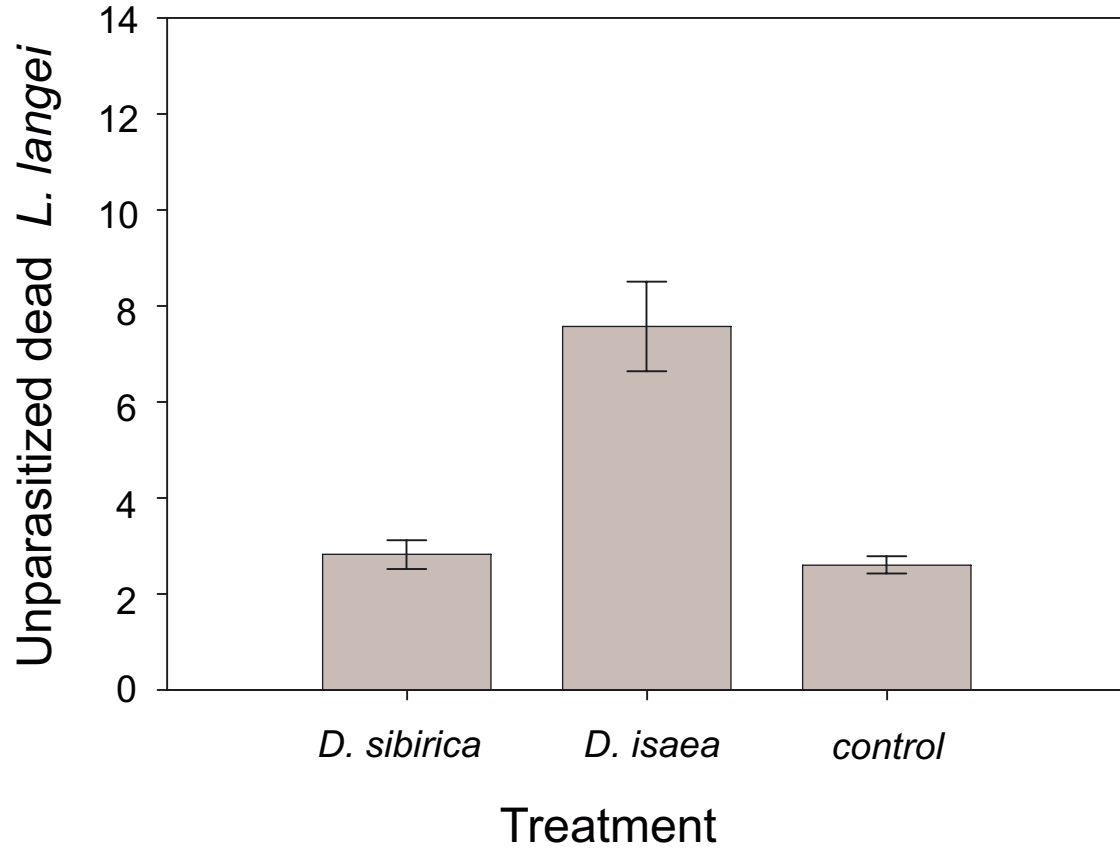


Fig. 1. Mean number of unparasitized dead leafminer larvae in the control, *D. isaea*, and *D. sibirica* treatments. There was a significant difference ($F = 0.075$, $df = 2, 35$, $P < 0.001$) among the three treatments was detected with the use of a two-way ANOVA. Values, expressed as the means ± 1 SE ($N = 12$ replicates per treatment), with the same letter are not significantly different at $P = 0.05$.

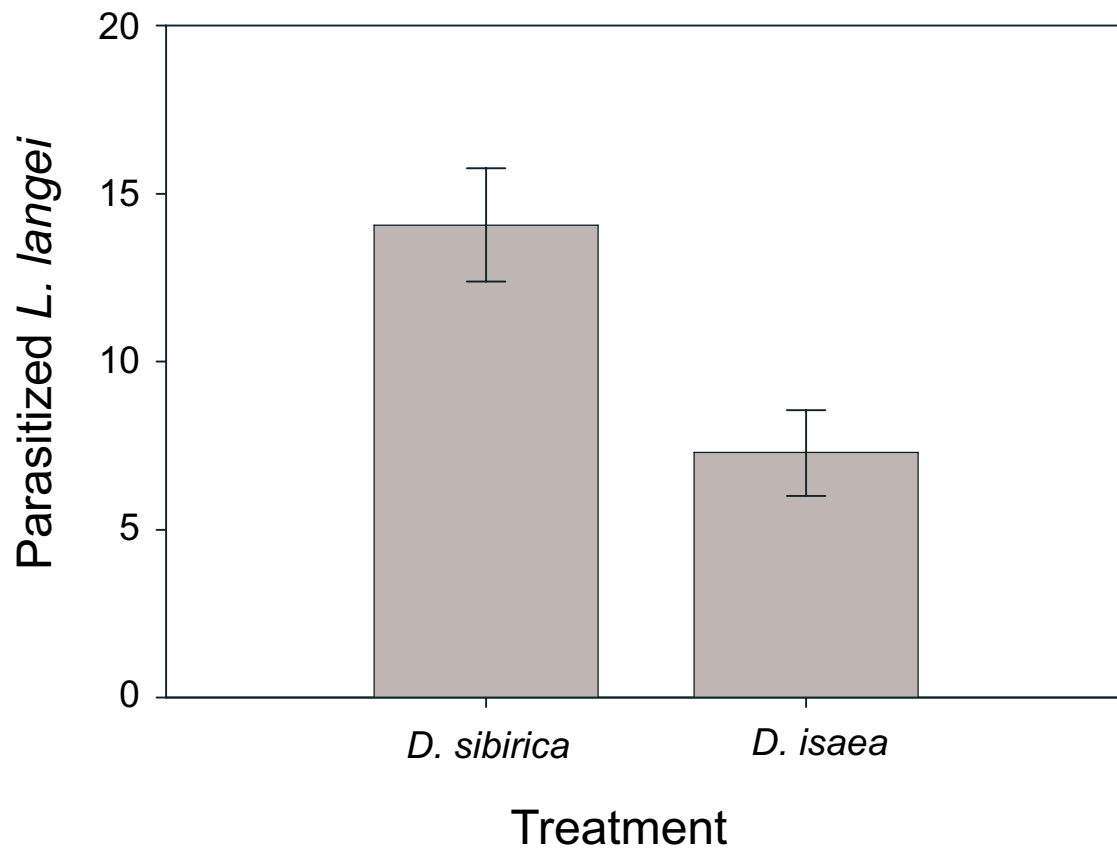


Fig. 2. Mean number of parasitized leafminer larvae in the *D. isaea* and *D. sibirica* cages. There was a significant difference ($F = 14.43$, $df = 1, 112$, $P < 0.001$) between the average number of parasitized dead leafminers in the *D. isaea* and *D. sibirica* release treatments. Values represent means (± 1 SE), $N = 12$ replicates per treatment.

