FACTORS POTENTIALLY LIMITING COLONY GROWTH, FORAGING EFFORT, AND POLLINATION EFFICIENCY OF BUMBLE BEES IN COMMERCIAL TOMATO GREENHOUSES

by
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Factors potentially limiting colony growth, foraging effort, and pollination efficiency of bumble bees in commercial tomato greenhouses.

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ABSTRACT

I examined several factors potentially limiting bumble bee colony population growth in commercial tomato greenhouses. I investigated whether bumble bees are obtaining adequate nutrition and growing to their maximum population size in commercial tomato greenhouses. As well, I compared *Bombus occidentalis* Greene and *Bombus impatiens* Cresson as pollinators and examined inter-specific competition between the two species. I also investigated how often bumble bees forage outside greenhouses and studied the effects of the protozoan parasite *Nosema bombi* and its treatment fumagillin in *B. occidentalis* colonies.

B. occidentalis colonies obtain adequate nutrition in commercial tomato greenhouses, but there is high loss of workers so colonies are not growing to their maximum population size. *B. impatiens* colonies are more effective pollinators than *B. occidentalis* colonies likely because they grow to larger population sizes and forage more frequently. An average of 14% of pollen foraging flights between January and September are outside of tomato greenhouses, with peaks up to 45% in May and July. This may contribute to a decreased amount of tomato pollination per colony. *N. bombi* did not affect bumble bee colony size and fumagillin was not an effective treatment against this parasite. Further research investigating the detrimental effects of higher *N. bombi* intensities on bumble bees and the threshold infections of *N. bombi* intensities for these effects would be helpful to greenhouse managers. Bumble bees are effective pollinators of greenhouse tomatoes, but there is still considerable research to be conducted to improve bumble bee colony growth and tomato pollination in greenhouses.

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Table of Contents

List of Figures

Chapter I
General Introduction

Bumble bees (Hymenoptera: Apidae) have become important agricultural pollinators in the 30 years since they have been domesticated (Plowright and Jay 1966). In particular, the greenhouse tomato (*Lycopersicon esculentum* Miller, Solanaceae) industry has come to rely on bumble bees for crop pollination instead of the labour-intensive method of vibrating each truss by hand (Banda and Paxton 1991, Kevan et al. 1991, van Ravestijn and van der Sande 1991, Straver and Plowright 1991, Dogterom et al. 1998, Pressman 1999). Tomato flowers are self-pollinating, but result in a larger more commercially attractive fruit if supplemental pollination is provided (van Koot and van Ravestijn 1962, Picken 1984).

Greenhouse growers usually use bumble bee species native to the area, and this was the case in western North America until the late 1990's when bumble bee producers had difficulties meeting the demand for native *Bombus occidentalis* Greene. *Bombus impatiens* Cresson was imported from eastern North America to relieve the pollination shortfall. At that time, tomato growers also became concerned about the effectiveness of *B. occidentalis* as a commercial pollinator. They were concerned that pollination by *B. occidentalis* was inconsistent and would result in periods of low pollination and hence a loss in tomato production.

This group of experiments examined bumble bee colony population growth in commercial tomato greenhouses and examined factors that may lead to increased colony growth and tomato pollination by bumble bees. Initially, I examined whether *B. occidentalis* colonies are obtaining adequate nutrition in the greenhouse environment. In

tomato greenhouses, bees have access only to tomato pollen and therefore may not be obtaining the full complement of nutrients needed for optimal colony growth. I compared the growth of *B. occidentalis* colonies fed supplemental multi-floral pollen to that of colonies that had access only to tomato pollen. As I was conducting this study, I noticed some bees returning with non-tomato pollen from outside the greenhouse, and conducted a survey throughout the following year to determine how much pollen *B. occidentalis* workers were collecting from outside the greenhouses and therefore how much pollination capacity greenhouse growers were potentially losing.

Thirdly, greenhouse growers began to suspect that *B. impatiens* may be a better pollinator than *B. occidentalis*. I compared the ability of the two species as pollinators by comparing colony growth and foraging effort, and examined potential competition between the species. I also investigated if *B. occidentalis* colonies grow to their full population potential in greenhouses by comparing growth of colonies in commercial greenhouses to colonies that were completely enclosed and protected from external mortality, and to colonies outside in a more natural environment.

Lastly, the bumble bee parasite *Nosema bombi* (Microsporidia: Nosematidae) has been suggested as a potential cause of poor colony performance. I examined whether *N. bombi* affects colony populations, and evaluated the effectiveness of fumagillin against *N. bombi* in bumble bee colonies, used to control the closely related *N. apis* in honey bees (Woyke 1984, Furgala and Sugden 1985, Szabo and Heikel 1987, Webster 1994). As well, I examined techniques for sampling *N. bombi* in bumble bee colonies, because the traditional method of destructively sampling 30 workers in honey bee colonies (Cantwell 1970) is not practical in smaller bumble bee colonies.

The overall objectives of this group of experiments were to examine the bumble bee species used in western North America for their effectiveness as pollinators of commercial greenhouse tomatoes, and to investigate methods of enhancing bumble bee colony population growth and foraging effort.

Chapter II

Are bumble bee colonies in tomato greenhouses obtaining adequate nutrition?

Introduction

Bumble bees have become an important managed pollinator in recent years, joining honey bees, leaf cutter bees, and orchard mason bees in providing commercial pollination services. Bumble bee colonies are particularly effective pollinators for tomatoes grown in greenhouses, which historically were pollinated mechanically by vibrating the tomato flower trusses (van Koot and van Ravestijn 1962, Picken 1984). However, this is an expensive, time-intensive practice, and pollination by bumble bees is more cost-effective and results in fruit of similar size and set (Banda and Paxton 1991, Kevan et al. 1991, van Ravestijn and van der Sande 1991, Straver and Plowright 1991, Dogterom et al. 1998, Pressman et al. 1999). Consequently, the greenhouse tomato industry has come to rely upon bumble bees for the production of large, commercially attractive fruit.

Bumble bees are excellent pollinators, but as a relatively new industry (Plowright and Jay 1966, van Heemert et al. 1990) there is still much to be learned about how to develop and maintain large, healthy colonies. Research to date has focussed on colony rearing methods, but few studies have examined colony maintenance once bumble bees are placed into greenhouses.

Bumble bee colonies typically are not managed once they are placed in commercial greenhouses, where the colonies presumably grow and bees pollinate for eight to ten weeks before colony populations decline. Perhaps relatively simple

management techniques could increase colony populations and individual bee life-spans in the greenhouse. One concern of growers is that the nutrition available to bumble bees from pollen of a single species of flower in tomato greenhouses may be inadequate to support long-lived bees and large colonies. Honey bees develop poorly if they are fed pollen lacking one or more essential amino acids (de Groot 1953), and those fed unifloral pollen or pollen containing low protein concentrations have short life-spans (Schmidt et al. 1987). Similarly, limited pollen availability increases larval development time in *Bombus terricola* K. (Sutcliffe and Plowright 1990) and decreases adult worker size (Sutcliffe and Plowright 1988). These studies suggest that supplementing the diet of bumble bee colonies with diverse pollens could extend worker life-spans and increase colony populations.

The objectives of my experiments were to examine colony growth of *Bombus occidentalis* Greene (Hymenoptera: Apidae) in commercial tomato greenhouses, and to determine if growth could be enhanced by supplementing their food supply with multi-floral pollens. Two experiments were conducted to determine the effects of differing diets. The first experiment was conducted in summer when both control colonies and colonies fed supplemental pollen had access to diverse flower species outside the greenhouse. The second experiment occurred in winter when external forage was unavailable, and in this experiment I also examined the average life-span of worker bees. In addition, I compared three methods of assessing colony size.

Materials and Methods

The two experiments were conducted in a greenhouse in Ladner, British Columbia during summer 2000 and winter 2001. The summer experiment was conducted with one

set of colonies in a 1.8 ha cherry tomato (*Lycopersicon esculentum* Miller var. Conchita) crop. The winter experiment was conducted with a different set of colonies in a 7.2 ha beefsteak tomato (*Lycopersicon esculentum* Miller var. Rhapsody) crop. Bumble bee hive density was between four and five hives per hectare in the summer experiment, and between 0.5 and 1.2 hives per hectare in the winter experiment.