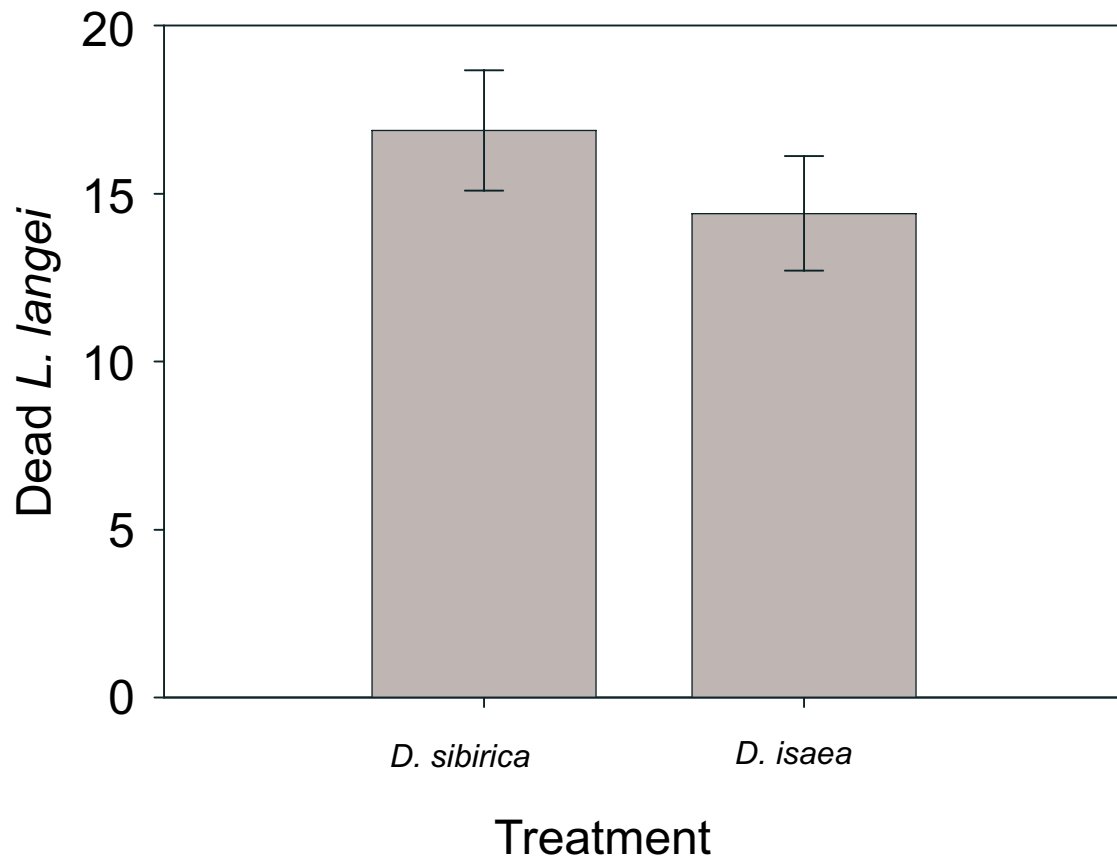


Fig. 3. Mean daily mortality of *L. langei* larvae resulting from attacks by *D. isaea* and *D. sibirica*. There was not a significant difference ($F = 1.46$, $df = 10, 113$, $P < 0.23$) between the average number of dead leafminers in the *D. isaea* and *D. sibirica* release treatments when compared using a two-way ANOVA. Values represent means (± 1 SE), $N = 12$ replicates per treatment.

Discussion

Reproductive potential is a valuable tool when comparing parasitoids for their potential as biological control agents (Minkenberg and van Lenteren 1987, 1990; Scholler and Hassan 2001; Haile et al. 2002). Reproductive potential can be used as an index to compare the population growth of a natural enemy to its target host and in comparing population growth among potential natural enemies (Minkenberg and van Lenteren 1987). In this study we found that *D. isaea* and *D. sibirica* can complete development and generate viable offspring with the pest *L. langei* as their host species. In addition, we showed that *D. isaea* and *D. sibirica* lived for relatively the same amount of time; however, *D. sibirica* has higher total offspring production. An average female of *D. sibirica* will produce over twice the number of offspring than an average female of *D. isaea*. Based on this parameter alone, we predict that *D. sibirica* exhibits a greater potential as a biological control agent of *L. langei* compared to *D. isaea*, and when they co-occur, *D. sibirica* would likely out-compete *D. isaea*.

If the reproductive potential of the parasitoids exceed those of *L. langei* they can be considered promising candidates for biological control of this host. Currently there is no information on the life history characteristics of *L. langei*. However, several studies using other *Liriomyza* species as hosts have shown the reproductive capacity of *D. isaea* (Minkenberg and van Lenteren 1987, 1990; Minkenberg 1989; Sampson and Walker 1998) and *D. sibirica* (Minkenberg 1990) to be greater than the pest at optimal temperatures.

The average longevity and lifetime offspring production of *D. sibirica* females found in this study were similar to values reported from other laboratory studies of *D. sibirica* on different hosts. Minkenberg (1990) found the longevity of *D. sibirica* attacking *Liriomyza bryoniae* (Kaltenbach) (Diptera: Agromyzidae) to be 7.4 ± 1.0 days (mean \pm MSE, N not given) at temperatures similar to those observed in our study. However, Croft and Copland (1994) found the longevity of *D. sibirica* to be 3.5 ± 1.0 days (mean \pm MSE N = 25 wasps) at a temperature of 20°C with *Chromatomyia syngenesiae* Hardy (Diptera: Agromyzidae) as host. Minkenberg (1990) found the number of offspring produced by *D. sibirica* lower (mean \pm MSE of 48 ± 7 , N = not given) at 25°C and slightly higher (mean \pm MSE of 94 ± 24 N = not given) at 20°C with *L. bryoniae* as host than was found in the current study. In comparison to another braconid leafmining parasitoid, *Opius dissitus* Muesebeck (Hymenoptera: Braconidae), *D. sibirica* has a similar life span, but produces significantly fewer offspring than *O. dissitus*. *Opius dissitus* has been found to live for an average of seven days and produce 169 offspring with *Liriomyza sativae* (Kaltenbach) as host (Pettit and Wietlisbach 1993).

The average longevity and total offspring production of *D. isaea* females were substantially lower than values reported from other laboratory studies of *D. isaea* and other eulophid parasitoids on different hosts. Minkenberg (1989) reported total offspring production by *D. isaea* to be 209 and longevity to be 10 days at 25°C (means not given) with *L. bryoniae* as the host species. The mean offspring production of *Diglyphus poppoea* Walker (Hymenoptera: Eulophidae) has been found to be 88.7 ± 4 (mean \pm MSE N = 10 wasps) and longevity 14 ± 1.5 (mean \pm MSE, N = 25 wasps) days

with *L. trifolii* as host (Goncalves and Almeida 2005). In this same study *D. isaea* total offspring production was 183.2 ± 17 (mean \pm MSE, N = 10 wasps) and longevity 38.4 ± 5 (mean \pm MSE, N = 25 wasps) days. Christie and Parrella (1987) examined longevity and fecundity of *Chrysocharis parksi* Crawford (Hymenoptera: Eulophidae) attacking *L. trifolii* and found that *C. parksi* produced an average of 134.6 ± 26.8 (mean \pm MSE, N = 16 wasps) offspring during a mean lifespan of 18.4 ± 2 (mean \pm MSE, N = 20 wasps) days. *Diglyphus intermedius* (Girault) (Hymenoptera: Eulophidae) a common parasitoid of *Agromyza frontella* (Rondani) (Diptera: Agromyzidae) has been found to live within a range of 21-28 days with an average of 40.2 (mean, N = 6 wasps) offspring produced per female at 25.5 ± 1.1 °C (mean \pm MSE) (Hendrickon and Barth 1978). Heinz and Parrella (1990a) showed that *D. begini* (Ashmead) had an average life expectancy of 6.3 days (N = 55 wasps) and an average lifetime reproductive capacity of 5.9 (N = 55 wasps) with *L. trifolii* as the host in a study conducted within a greenhouse setting.

Several factors could cause the discrepancy of longevity and offspring production between our study and other published studies. Temperature (Minkenberg 1990; Patel and Schuster 1991; Bordat et al. 1995; Bazzocchi et al. 2003) and humidity (Croft and Copland 1994) variations have been shown to alter life history characteristics of parasitoids. Each experiment discussed above was conducted under slightly different environmental conditions. In addition species-specific differences in longevity and total offspring production are common and making direct comparisons among parasitoids

attacking an array of different host species can be inconclusive within the context of this study.