In the summer experiment, 23 *B. occidentalis* colonies, approximately 12 weeks old, were received from Biobest Canada Ltd. over a three-week period starting the first week of June 2000. The colonies were sorted by size and then colonies of each size class were randomly assigned to two treatments, Pollen Added (n = 11) and Sham-Manipulated (n = 12). Pollen Added colonies were given excess multi-floral pollen, collected from honey bee field colonies with pollen traps, mixed with honey water (50:50 by volume), and replaced twice a week. Sham-Manipulated colonies were not fed pollen, but pollen addition was simulated by exchanging food dishes. I assessed colony populations (queens, drones, and workers), amount of brood (number of egg masses, larvae and pupae) and amount of stores (honey pots and pollen pots). Colonies were randomly placed along the center aisle of the greenhouse and assessed weekly for ten weeks.

In the winter experiment, 24 *B. occidentalis* colonies were received over a five-week period starting the first week of January 2001. They were sorted by population size and colonies from each size class were randomly placed into three treatment groups: Pollen Added (n = 8), Sham-Manipulated (n = 8) and Unmanipulated (n = 8). Colony age at time of introduction to the greenhouse and assessment regime were the same as for the summer experiment, except that colonies were only assessed for eight weeks and the pollen mixture was only replaced once a week. All bees in the Pollen Added and Sham-Manipulated colonies were tagged during the first two assessments on their thoraces,

using coloured, numbered, plastic tags, to determine bee survivorship. The unmarked bees that were tagged during the second week were either less than one week old, or had drifted into that colony from other colonies. Therefore, the bees were assumed to be half a week old at the time they were tagged, a slight underestimate of their average age. I conducted survivorship analysis on four Pollen-Added and three Sham-Manipulated colonies that were uniquely tagged. The Unmanipulated treatment was included to determine effects of our assessment methods and manipulations on colony growth. These colonies were assessed upon arrival at the greenhouse and then were not examined again until week eight.

Three methods of assessing the number of bees in each colony were compared during the winter experiment: 1) counting the number of bees on top of the cotton insulation (number of bees on cotton), 2) opening the cotton so that the brood nest could be seen and then counting or estimating the number of bees in the colony (visual estimate), and 3) removing every bee from the colony, counting them, and then returning the bees to the colony (individual count).

A repeated measures analysis of variance was used to determine if there was any effect of treatment on colony size (SPSS Inc. 1999). Colony size of Sham-manipulated and Pollen Added colonies was compared across all weeks and a comparison of colony size of all treatments at weeks 0 and 8 was conducted separately. Means were compared using the Tukey-Kramer HSD multiple comparison test. A simple linear regression was used to examine the relationship between different assessment methods (SAS Institute 2000a). The lifespan of tagged bees was calculated by averaging the age at which each bee was last observed in the greenhouse. A one-way analysis of variance was used to

examine the effects of treatment on lifespan (SAS Institute 2000a). Results are reported with the mean and standard error.

Results

Colony size did not differ between Pollen Added and Sham-Manipulated groups for worker populations or amount of brood during the summer (workers, $F_{1,21} = 0.03$, $P = 0.87$; brood, $F_{1,21} = 0.03$, $P = 0.88$; Fig. 2.1) or during the winter (workers, $F_{1,14} = 0.52$, $P = 0.48$; brood, $F_{1,14} = 0.61$, $P = 0.45$; Fig. 2.2) experiment. There also were no differences in colony size when all treatments were examined at weeks 0 and 8 (workers, $F_{2,21} = 0.04$, $P = 0.96$; brood, $F_{2,21} = 0.69$, $P = 0.51$). Hence, there was no effect of nutritional supplements, colony assessments, or tagging of bees on colony size.

During the winter experiment, Sham-Manipulated and Pollen Added colonies had significantly more stores (Sham-manipulated, 96.1 ± 4.0 pollen and honey pots; Pollen Added, 92.6 ± 4.2 pollen and honey pots) than the Unmanipulated colonies (55.4 ± 4.8 pollen and honey pots) ($F_{2,157} = 9.84$, $P < 0.001$). As expected, time had a significant effect on brood production (summer, $F_{12,222} = 2.32$, $P = 0.008$; winter, $F_{8,119} = 8.71$, $P < 0.001$), with a dip at week one and a peak at week four in Pollen Added and Sham-Manipulated treatments (Figs. 2.1-2.2). Interestingly, brood production increased 25 to 50 percent in both experiments, while worker populations remained steady (summer) or declined (winter) (Figs.2.1-2.2).

There was no difference in queen or drone production between Pollen Added and Sham-Manipulated treatments either during summer (queens, $F_{1,21} = 0.73$, $P = 0.401$; drones, $F_{1,21} = 1.90$, $P = 0.183$) or winter (queens, $F_{1,14} = 2.24$, $P = 0.157$; drones, $F_{1,14} = 1.35$, $P = 0.265$) experiments, although both queen and drone production were higher

during the winter experiment than in the summer experiment (Fig. 2.3). Drone production began in week four while queen production began in week two (Fig. 2.3). The average life-span of workers was unaffected by treatment ($F_{1,5} = 2.87$, $P = 0.15$), with bees from Pollen Added and Sham-manipulated treatments living 13.3 ± 2.7 days and 20.1 ± 3.1 days, respectively.

Visual estimates of the number of bees in each colony were positively correlated with the true number of bees present (individual count=1.75(visual estimate)–7.87), as determined by removing and counting every individual in the colony ($F_{1,158} = 62.00$, $P < 0.001$, $r^2 = 0.80$; Fig. 2.4). The number of bees on top of the cotton also was significantly correlated to the individual counts (individual count=1.73(bees on cotton)+62.92), although this method of estimating colony populations was not as strongly correlated as the visual estimate ($F_{1,158} = 90.36$, $P < 0.001$, $r^2 = 0.36$; Fig. 2.4).

Discussion

These experiments indicate that nutrition was not a limiting factor for *B. occidentalis* colony growth. Adding multi-floral pollens did not increase colony size in either the summer or the winter experiment. The lack of difference between treatments in the summer experiment could have been due to availability of pollen from plant species outside the greenhouse. However, I also found no differences in colony growth between treatments in the winter experiment when alternate pollen sources were not available. I conclude that tomato pollen is adequate for bumble bee colony growth, a conclusion supported by the similar patterns of brood production I found in all treatments.

Brood production increased while adult worker populations were stable or declined, suggesting that adult worker mortality may be the cause of poor colony growth.

Brood increased by 25% and 50% in the summer and winter experiments, respectively, but adult populations did not exhibit similar increases. A consistent dip in brood production in week one likely was due to the stress of travel and the establishment of the colony in a new environment.

Worker life-spans of 13.3 and 20.1 days for Pollen Added and Sham-Manipulated colonies respectively also support the hypothesis of high adult mortality when compared with life-spans of other bumble bee species. In their natural environment, life expectancies are 33 days in *Bombus pennsylvanicus*, 21.8 and 34.1 days in two different years of data from *Bombus fervidus* (Goldblatt and Fell 1987), 13.2 days in *Bombus terricola* (Rodd et al. 1980), and 36.4 days in *Bombus* (*fervidobombus*) *mario* (Garófalo 1978). The life-spans observed in this study are shorter on average than those of typical bumble bees. There was a tendency for longer worker life-spans in Sham-manipulated colonies than in Pollen Added colonies, but a low number of replicates (three and four colonies respectively) did not provide sufficient statistical power to draw firm conclusions about the effects of nutrition on *B. occidentalis* life-spans.

Drone and queen production was not affected by access to a multi-floral pollen. However, because the production of reproductives (queens and drones) is correlated with a decrease in worker production (Duchateau and Velthuis 1988), investigation of factors that influence reproductive production such as temperature, colony congestion, changes in queen pheromone composition, and worker-larva ratio might reveal management techniques that would promote worker production.