Another factor contributing to the variation could be the use of an alternative food source. Unlike the conditions of our studies, a carbohydrate food source in the form of honey was made available to the wasps studied in many of the previously published studies. This availability of honey may have contributed to the longer life span and greater offspring production values compared to those observed in our studies. Christie and Parrella (1987) showed that a diet of honey increased parasitoid longevity by 500% compared to a diet of only water. Finally, in all of the previous studies the female parasitoids were newly emerged, whereas the adults used in our study were shipped to the testing laboratory by a commercial vendor and hence were of an unknown age.

Host kill comparisons between *D. isaea* and *D. sibirica* demonstrated that *D. isaea* kills significantly more hosts than *D. sibirica* without ovipositing in them. This result is likely due to the ability of *D. isaea* to host feed upon all the larval instars of *Liriomyza* species (Ibrahim and Madge 1978; Minkenberg and van Lenteren 1987; Minkenberg 1989). By comparison, no evidence exists to document the abilities of *D. sibirica* to host feed upon any stage of a leafminer host (Minkenberg and van Lenteren 1987; Dicki and Minkenberg 1991), and this is verified in the present study.

Comparisons also demonstrated that *D. sibirica* killed significantly more hosts by oviposition than *D. isaea*, which would be expected due to its higher offspring production. However, total host mortality achieved by both host feeding and

parasitization were similar between the two species. *Diglyphus isaea*'s host feeding capability significantly increases the potential number of hosts *D. isaea* can kill. Previous studies have shown that host feeding by *D. isaea* (Ibrahim and Madge 1979), *D. begini* (Heinz and Parrella 1989, 1990a) *Diglyphus puztensis* (Erdös and Novicky) (Sugimoto et al. 1982), *Chrysocharis pentheus* (Walker) (Hymenoptera: Eulophidae), and *C. parksi* (Christie and Parrella 1987) contributed to the overall mortality exerted on the target host population.

In summary, when comparing the total number of hosts killed by the two parasitoids the ability of *D. isaea* to host feed and oviposit into hosts previously parasitized by *D. sibirica* (Mitsunaga and Yano 2004) may offset the advantage of a larger offspring production potential of *D. sibirica*. This result may suggest that both species could possibly coexist in areas where they co-occur. Verification of this prediction will require specific experiments to be conducted, hopefully in the field, to ascertain the outcome of interspecific interactions between these species, and the effects of these interactions on biological control of *Liriomyza* leafminers.

CHAPTER III
ASSESSMENT OF INTERSPECIFIC INTERACTIONS AMONG PARASITOIDS
ON THE OUTCOME OF INOCULATIVE BIOLOGICAL CONTROL OF
LEAFMINERS ATTACKING CHRYSANTHEMUM

Introduction

The practice of biological control of arthropod pests infesting ornamental crops has long resided within the 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 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 forty 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 centimeters (32 inches) of the plant is harvested with the bottom 41 centimeters (16 inches) 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; Godfry 1994).

The importance of interspecific competition in regulating population dynamics has long been discussed within population ecology (Connell 1983; MacNally 1983; Briggs 1993; and Walter and Paterson 1995), and its importance pest management has been discussed in the biological control literature since the early 1900s (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 (Godfry 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

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 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 is already occurs.

Methods

Parasitoid-host system

The validity of the names used for the species referred to as *L. huidobrensis* have recently been clarified (Scheffer 2000; and Scheffer et al. 2001). Frick (1951) described *L. langei* found primarily on peas and later described *Liriomyza dianthi* (Frick 1958) found on carnations. Frick (1964) synonymized *L. dianthi* with *L. langei*, and Spencer (1973) synonymized the North American *L. langei* with the South American *L. huidobrensis* (Scheffer 2000). In different areas of the world *L. huidobrensis* appears to have different preferred hosts and tolerances to insecticides, giving rise to the possibility of the existence of two or more cryptic species (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*. Currently there are no morphological differences to distinguish the two clades. As of 2001, *L. langei* has not been recorded anywhere outside of North America (Scheffer et al. 2001). Populations from South and Central America, as well as those from the Old World, belong to *L. huidobrensis* sensu stricto. 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 U.S. 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 langei* 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). *Dacnusa sibirica* is a synovigenic endoparasitoid without a preoviposition period (Minkenbergh 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 (Minkenbergh and van Lenteren 1987; Dicki and Minkenbergh 1991). The reproductive rate of *D. sibirica* decreases with increasing temperature (Minkenbergh 1990) and is most commonly released at the beginning of the season against increasing host infestations (van der

Linden 2004). *Diglyphus isaea* is a synovigenic ectoparasitoid with a preoviposition period of 1 to 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 (Heinz and Parrella 1989). 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 adult 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.

Field site and insect material

Experiments were conducted at the Rangeland Field Site on the Texas A&M University campus in College Station, Texas, U.S.A. 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 3.3m (length) by 3.3m (width) by 2.0m (height) and was covered with 20 X 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 1m by 2m beds were planted with rooted chrysanthemum cuttings provided by a commercial propagator (Yoder Bros. Inc., Barberton, Ohio, USA) with 20 x 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 two 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. *Liriomyza 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.

Inoculation rates of flies and parasitoids in field cages

Cages were inoculated with adult leafminers at a rate of one female and two males on four alternating days a week for the duration of the crop. Twelve hours before making releases into the field cages, the adult groups of three adult flies were placed in plastic tubes without a food source to help ensure mating. Parasitoid releases followed per area recommended rates established by Koppert Biological Systems (<http://www.koppert.com/>). In each treatment in which wasps were released, two females and two males were released beginning two weeks after the initial inoculation of flies (Table 2). This was the same for each parasitoid species. 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 2. 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	First Release	Second and all Subsequent Releases
<i>D. isaea</i> only	2 female & 1 male of <i>D. isaea</i>	2 female & 1 male of <i>D. isaea</i>
<i>D. sibirica</i> only	2 female & 1 male of <i>D. sibirica</i>	2 female & 1 male of <i>D. sibirica</i>
Both	2 female & 1 male of <i>D. isaea</i> and <i>D. sibirica</i>	2 female & 1 male of <i>D. isaea</i> and <i>D. sibirica</i>
<i>D. isaea</i> first, <i>D. sibirica</i> second	2 female & 1 male of <i>D. isaea</i>	2 female & 1 male of <i>D. isaea</i> and <i>D. sibirica</i>
<i>D. sibirica</i> first, <i>D. isaea</i> second	2 female & 1 male of <i>D. sibirica</i>	2 female & 1 male of <i>D. sibirica</i> and <i>D. isaea</i>

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 parasitoid 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 parasitoid eggs oviposited 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.

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:

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

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:

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

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 goodness of fits test (G-statistic). Equations 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 three would suggest that releasing either parasitoid into a population of the other would disrupt host suppression.

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 when a flower was completely opened, meaning the bottom petals were flat. In each cage, 30 plants were harvested, where the top 33 centimeters (13 inches) 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.

Results

Within-cage air temperatures closely tracked temperature recorded outside of the cages (Figure 4), 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 (Figure 4) was consistently and significantly 1 to 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 ($F_{1, 35} = 1.835$, $P = 0.667$) or treatments ($F_{5, 35} = 1.170$, $P = 0.343$). Thus, any observed differences among treatments were most likely due to treatment effects and not differences in temperature or starting conditions (see Table 3 for sample counts).

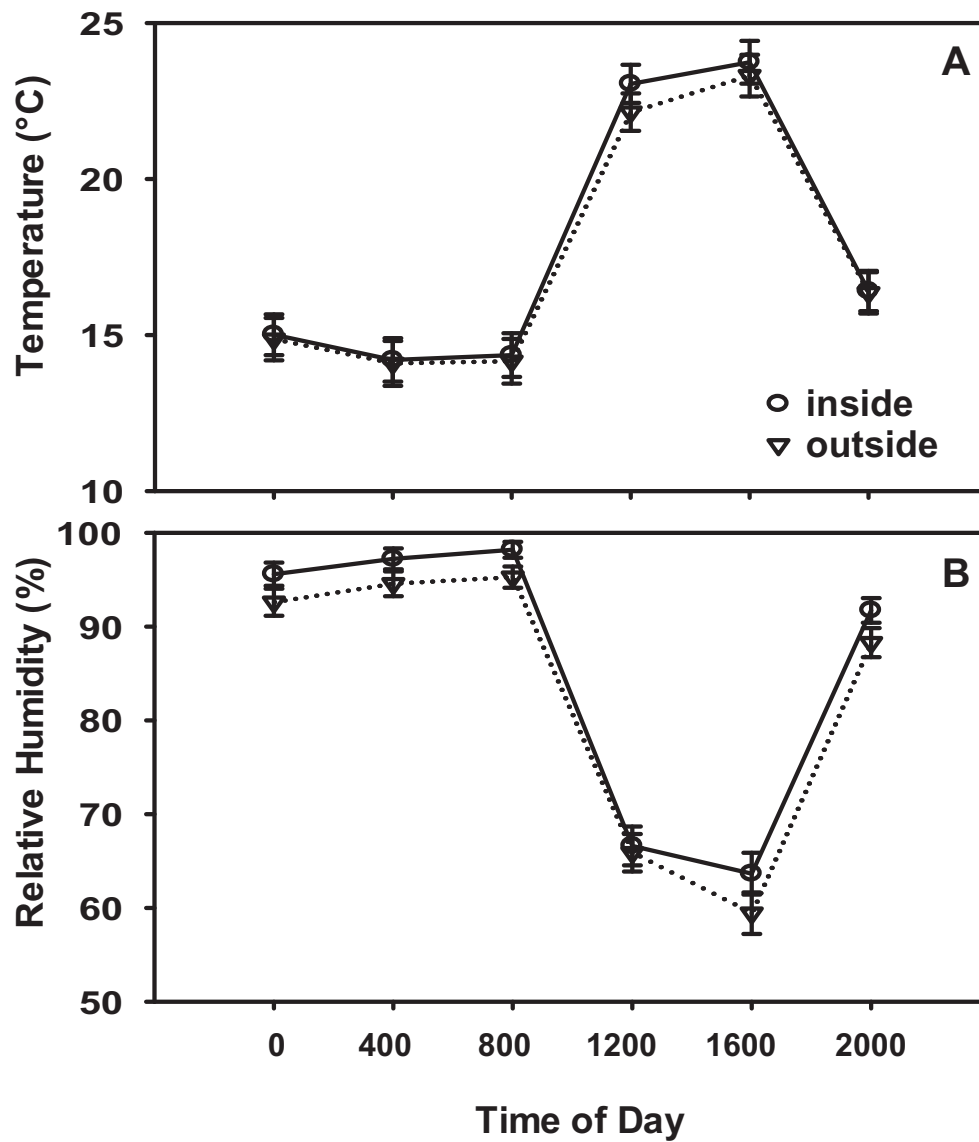


Fig. 4. Mean air temperature (°C) and relative humidity (%) inside and outside the cages during 2003. Comparisons were conducted using paired t tests (A) $t = -2.362$, $df = 5$, $P = 0.065$ and (B) $t = -5.963$, $df = 5$, $P = 0.002$. Values represent means (\pm SE) of recordings every 4 hours.

Table 3. Summary of the total number of immature *L. langei*, *D. isaea*, and *D. sibirica* collected over the growing season per treatment in 2003 and 2004.

Treatment	Year		2004			
	2003					
	<i>Liriomyza langei</i>	<i>Diglyphus isaea</i>	<i>Dacnusa sibirica</i>	<i>Liriomyza langei</i>	<i>Diglyphus isaea</i>	<i>Dacnusa sibirica</i>
Control	74	0	0	26	0	0
<i>D. isaea</i>	46	5	0	16	11	0
<i>D. sibirica</i>	8	0	2	18	0	4
Both	11	2	2	12	0	2
<i>D. isaea</i> first	19	4	3	33	24	4
<i>D. sibirica</i> first	18	0	2	18	8	4
Total	176	11	9	123	43	14

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 *Diglyphus 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 ($F_{1,11} = 1.472, P = .251$), or in the year-by-treatment interaction ($F_{1,11} = 1.163, P = .304$) (Figure 5A). When comparing the mean seasonal abundance of *D. sibirica* in the simultaneous release treatment to the single release treatment, there were no significant year ($F_{1,10} = .918, P = .360$), treatment ($F_{1,10} = .283, P = .606$), or year-by-treatment interaction effects ($F_{1,10} = .102, P = .756$) (Figure 5B). The population

densities of each parasitoid when they were released together were not significantly different from their population densities when released alone. This suggests that the presence of one parasitoid species did not negatively impact the population density of the other.

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 ($F_{1,10} = 1.541, P = .243$), treatments ($F_{1,10} = 2.877, P = .121$), or year-by-treatment interaction ($F_{1,10} = 4.116, P = .070$) (Figure 5C). 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 ($F_{1,11} = 1.202, P = .296$), treatments ($F_{1,11} = .371, P = .555$), or year and treatment interaction ($F_{1,11} = .134, P = .722$) (Figure 5D). Because the population densities of each parasitoid were similar between the two release treatments, the results suggest the pre-existing occurrence of either parasitoid species does not prevent the invasion, successful establishment, and increase in population density of the second species.

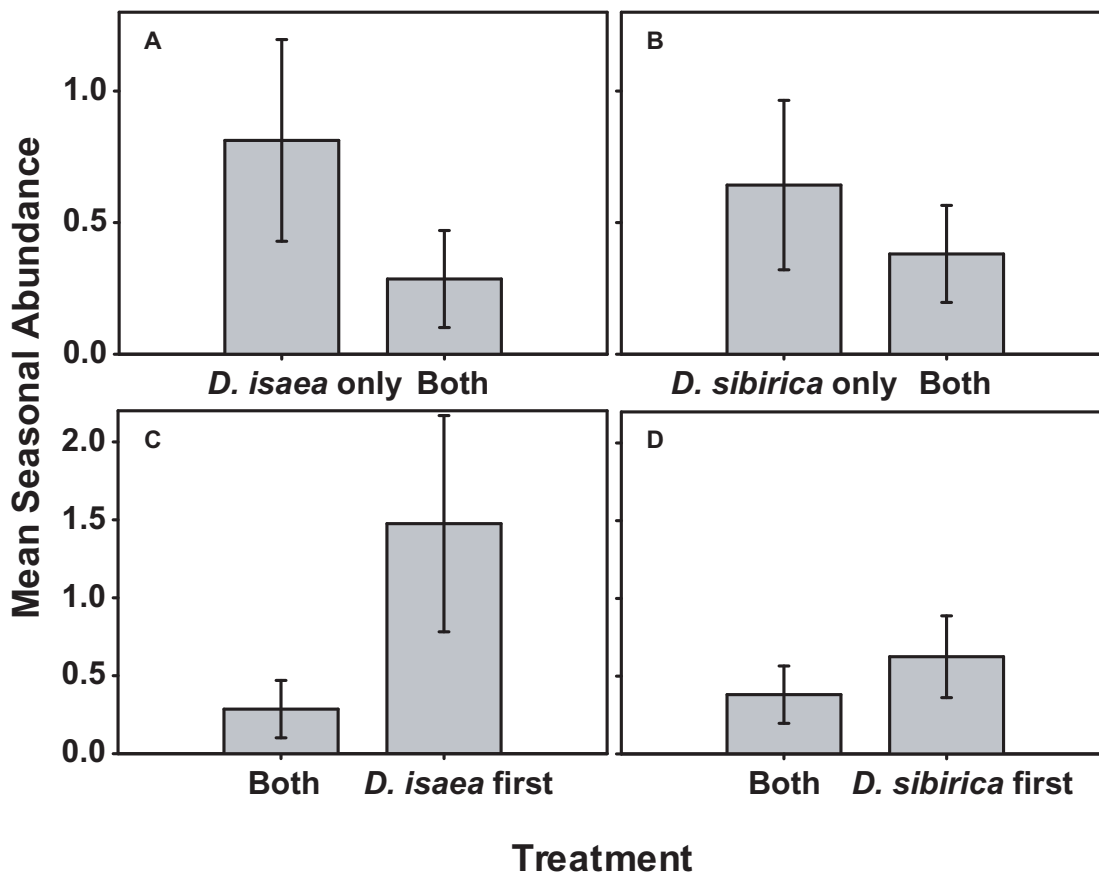


Fig. 5. Mean population density of (A) *D. isaea* in the *D. isaea* only and the simultaneous release treatments and of (B) *D. sibirica* in the *D. sibirica* only and the simultaneous release treatments. No significant differences among the treatments were found for (A) $F_{1,11} = 1.472$, $P = 0.251$ 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.