The increase in pollen and nectar storage in Sham-Manipulated and Pollen Added colonies compared to Unmanipulated colonies in winter 2001 suggests an effect of weekly assessments or worker tagging. Previous research on stress in both bumble bees

and honey bees indicates increased foraging in response to food deprivation (Cartar 1992, Fewell and Winston 1992, Plowright et al. 1993). However, honey bees decrease foraging effort in response to disease (Anderson and Giacon 1992, Janmaat and Winston 2000), and it is not clear which of these factors might have affected pollen and nectar storage in these experiments.

The most likely explanations for the lack of colony growth in this study are disorientation and disease. Tagged bees were observed entering hives that were not their own, and bee mortality may be increased by inter-colony aggression or by disease transfer among colonies due to drifting.

The most efficient and accurate assessment method was counting the number of bees once the insulating cotton was opened, and calculating the bee populations from this visual estimate. This method was faster than counting every individual, and more highly correlated to the true number of bees in the colony than estimates from counting the number of bees on top of the cotton.

My research suggests that future studies should focus on determining why *B. occidentalis* colonies do not grow once they are placed into commercial tomato greenhouses. Inadequate nutrition does not appear to be a factor because pollen supplements did not affect colony populations, brood production, or the production of queens and drones. The placement of orientation markers in the greenhouse may decrease inter-colony drift and subsequent worker mortality. As well, greater knowledge about bumble bee diseases and how to manage them may result in longer-lived workers and healthier colonies. Research investigating methods of suppressing queen and drone production also may lead to increased worker populations in tomato greenhouses.

The stable or declining populations in study colonies indicate a concern for growers regarding the effectiveness of *B. occidentalis* and possibly other bumble bee species for greenhouse pollination, and suggests that further research directed at improving management for this species would be desirable.

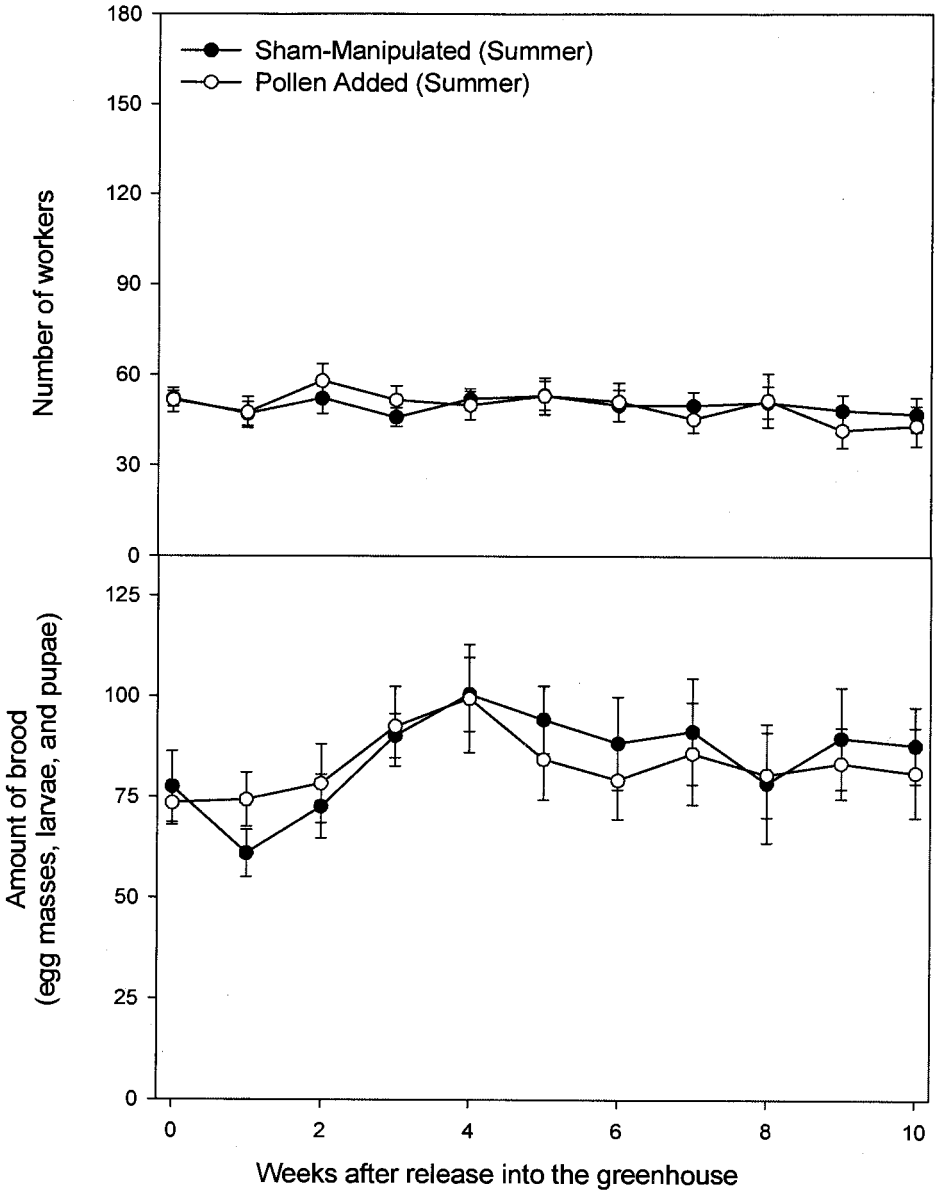

Figure 2.1. Number of workers and amount of brood in Pollen Added and Sham-Manipulated colonies during summer 2000. Error bars = $\pm$ 1 SE.

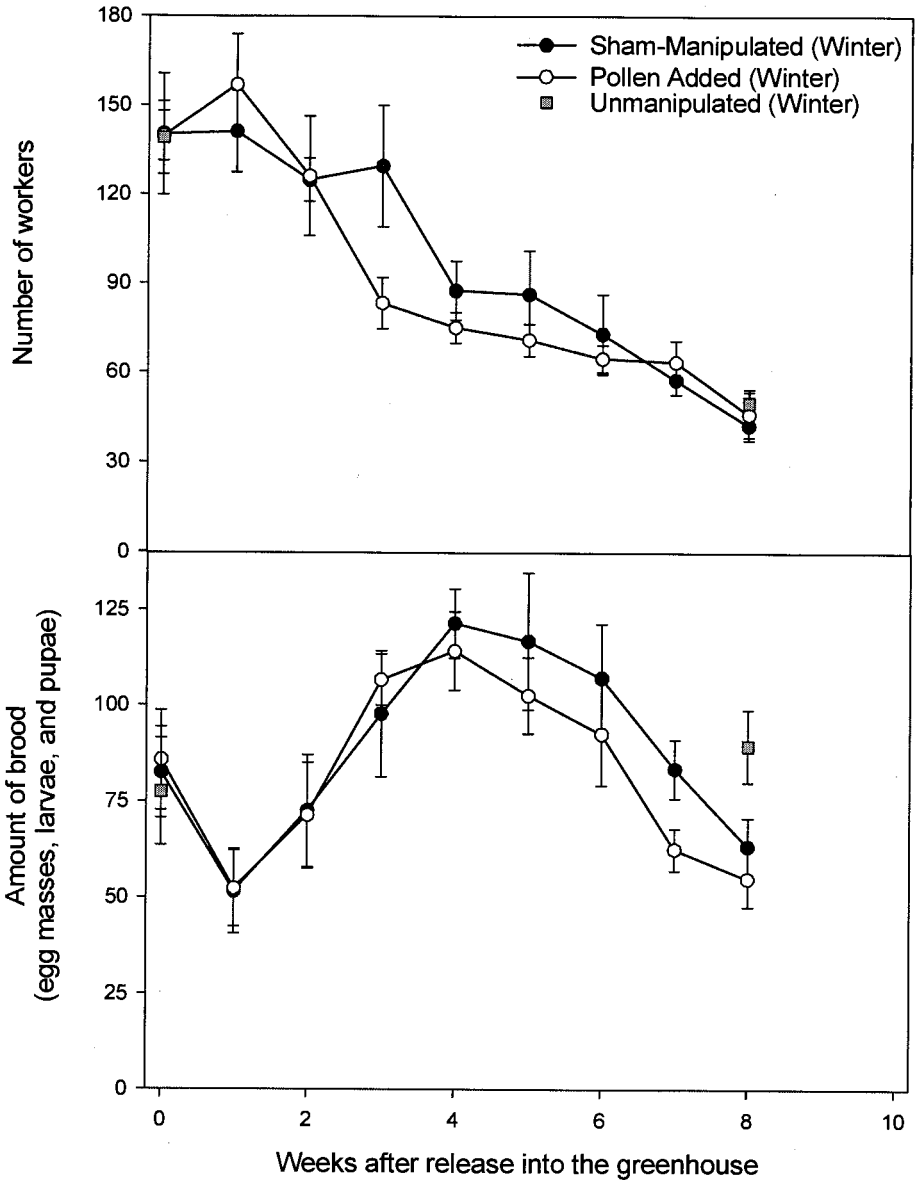

Figure 2.2. Number of workers and amount of brood in Pollen Added, Sham-Manipulated, and Unmanipulated colonies during winter 2001. Error bars = $\pm$ 1 SE.