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 Figure 6A, C, 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 Figure 3B, D, 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. Results from replicated G-tests comparing the observed and expected levels of leafminer suppression in 2003 and 2004 appear in Table 4.

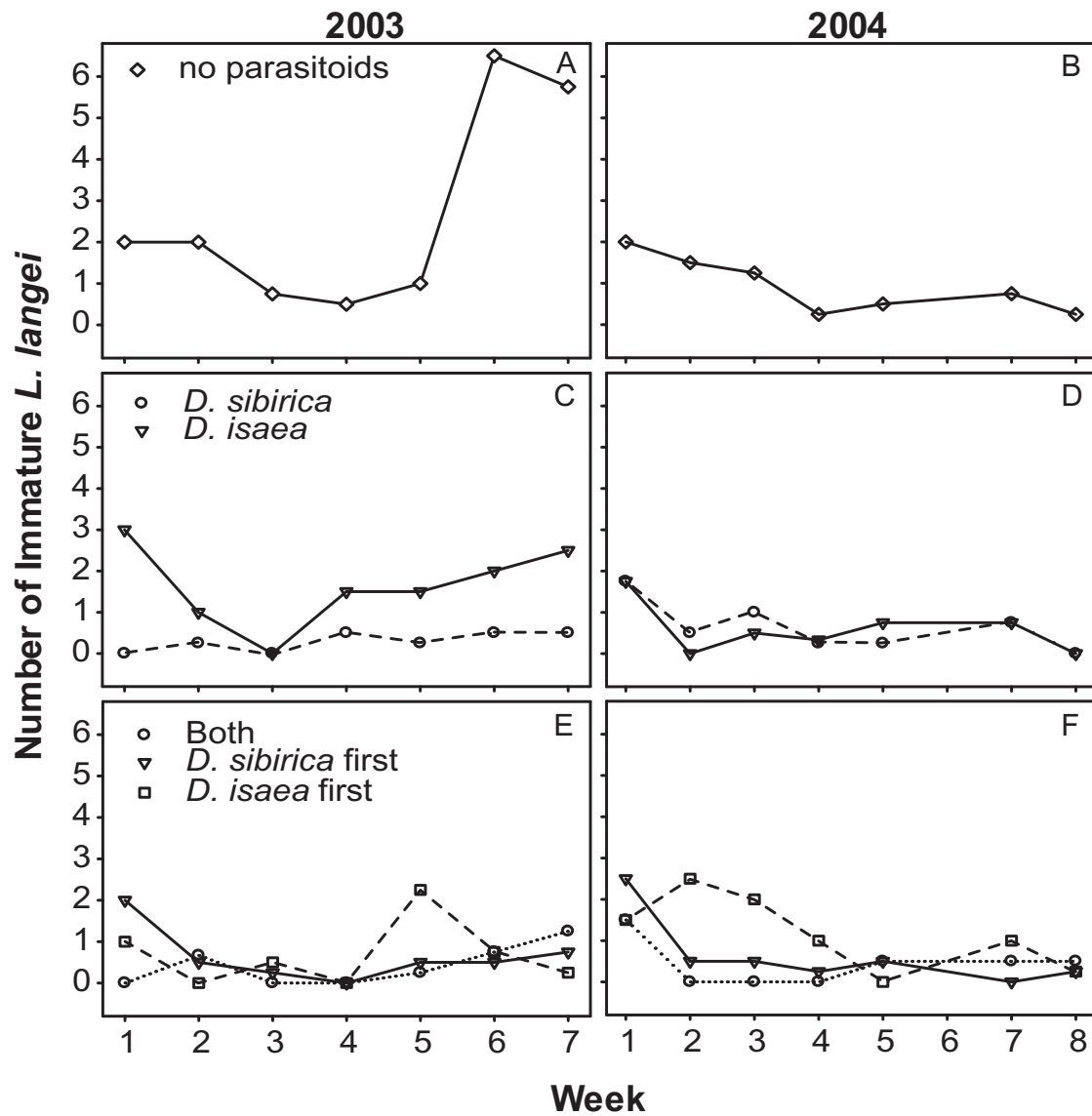


Fig 6. Leafminer population density over time in the no parasitoid release control (A, B), the individual releases (C, D), and the combination releases (E, F) in 2003 and 2004 respectively.

Table 4. Summary of replicated G test comparing the level of suppression in each of the release combinations tested for each week in 2003 and 2004. In all cases, the G-statistics were not statistically significant ($P > 0.05$).

A. Observed suppression in the simultaneous release and the expected suppression based on the individual releases								
Week	Test	df	2003			2004		
			G	P		df	G	P
1	Pooled	1	0	n.s.	1	0.128	n.s.	
	Heterogeneity	3	0	n.s.	3	0.300	n.s.	
	Total	4	0	n.s.	4	0.428	n.s.	
2	Pooled	1	0.052	n.s.	1	0	n.s.	
	Heterogeneity	2	0.155	n.s.	3	0	n.s.	
	Total	3	0.207	n.s.	4	0	n.s.	
3	Pooled	1	0	n.s.	1	0.042	n.s.	
	Heterogeneity	3	0	n.s.	3	0.16	n.s.	
	Total	4	0	n.s.	4	0.202	n.s.	
4	Pooled	1	0	n.s.	1	0	n.s.	
	Heterogeneity	3	0	n.s.	3	0	n.s.	
	Total	4	0	n.s.	4	0	n.s.	
5	Pooled	1	0.067	n.s.	1	0.077	n.s.	
	Heterogeneity	3	0.273	n.s.	3	0.263	n.s.	
	Total	4	0.340	n.s.	4	0.340	n.s.	
6	Pooled	1	0	n.s.	1	0.077	n.s.	
	Heterogeneity	3	0.010	n.s.	3	0.263	n.s.	
	Total	4	0.010	n.s.	4	0.340	n.s.	
7	Pooled	1	0.032	n.s.	1	0	n.s.	
	Heterogeneity	3	0.116	n.s.	3	0	n.s.	
	Total	4	0.148	n.s.	4	0	n.s.	

Table 4 cont.

B. Observed suppression in the simultaneous release and the <i>D. isaea</i> released first							
Week	Test	df	2003		2004		
			G	P	df	G	P
1	Pooled	1	0.155	n.s.	1	0.024	n.s.
	Heterogeneity	3	0.256	n.s.	3	0.048	n.s.
	Total	4	0.411	n.s.	4	0.072	n.s.
2	Pooled	1	0.287	n.s.	1	0	n.s.
	Heterogeneity	2	0.393	n.s.	2	0	n.s.
	Total	3	0.680	n.s.	3	0	n.s.
3	Pooled	1	0.067	n.s.	1	0.067	n.s.
	Heterogeneity	3	0.273	n.s.	3	0.273	n.s.
	Total	4	0.340	n.s.	4	0.340	n.s.
4	Pooled	1	0	n.s.	1	0.067	n.s.
	Heterogeneity	3	0	n.s.	3	0.273	n.s.
	Total	4	0	n.s.	4	0.340	n.s.
5	Pooled	1	0.335	n.s.	1	0.287	n.s.
	Heterogeneity	3	0.345	n.s.	3	0.393	n.s.
	Total	4	0.680	n.s.	4	0.680	n.s.
6	Pooled	1	0.002	n.s.	1	0.077	n.s.
	Heterogeneity	3	0.030	n.s.	3	0.263	n.s.
	Total	4	0.032	n.s.	4	0.340	n.s.
7	Pooled	1	0.032	n.s.	1	0	n.s.
	Heterogeneity	3	0.115	n.s.	3	0	n.s.
	Total	4	0.147	n.s.	4	0	n.s.

Table 4 cont

C. Observed suppression in the simultaneous release and the <i>D. sibirica</i> released first							
Week	Test	df	2003		2004		
			G	P	df	G	P
1	Pooled	1	0.465	n.s.	1	0	n.s.
	Heterogeneity	3	0.286	n.s.	3	0.512	n.s.
	Total	4	0.751	n.s.	4	0.512	n.s.
2	Pooled	1	0.077	n.s.	1	0	n.s.
	Heterogeneity	2	0.435	n.s.	2	0	n.s.
	Total	3	0.512	n.s.	3	0	n.s.
3	Pooled	1	0.067	n.s.	1	0.178	n.s.
	Heterogeneity	3	0.273	n.s.	3	0.226	n.s.
	Total	4	0.340	n.s.	4	0.404	n.s.
4	Pooled	1	0	n.s.	1	0	n.s.
	Heterogeneity	3	0	n.s.	3	0	n.s.
	Total	4	0	n.s.	4	0	n.s.
5	Pooled	1	0	n.s.	1	0	n.s.
	Heterogeneity	3	0	n.s.	3	0.680	n.s.
	Total	4	0	n.s.	4	0.680	n.s.
6	Pooled	1	0	n.s.	1	0.287	n.s.
	Heterogeneity	3	0.012	n.s.	3	0.393	n.s.
	Total	4	0.012	n.s.	4	0.680	n.s.
7	Pooled	1	0.008	n.s.	1	0.077	n.s.
	Heterogeneity	3	0.155	n.s.	3	0.942	n.s.
	Total	4	0.163	n.s.	4	1.019	n.s.

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) (Figure 7.) This suggests that releasing both parasitoids at the same time does not disrupt host suppression. In both 2003 and 2004 the cages in which *D. isaea* was released first (Figure 8A, B) and the cages in which *D. sibirica* were released first (Figure 8C, D) had similar levels of suppression as did the simultaneous releases. This suggests that releasing either parasitoid into a population of the other would not disrupt host suppression.

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 ($F_{1,24} = 140.720$, $P \ll 0.01$) and when comparing the control, simultaneous, *D. isaea* first, and *D. sibirica* first treatments ($F_{1,24} = 138.183$, $P \ll 0.01$). Therefore, subsequent analyses of variance on days until harvest were performed separately for the two years using a one-way ANOVA. There were no significant among-treatment differences in 2003 ($F_{3,12} = 0.951$, $P = 0.447$) or in 2004 ($F_{3,12} = 0.482$, $P = 0.701$) when comparing the control, *D. isaea* only, *D. sibirica* only, and the simultaneous release treatments. The same was true when comparing the control, simultaneous, *D. isaea* first, and *D. sibirica* first treatments, no significant year effects in 2003 ($F_{3,12} = 0.670$, $P = 0.587$) or in 2004 ($F_{3,12} = 2.108$, $P = 0.153$). The

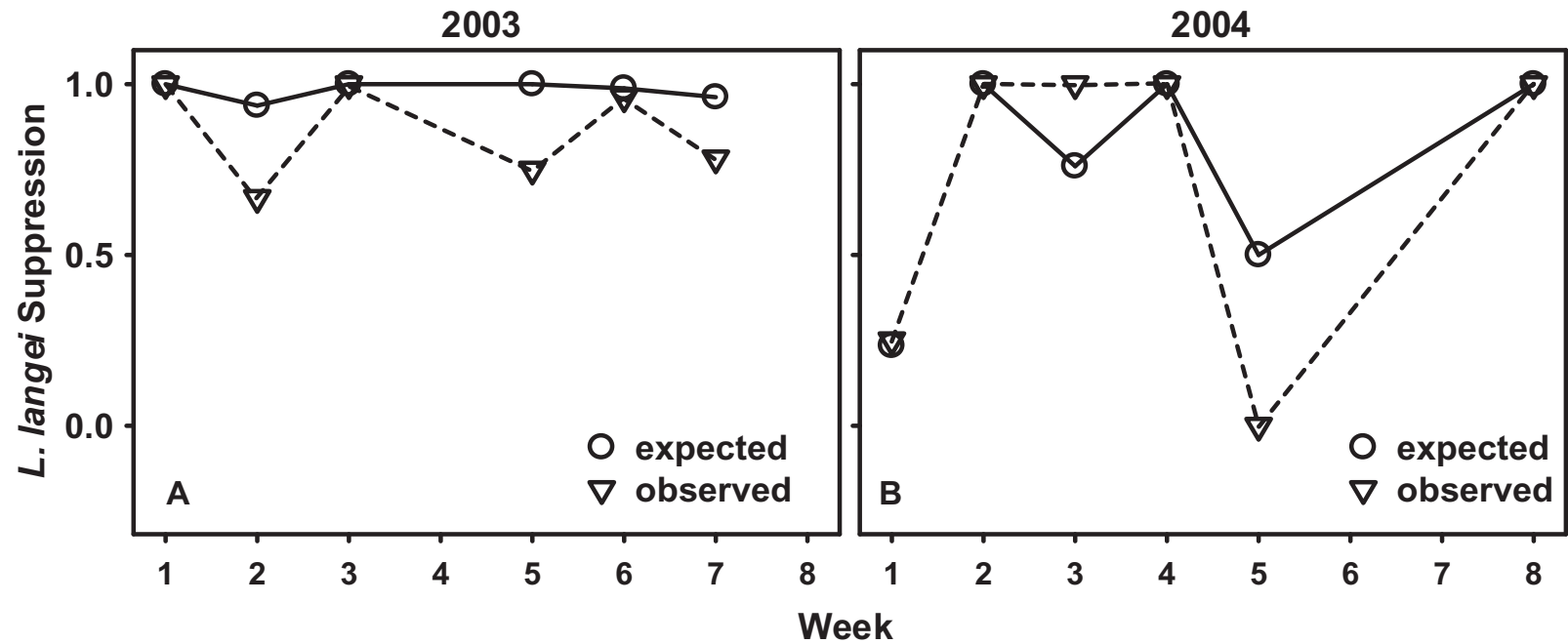


Fig. 7. Observed and expected levels of leafminer suppression in the treatment where *D. isaea* and *D. sibirica* were released simultaneously in (A) 2003 and (B) 2004. Expected mortality for the simultaneous release was calculated using the mortality caused by individual species in the absence of competitors and observed values are leafminer means for all replicates. Values represent cage means.