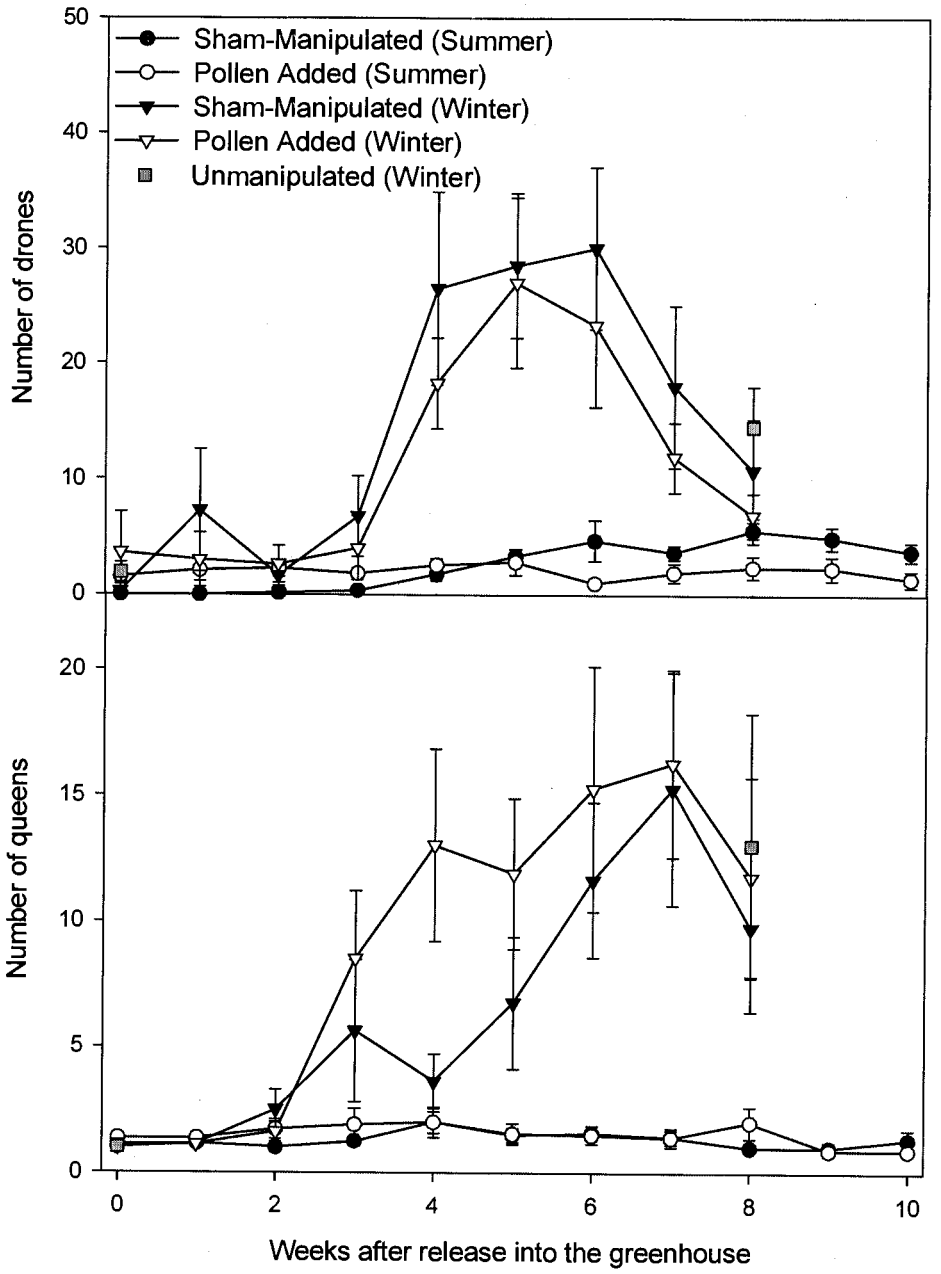

Figure 2.3. Number of queens and drones in Pollen Added, Sham-Manipulated, and Unmanipulated colonies in winter and summer. Error bars = $\pm$ 1 SE.

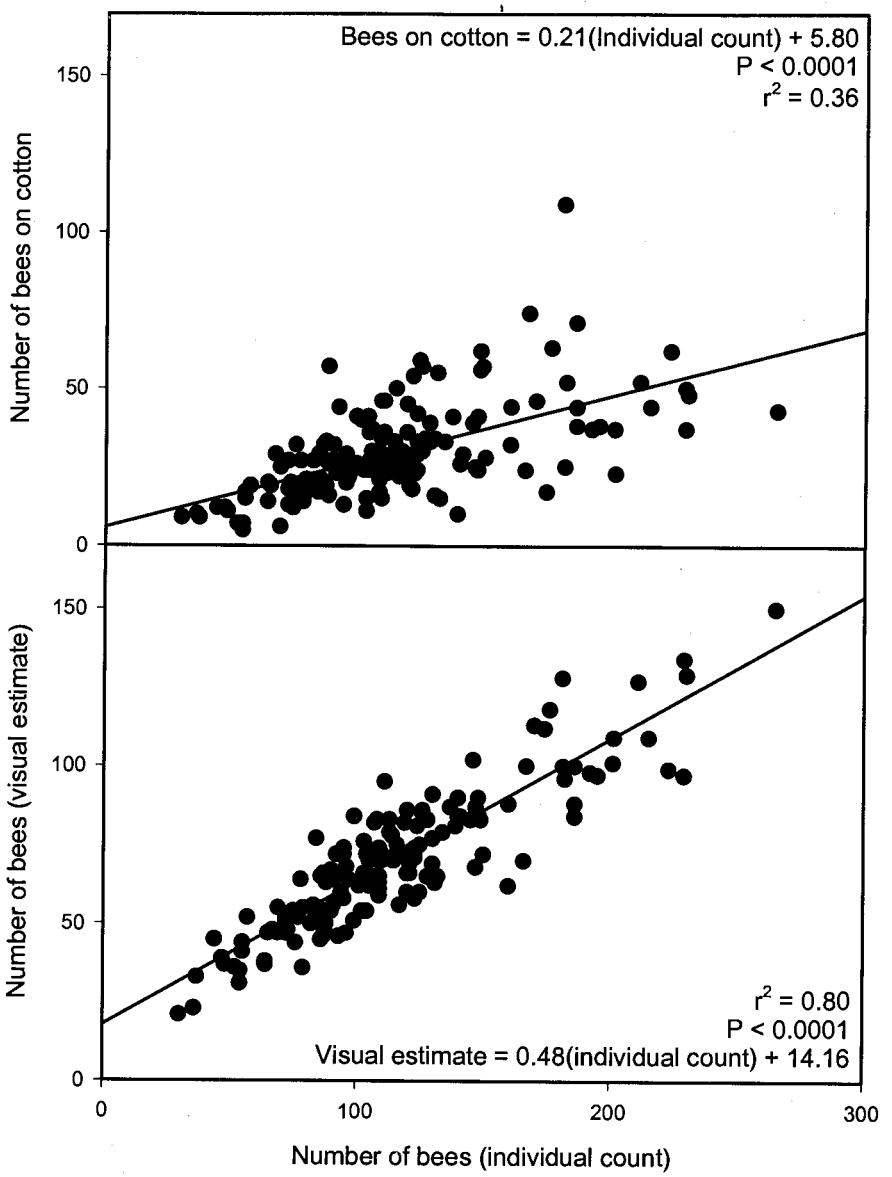

Figure 2.4. Correlation between the total number of bees in the colony (individual count) and the visual estimate of the number of bees after the insulating cotton is opened, or by counting the number of bees on top of the cotton (winter 2001 data only).

Chapter III

Pollen species collected by greenhouse bumble bees

Introduction

Bumble bees recently have been domesticated and used to pollinate greenhouse tomatoes in many parts of the world because they are less expensive and more efficient than manual pollination (Dogterom et al. 1998, Pressman et al. 1999). However, pollination efficacy fluctuates throughout the year, so growers compensate by increasing or decreasing the number of colonies used per acre. I investigated seasonal differences in the number of bumble bees foraging on plants outside the greenhouse that could contribute to pollination fluctuations. I surveyed the pollen collected by bees throughout the year to determine what plant species bumble bees forage on, and the proportion of the pollinating force foraging outside the greenhouse at different times of the year.

Materials and Methods

Pollen loads were collected monthly from 15 or more bees from each of three tomato greenhouses in Ladner (9.2 ha), Surrey (4.2 ha), and Pitt Meadows (2.5 ha), in south-western British Columbia, Canada. Pollen loads were collected from both *Bombus occidentalis* Greene and *Bombus impatiens* Cresson, because both species were present in Surrey and Pitt Meadows, but only *B. occidentalis* was present in the Ladner greenhouse. Bees were captured at the entrances of randomly selected colonies, cooled on ice, and pollen loads then were removed. Hive density ranged from 0.5 hives per hectare in late winter to fives hives per hectare in mid-summer. Because tomato flowers do not produce nectar, colonies were supplied with sugar syrup as a substitute. Cherry (*Lycopersicon*

esculentum Miller var. Conchita) and beefsteak (*L. esculentum* Miller var. Rhapsody) tomatoes were the primary varieties grown in the greenhouses.

Pollen loads were mounted on fuschin gel (Kearns and Inouye 1993) and examined with light microscopy. One hundred pollen grains from each pollen load were randomly selected and identified (Sawyer 1981, Moore et al. 1991, Crompton and Wojtas 1993), and verified against a set of reference slides prepared from known local flora. The size and morphology of pollen grains were used as primary taxonomic characters. However, pollen load color also provided a preliminary indication of species composition, particularly for unknown or closely related species (Hodges 1984).

For the Ladner and Surrey greenhouses where little non-tomato pollen was collected, Fisher's exact test was used to determine if the proportions of non-tomato pollen collected varied throughout the year, while a Chi-square test was used for Pitt Meadows where a relatively large amount of non-tomato pollen was collected (SAS Institute 2000a). The Cochran-Mantel-Haenszel test was used to determine if the pattern of pollen collection throughout the year differed among greenhouses (SAS Institute 2000b).

Results

The proportion of non-tomato pollen collected by bees each month varied significantly during our eight month study (Ladner $df = 127$, $P = 0.013$; Surrey $df = 132$, $P < 0.001$; Pitt Meadows ($\chi^2 = 41.69$, $df = 7$, $P < 0.0001$), with the greatest proportion of non-tomato pollen collected in May (23%) and July (44%) (Fig. 3.1). The pattern of pollen collection also varied significantly among greenhouses ($\chi^2 = 24.75$, $df = 1$, $P < 0.0001$), with more non-tomato pollen collected by bumble bees in Pitt Meadows ($24 \pm$

9% SE), than in Surrey (7 ± 5% SE) or Ladner (5 ± 3% SE). An average across all greenhouses of 14% of bees foraged outside between February and September. Foreign pollen was composed primarily of *Rubus* species (47%) and dandelion (*Taraxacum officinale*) (5%), although other species (19.3%) such as bull thistle (*Cirsium vulgare*), buttercup (*Ranunculus acris* and *R. repens*), fireweed (*Epilobium angustifolium*), foxglove (*Digitalis purpurea*), and pink spirea (*Spirea douglasii*) also were observed. The identity of 28.8% of foreign pollen was undetermined. Several *Rubus* species were present near the greenhouses, including Himalayan blackberry (*R. discolor*), evergreen blackberry (*R. lactiniatus*), trailing blackberry (*R. ursiaus*), common raspberry (*R. idaeus*), thimbleberry (*R. parviflorus*), and salmonberry (*R. spectabilis*). Pollen loads were composed primarily of pollen from only one plant species (94%), while 6% of the loads were mixed. Seventeen percent of these mixed loads contained primarily tomato pollen with minor amounts of other pollens, while 83% were composed primarily of pollen from other plant species with minor amounts of, or no tomato pollen.

Discussion

Fourteen percent of the pollen collected throughout our study was non-tomato, and 23% of the pollen collected during May and 44% during July was obtained outside the greenhouse. Assuming the amount of pollen collected reflects the time spent foraging on each plant species, the average annual financial loss to greenhouse growers in the south-western British Columbia from bees foraging outside was approximately $1,500 to $3,000/ha/yr (CDN), based on the proportion of non-tomato pollen collected each month, the number of new bumble bee colonies released into the greenhouse each month, and the cost per colony ($250 CDN).

This estimated pollination loss is a minimum value, because I collected only bees returning with pollen loads, and therefore underestimated the number of bees returning without pollen that may have been foraging outside for nectar. As well, some bees may have left the greenhouse and failed to return (Morandin et al. 2001). Greenhouse growers could reduce the loss of pollinators by screening the greenhouse vents to prevent bees from leaving, which would have the added benefit of preventing pest species from entering the greenhouse through the vents, although there may be detrimental effects such as decreased light or poorer ventilation. Growers also could predict the timing of pollination shortfall inside greenhouses by monitoring the bloom of adjacent patches of plants such as *Rubus* species.

The surrounding habitats may explain differences in foraging patterns of bumble bees between the greenhouses I studied. The Surrey and Ladner greenhouses were in flat agricultural areas, while the Pitt Meadows greenhouse was adjacent to a river and parklands. This habitat may promote greater plant species diversity and more foraging opportunities. Thus, adjacent vegetation should be a factor when considering where to locate commercial greenhouses.

The large diversions of bumble bee pollination to plants external to the greenhouses may explain in part the fluctuations in pollination levels that greenhouse growers experience. Methods of preventing bee loss from greenhouses should be investigated because outside foraging results in considerable financial loss to commercial greenhouse tomato operations.