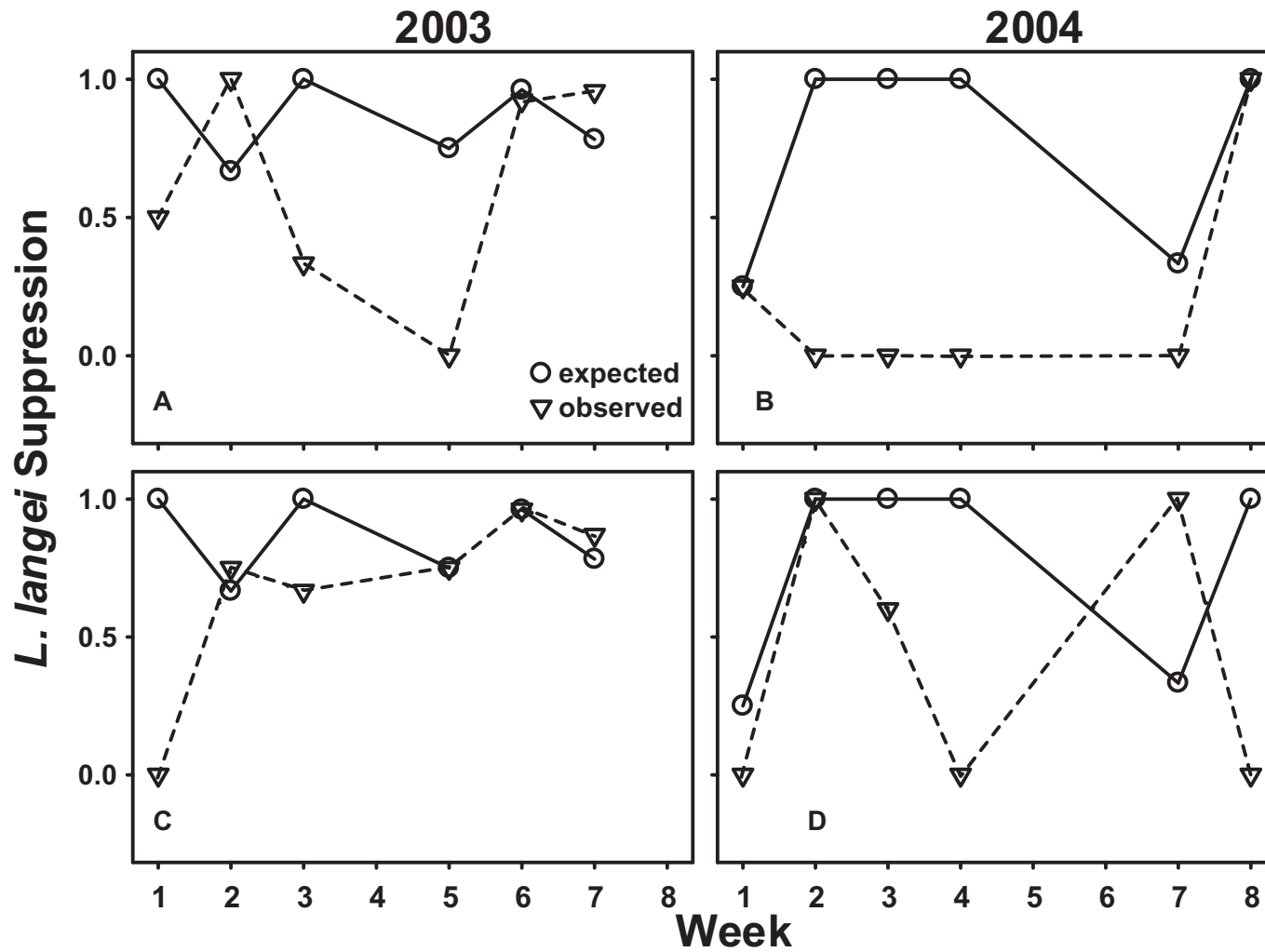


Fig. 8. Observed and expected levels of leafminer suppression in the treatment where *D. isaea* was released first in (A) 2003 and (B) 2004 and where *D. sibirica* was released first in (C) 2003 and (D) 2004. Expected mortality was calculated using the mortality caused when both species were released simultaneously and observed values are leafminer means for all replicates. Values represent cage means.

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

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 ($F_{1,24} = 10.419$, $P = 0.004$) and when comparing the control, simultaneous release, *D. isaea* first, and *D. sibirica* first treatments ($F_{1,24} = 14.050$, $P = 0.001$). Therefore, subsequent analyses of variance on proportion of damaged leaves at harvest were performed separately for the two 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 ($F_{3,12} = 4.808$, $P = 0.020$) and 2004 ($F_{3,12} = 3.746$, $P = 0.041$) (Figure 9A, B). In both 2003 and 2004 (Fig 6A and 6B), 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 9A, 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 ($F_{3,12} = 9.079$, $P = 0.002$) and 2004 ($F_{3,12} = 5.996$, $P = 0.010$) (Figure 9C, D). In 2003, the no parasitoid release control had significantly more damage than the other treatments (Figure 9C). In 2004, the no parasitoid control had significantly more damage than the

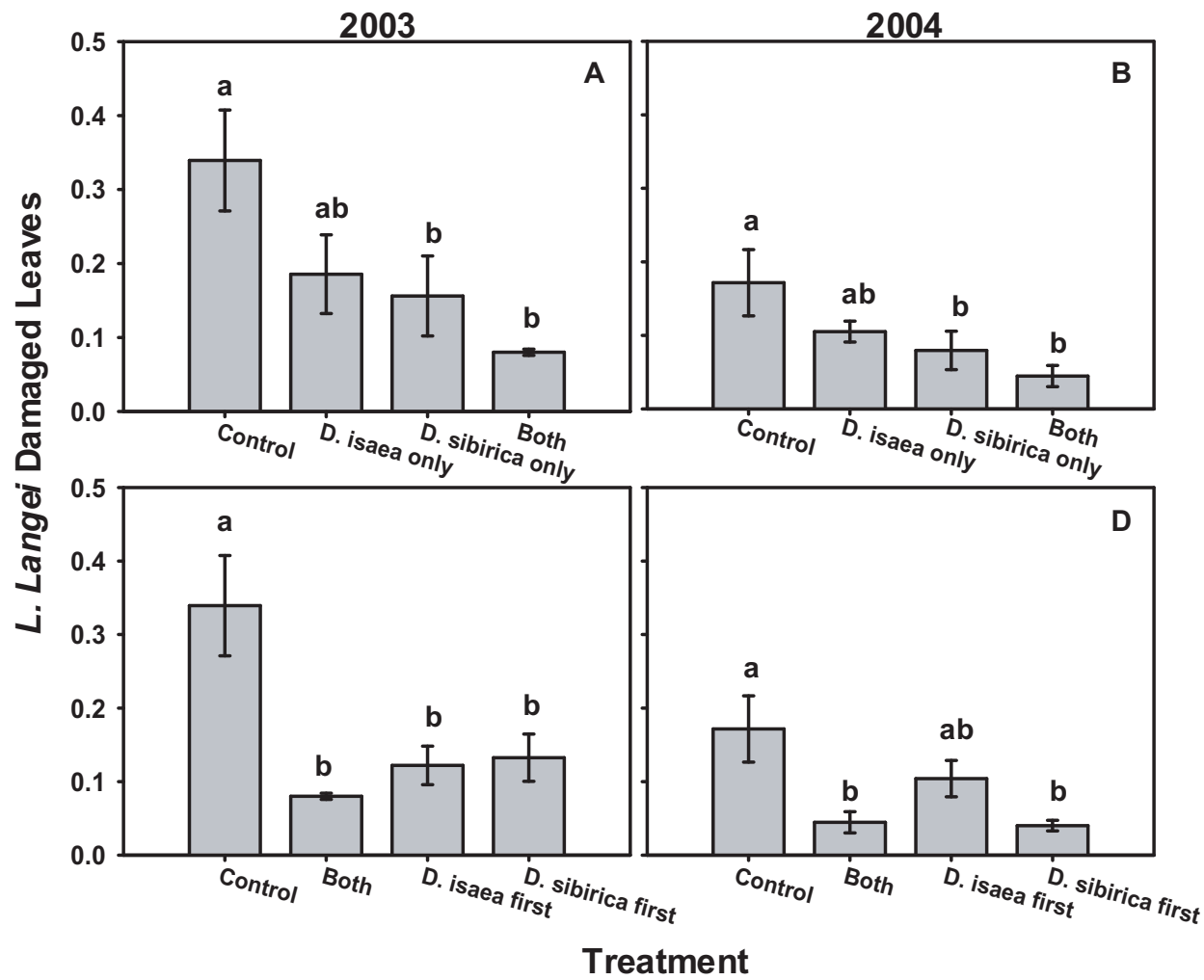


Fig. 9. Mean proportion of damaged leaves in the control, *D. isaea* only, *D. sibirica* only, and the simultaneous release treatments in (A) 2003 and (B) 2004. Mean proportion of damaged leaves in the control, simultaneous, *D. isaea* first, and *D. sibirica* first treatments in (C) 2003 and (D) 2004. 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.