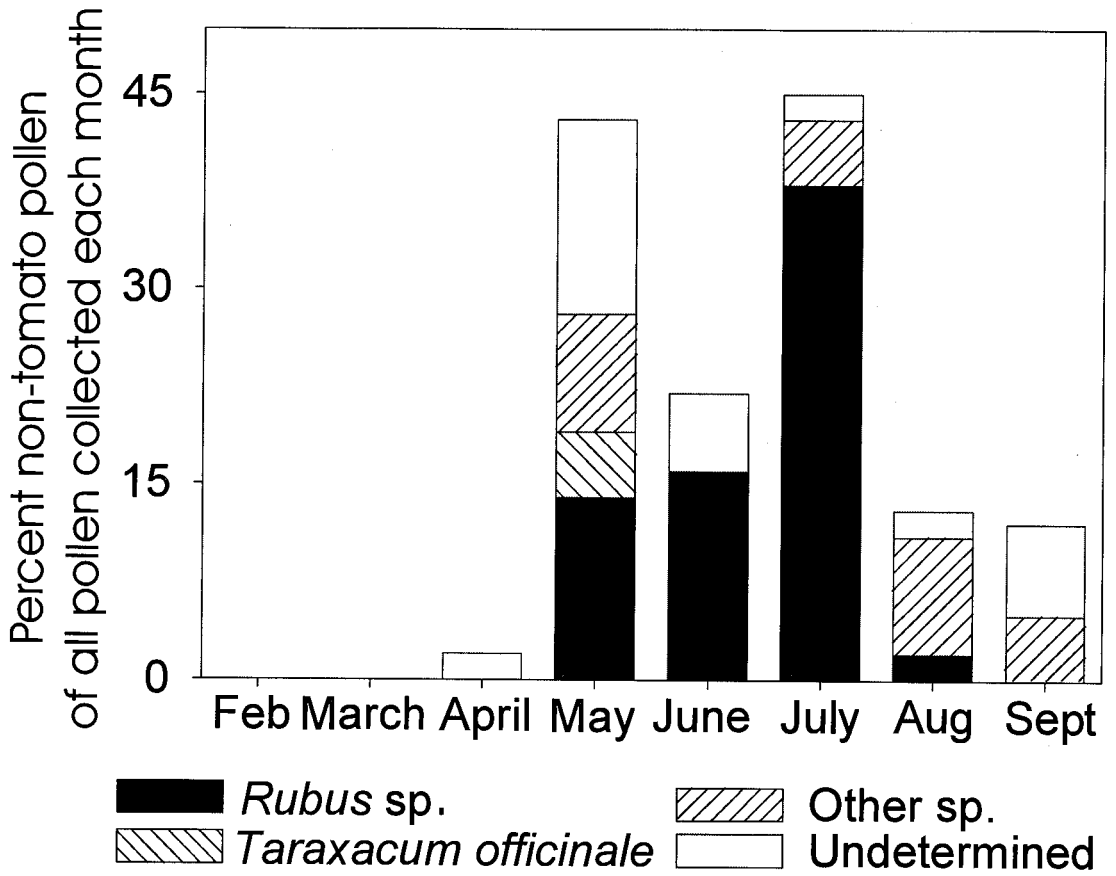

Figure 3.1. Percentage of non-tomato pollen of all pollen collected each month. In February and March, only tomato pollen was collected. 'Other sp.' include *Cirsium vulgare, Digitalis purpurea, Epilobium angustifolium, Ranunculus* sp., and *Spirea douglasii.*

Chapter IV

Comparison and examination of *Bombus occidentalis* and *Bombus impatiens* as pollinators of greenhouse tomatoes

Introduction

In the 30 years since bumble bees were initially reared in captivity (Plowright and Jay 1966), research has shown that pollination by bumble bees in tomato (*Lycopersicon esculentum* Miller, Solanaceae) greenhouses results in fruit that is comparable or superior to that from the traditional, time-intensive practice of manually vibrating tomato trusses (van Koot and van Ravestijn 1962, Picken 1984, Banda and Paxton 1991, Kevan et al. 1991, van Ravestijn and van der Sande 1991, Straver and Plowright 1991, Dogterom et al. 1998). Growers have come to rely upon bumble bees for crop pollination and usually use bumble bee species native to their area. However, in the late 1990's in western Canada and the United States producers of native *Bombus occidentalis* Greene were unable to meet the demand for bumble bee services, and *Bombus impatiens* Cresson colonies from eastern North America were imported under emergency permits.

Some growers used both species for pollination of their tomato crops, but became concerned about the effectiveness of *B. occidentalis* and perceived that *B. impatiens* might be a better pollinator. However, they have been wary of committing solely to using *B. impatiens* due to the environmental risk associated with potentially introducing a foreign species if *B. impatiens* queens escape from their greenhouses.

This study compared the effectiveness of *B. occidentalis* and *B. impatiens* as pollinators by monitoring colony growth and foraging effort over the time they are in the greenhouse. As well, I investigated whether *B. occidentalis* colonies are growing to their

full potential in greenhouses by comparing greenhouse colony growth to that of completely enclosed colonies protected from external mortality, and to colonies placed outside in a more natural environment. As well, the potential effect of inter-specific competition between *B. impatiens* and *B. occidentalis* was investigated by comparing the growth of *B. occidentalis* colonies in a greenhouse with *B. impatiens* to that of *B. occidentalis* in greenhouses where it was the sole pollinator.

Materials and Methods

Five treatment groups were included: 1) *B. impatiens* observed in the same greenhouse as *B. occidentalis* (Imp-together); 2) *B. occidentalis* observed in the same greenhouse as *B. impatiens* (Occ-together); 3) *B. occidentalis* in greenhouses containing no other bee species (Occ-alone); 4) *B. occidentalis* colonies placed outside the greenhouse in their natural environment (Occ-outside); and 5) *B. occidentalis* colonies in greenhouses but completely enclosed (Occ-enclosed). Imp-together, Occ-together, and Occ-enclosed colonies were placed along the south side of the main aisle of a 2.5 ha greenhouse in Pitt Meadows, British Columbia, Canada. Due to greenhouse management decisions, there were approximately twice as many *B. impatiens* as *B. occidentalis* colonies present at any one time, and the study hives were part of a larger population of colonies in the greenhouse. Hive density ranged between four and five hives per hectare.

The entrances of Occ-enclosed colonies were screened so there was air circulation, but no bees could enter or leave the hives. These colonies were provided with excess water and multi-floral pollen that was field-collected from honey bee colonies and mixed with honey water (50:50 by volume). Water and pollen were replaced once a

week. As well, all colonies in the experiment were supplied with a sugar syrup nectar substitute because tomato flowers do not produce nectar.

The Occ-outside colonies were placed adjacent to the Pitt Meadows greenhouse in wooden boxes where they had access to agricultural areas and woodlands. Five Occ-alone colonies were in a 9.2 ha greenhouse in Ladner, British Columbia and three were in a 4.2 ha greenhouse in Surrey, British Columbia. All other treatment groups had seven colonies each. The primary tomato varieties used in the greenhouses were beefsteak (var. Rhapsody) and cherry (var. Conchita).

Colonies were received from Biobest Canada Ltd. during the first week of May 2001, except for two Occ-alone colonies that were obtained two weeks later. *B. occidentalis* colonies were approximately twelve weeks old upon arrival at the greenhouse, whereas the *B. impatiens* colonies were approximately ten weeks old. Colonies were assessed for their population composition (queens, drones, and workers), amount of brood (number of egg masses, larvae and pupae), and stores (honey pots and pollen pots) upon arrival at the greenhouse and then every other week for eight weeks. After the initial assessment, colonies were blocked by size, randomly assigned to treatments, and randomly placed along the center aisles of the greenhouses.

Foraging effort of Imp-together, Occ-together, and Occ-outside colonies were assessed by observing the number and species of bees entering and exiting each colony for thirty minutes in a random order, between 0800 h and 1500 h (PDT). Foraging effort was assessed every other week, commencing one week after the start of the experiment. The presence of pollen loads also was noted.

A components of variance analysis was conducted with the two Occ-alone greenhouses to determine if the variation in bumble bee colony size within each

greenhouse was representative of the variation found in all greenhouses (SPSS Inc. 1999). The components of variance analysis showed that variation due to greenhouse was negligible (variance ratio (greenhouse/colony) ≈ 0) and that the variation among colonies within each greenhouse was greater than colony variation between greenhouses. Therefore all treatment groups could be compared at the colony level and hence analysis was conducted at the colony level. A repeated measures analysis of variance was used to examine the effect of treatment on worker numbers, amount of brood, and foraging effort over time (SPSS Inc. 1999). The effect of time of day on foraging activity, pooled across weeks, also was analyzed with a repeated measures analysis of variance (SPSS Inc. 1999). Tukey's honestly significant difference test was used as a post hoc multiple comparison test.

Results

There was a significant effect of treatment on number of workers ($F = 14.9$; $df = 4,30$; $P < 0.05$) and amount of brood ($F = 14.7$; $df = 4,12$; $P < 0.05$) over time with Imp-together, Occ-outside, and Occ-enclosed colonies having more workers than Occ-together and Occ-alone colonies (Fig. 4.1). Honey bees from nearby colonies began to rob the Occ-outside colonies of sugar syrup in week five, resulting in high bumble bee mortality. Therefore observed Occ-outside populations may have been lower than they would have been without honey bee attack. Occ-enclosed colonies had significantly lower amounts of brood than all other treatments except Occ-together (Fig. 4.1). Imp-together colonies had significantly more brood than any other treatment group (Fig. 4.1).

Foraging effort was significantly affected by treatment ($F = 28.0$; $df = 2,17$; $P < 0.05$) with Imp-together colonies making the most trips per colony per hour, and Occ-

together the fewest (Fig. 4.2). However, *B. impatiens* workers left colonies without depositing their pollen loads 13 ± 3% (SE) of the time, while Occ-together and Occ-outside bees left colonies with pollen loads 4 ± 4% (SE) and 0% of the time, respectively. Imp-together bees entered the colony without pollen loads, and may have been foraging for nectar outside the greenhouse (Chapter 3), 39 ± 3% (SE) of the time, but Occ-together and Occ-outside bees entered the colony without pollen loads 7 ± 2% (SE) and 49 ± 8% (SE) of the time, respectively. The presence of pollen loads was recorded while observing bees entering and exiting the colonies from one to three meters away, hence we may have categorized some small pollen loads as not being present.

Time of day did not affect bumble bee foraging activity ($F = 1.9$; $df = 5,20$; $P = 0.13$), although there was a trend towards a peak in activity mid-morning, and a dip in activity at 1300 h (PDT) (Fig. 4.3).

Discussion

This study illustrates a number of important issues regarding the use of two species of bumble bees as pollinators in commercial tomato greenhouses. Most importantly, *B. occidentalis* colonies commonly used by greenhouses operators in western North America are not performing optimally in the greenhouse environment. Occ-enclosed colonies had more workers, but the same amount or less brood than Occ-together or Occ-alone colonies (Fig. 4.1), indicating that colonies reared workers to adulthood but there was a high level of adult bee loss or mortality in the greenhouse.

Occ-outside colonies had similar brood levels to Occ-alone, but more adult workers. Further, Occ-outside and Occ-enclosed colonies had similar numbers of workers, even though Occ-outside colonies had more brood (Fig. 4.1). These results

suggest that although there is greater worker loss in their natural environment than in enclosed colonies, the highest level of adult worker loss occurred in the greenhouse.

These data also suggest that inter-specific competition with *B. impatiens* may negatively affect *B. occidentalis* colony growth. When *B. occidentalis* colonies are in the same greenhouse as *B. impatiens* (Occ-together), there is a trend towards less brood and fewer workers than when *B. occidentalis* is alone in a greenhouse without *B. impatiens* (Occ-alone) (Fig. 4.1). Although this difference in colony growth may be either due to a location or a treatment effect, this result supports anecdotal evidence from greenhouse growers that *B. occidentalis* colonies do not fare well in the presence of *B. impatiens*.

B. occidentalis colony populations may be diminished in the presence of *B. impatiens* because of drift between colonies of different species. Ten to 20 dead *B. impatiens* workers were found in *B. occidentalis* colonies each week, and a similar number of dead *B. occidentalis* workers were found in *B. impatiens* colonies. However, because *B. impatiens* colonies were larger, they may have been able to absorb the loss of worker bees more easily than *B. occidentalis*. There also were dead bees of the same species found in each hive, possibly the result of hive guarding behaviour against intra-specific interlopers.

These data also show that Imp-together colonies are larger than Occ-alone colonies both in terms of number of workers and amount of brood, and hence *B. impatiens* provides more pollinators per colony than *B. occidentalis*. This has important implications for greenhouse growers, because the price per colony is the same for the two species and if *B. impatiens* colonies provide more bees per colony they will be more cost-effective than *B. occidentalis*. However, it is difficult to weigh this direct financial benefit against environmental costs from the potential accidental escape of this species.

Another bumble bee species, *B. terrestris*, that is also used extensively to pollinate greenhouse crops, has colonized areas in Japan, Tasmania, and Israel where it is not native. There are indications that *B. terrestris* out-competes many of the native bee species for resources and that when *B. terrestris* is the main pollinator there is a significant reduction in the seed production of some plant species (Dafni 1998).

I found additional evidence that *B. impatiens* bees provide more pollination than *B. occidentalis* because they made more foraging trips than either Occ-outside or Occ-together colonies (Fig. 4.2), even though Imp-together and Occ-outside colonies had the same number of workers (Fig. 4.1). However, my data suggest that individual *B. impatiens* workers may not be as efficient at pollination as *B. occidentalis* because some (13.2%) *B. impatiens* foragers left the colony with their pollen loads after bringing pollen in, while Occ-together bees left with pollen loads only 3% of the time, and Occ-outside bees did not exit colonies with pollen loads at all.

Bees that have not deposited their pollen loads are less likely to continue foraging. As well, Imp-together bees returned to their colony without pollen 39% of the time, while this occurred in Occ-together colonies only 7% of the time. These bees were most likely foraging on nectar-producing plants outside the greenhouse and not on tomatoes. The number of foraging trips per colony may not accurately reflect the amount of pollination provided by each species accurately, as it does not reflect the fidelity of each species to tomato flowers. This is a concern because bumble bees forage on flowers outside the greenhouse 10 to 40% of the time between May and September (Chapter 3). The amount of time spent foraging outside of the greenhouse by the two bee species could directly affect the amount of pollination provided for greenhouse tomatoes.

The dip in foraging activity in early afternoon may reflect a dip in pollen production. Other species such as honey bees change their foraging activity in response to pollen and nectar production (Moore et al. 1989).

In conclusion, *B. impatiens* colonies grow larger, forage more, and likely provide more pollination per colony than *B. occidentalis* colonies. Growers who use only *B. occidentalis* for pollination have been pleased with the benefits of maintaining access to a second species, but need to use more colonies per acre than growers who use solely *B. impatiens*. *B. occidentalis* are adversely affected by the presence of *B. impatiens*, and are not growing to their potential population size in greenhouses. Further research should investigate limiting factors such as disease and orientation problems. Increased understanding of limiting factors could result in larger, more efficient colonies that would increase pollination levels in commercial tomato greenhouses.