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 (Figure 9D).

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 ($F_{1,24} = 0.339$, $P = 0.566$), the treatments ($F_{3,24} = 2.949$, $P = 0.053$), or the year-by-treatment interaction ($F_{3,24} = 0.422$, $P = 0.739$). 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 ($F_{1,24} = 0.702$, $P = 0.410$), treatment effect ($F_{3,24} = 0.779$, $P = 0.517$) or year-by-treatment interaction ($F_{3,24} = 0.830$, $P = 0.490$). Even though parasitoid release combination did affect the amount of damage at harvest, the amounts of flowers generated were not influenced by release treatment.

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 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 concurrently did not disrupt host

suppression. When parasitoids were released, either alone or in tandem, the leafminer 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 leafminer 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 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; Dicki 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 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 30 leaves sampled from each cage. This small sample does not fully represent the average scale of production, measured as 4.5 hectares (11 ac) and over 65,000 plants, planted by large U.S. 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 and 2004 combined using the equation:

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

where n is the number of replication, σ the true standard deviation, δ 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, α 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 (13020 plants) replicates would be needed. These calculations suggest that on a large-scale commercial production system (65000 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 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 verses 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 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 (Minkenburg 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). This 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).

CHAPTER IV

CONCLUSIONS

Summary

Diglyphus isaea and *Diglyphus sibirica* have successfully been released as biological control agents of several leafminer pests worldwide. Due to these introductions, both parasitoids are cosmopolitan and likely to be occurring in major ornamental production areas. Each species may be purchased in combination or individually with the possibility of being released into an existing population of natural enemies. In such situations competitive interactions are likely to occur. We examined life history traits of the two parasitoids, the impact of releasing both parasitoids simultaneously from the beginning of the growing season on parasitoid and host population dynamics, and the impact on parasitoid and host population dynamics by releasing a biological control agent into a field where an established natural enemy population occurs.

Life history traits

Dacnusa sibirica and *D. isaea* lived for approximately the same amount of days, but an average female of *D. sibirica* produced more offspring than an average female of *D. isaea*. *Diglyphus isaea* killed more hosts without ovipositing in them than *D. sibirica* does, which could be attributed to host feeding. When comparing the total number of hosts killed by the two parasitoids the ability of *D. isaea* to host feed and oviposit into hosts previously parasitized by *D. sibirica* (Mitsunaga and Yano 2004) may offset the

advantage of a larger offspring production potential of *D. sibirica*. This result may suggest that both species could possibly coexist in areas where they co-occur.

Verification of this prediction will require specific experiments to be conducted, hopefully in the field, to ascertain the outcome of interspecific interactions between these species, and the effects of these interactions on biological control of *Liriomyza* leafminers.

Release of both species simultaneously

Releasing both parasitoids simultaneously did not disrupt the mean seasonal abundance of either species. This suggests that the presence of one parasitoid species did not negatively impact the population density of the other. Thus, interspecific competition among these parasitoid species was undetectable at leafminer densities typical of field-grown ornamental crops. Interspecific interactions also did not have an impact on host suppression. The observed suppression levels when both species were released simultaneously were equal to what we were expecting to see if competition were not occurring.

Release of a biological control agent into an existing natural enemy population

The release of one parasitoid into a population of the other did not disrupt the mean seasonal abundance of the established natural enemy. Because the population densities of each parasitoid were similar between the two release treatments, the results suggest the pre-existing occurrence of either parasitoid species does not prevent the invasion, successful establishment, and increase in population density of the second species. Interspecific interactions also did not have an impact on host suppression. The

observed suppression levels in the field cages were equal to what we were expecting to see when both species were released simultaneously. Thus, the efficacy of one species released into a backdrop of potentially competing parasitoids did not negatively affect the outcome of the augmentative biological control.

Interspecific competition and biological control

All releases of *D. isaea* and/or *D. sibirica* reduced the population of leafminers to below those in the absence of parasitoids in one or both of the years that these experiments were conducted. Even though parasitoid releases did affect the amount of damage visible at harvest, there was no influence on the number of flowers produced (yield). The two commercially available parasitoids used for *Liriomyza* control were able to coexist throughout the chrysanthemum-growing season. However, *post hoc* sample size calculations showed that on a larger scale, representative of current commercial production, competitive interactions are likely to occur. This needs to be taken into consideration before mass releases of these two biological control agents. 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 the native populations.

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APPENDIX A

RELEVANCE TO HISPANIC COMMUNITIES

Currently Hispanics constitute the largest growing segment of farmer operators, which was an increase of 58% since 1978. By 1997 the number of counties containing Hispanic owned farms increased from 589 to 1,775 (57%) of the 3,128 counties in the 50 states. Pre Immigration Reform and Control Act state that (IRCA) 80% of the Hispanic population lives in the border states from Texas to Washington, Colorado and Florida, with 81% of the farms in that area Hispanic owned. Ten years after the IRCA the number of Hispanic owned farms in the rest of the states increased by 77% and Hispanic operated farms increased 50%. Even with this rapid increase in numbers, Hispanic farms constitute only 1.5% of all US farms. This increase in farm ownership occurred during a time when the total number of farms in the US was declining.

In addition to Hispanic owned farms increasing in number, Hispanics are a large segment of the agriculture work force. Nearly 88% of US farm workers are Hispanic. As the farm population across the US is decreasing, the average farm size is increasing. With this increases the need for hired workers, both domestic and immigrant, will become greater.

According to the American Public Health Association, the occurrence of work related injuries, illnesses, and fatalities continue to rise for Hispanic workers, whereas they have been decreasing for other US populations. This can be partially attributed to

un-enforced worker safety laws. Unfortunately, agricultural workers are not provided the same legal protections as other worker types. Hispanic agricultural workers face the greatest threat of pesticide – related illnesses, including cancer and birth defects. In many situations farm workers along the border lack pesticide safety training, go without proper cleansing equipment, not told when or where pesticides have been applied, and there are even press rumors of workers being directly exposed to pesticides.

Not only are Hispanic farm worker facing unsafe working conditions, some are also facing unsafe living conditions. Colonia's typically lack safe drinking water and waste facilities. Residents are forced to draw water form canals and wells possibly contaminated with insecticide residue, increasing the risk of giardiasis, hepatitis and cholera. For farm workers, inadequate washing facilities in both the home and work place lengthens the time exposed to chemicals, which leads to increased risks of lymphoma, prostate cancer and childhood cancers. Children are also exposed to pesticides because of the residues clinging to workers clothing, contaminated water, and pesticide drift. Many organizations are calling for EPA to ban the most hazardous pesticides, prohibit drift prone application methods, and require landowners to establish a larger buffer zone during application. In addition organizations are calling for EPA and state agencies to designate farm children a vulnerable population that must be considered and protected in pesticide registration and tolerance decisions under federal and state law.

My project examines alternative means to pesticide use for control of an insect pest found in both agricultural fields and greenhouses. One of the reasons biological

control is gaining interest is because of worker safety issues. Illnesses due to pesticide exposure and residues are a serious problem for farm workers and their families. The use of biological control will decrease the frequency of pesticide applications and in some situations do away with pesticides all together. Research in biological control is also important, because as EPA bans more and more chemicals, because of safety, farm owners will need to rely on non-chemical forms for pest control.

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