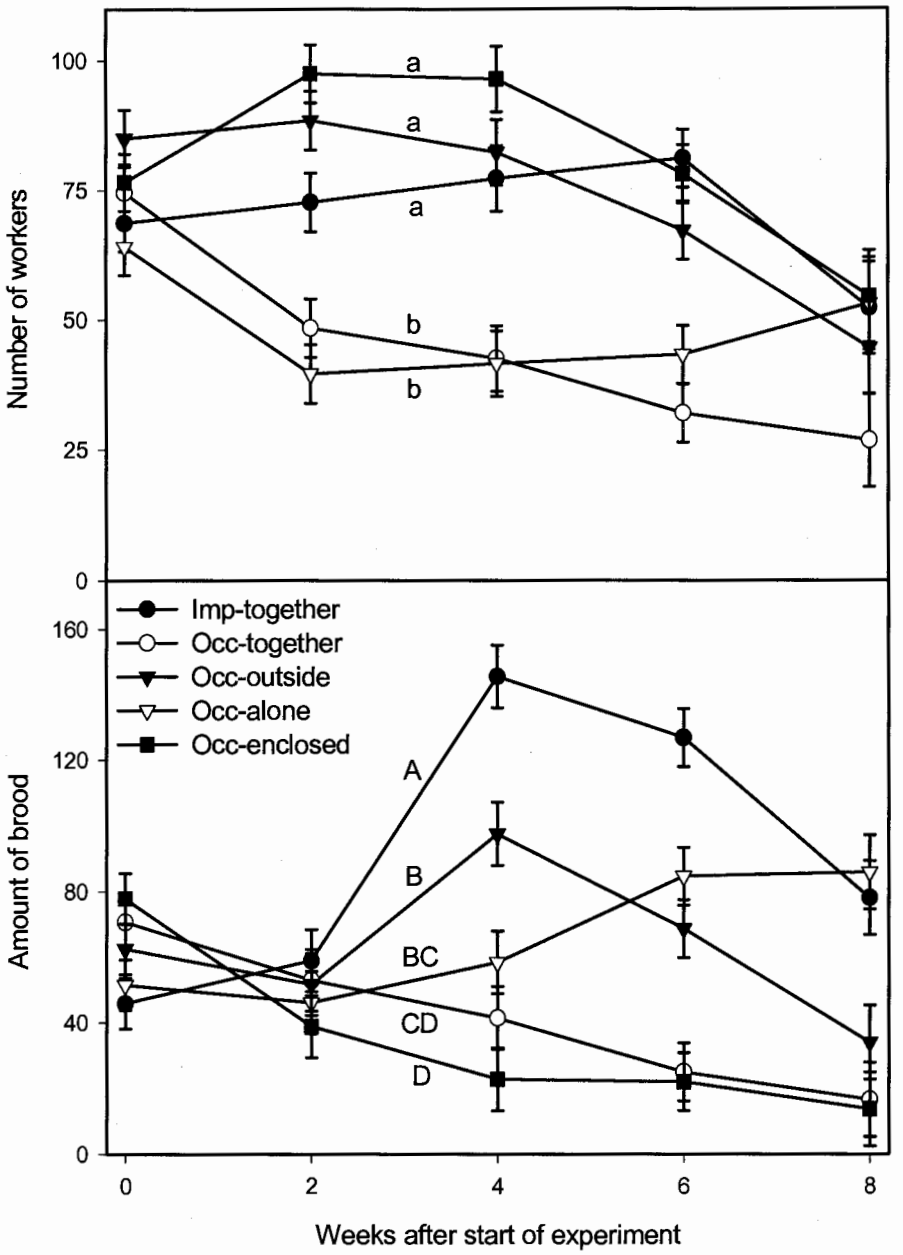

Figure 4.1. Effect of treatment on colony worker populations and amount of brood (number of eggs, larvae and pupae) over time. Treatments with the same letter are not significantly different ($P < 0.05$). Error bars = $\pm$ 1 SE.

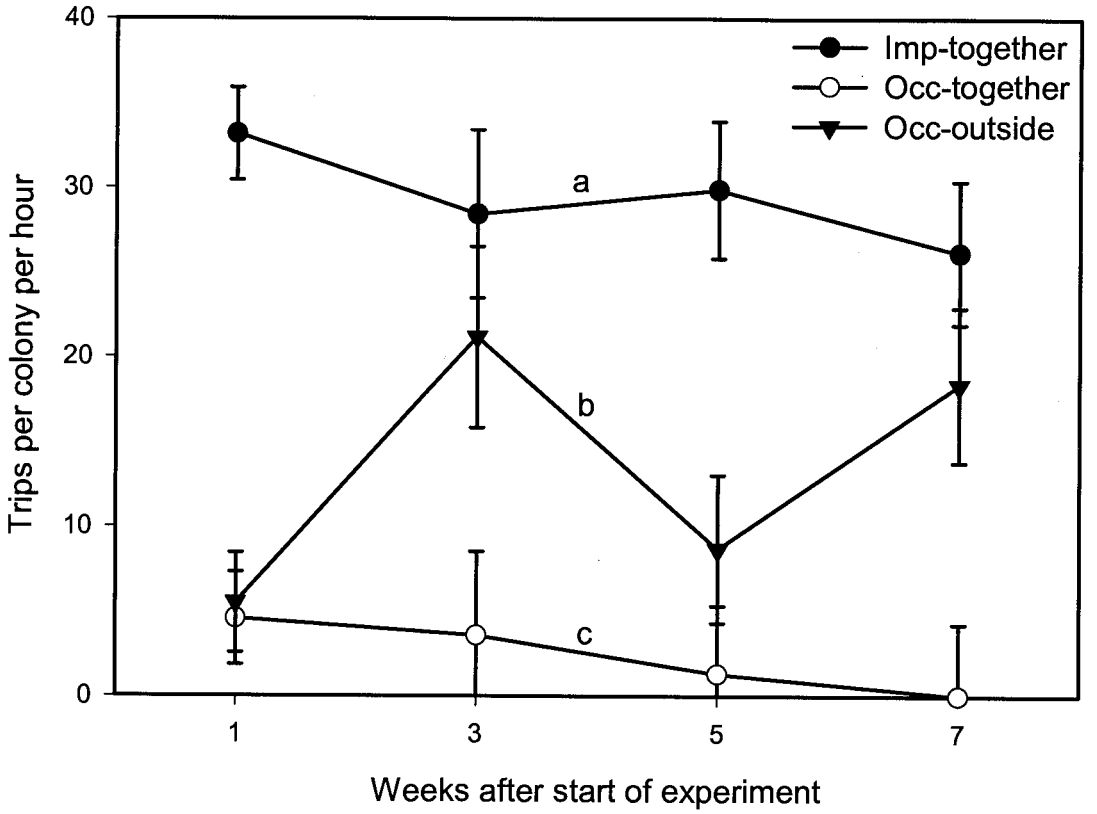

Figure 4.2. Effect of treatment and colony age on foraging effort. Treatments with the same letter are not significantly different ($P < 0.05$). Error bars = $\pm$ 1 SE.

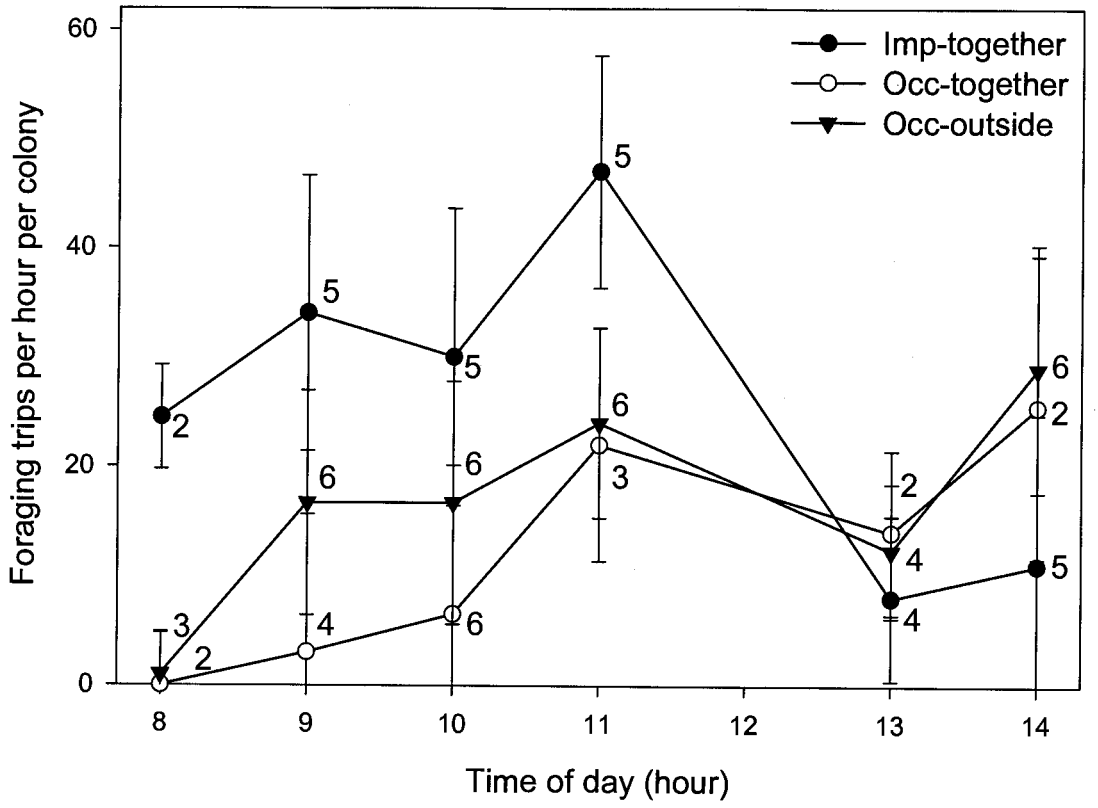

Figure 4.3. Effect of time of day and treatment on foraging effort. Numbers indicate sample size at each point in time. Error bars = $\pm$ 1 SE.

Chapter V

Effects of *Nosema bombi* and its treatment fumagillin in bumble bee (*Bombus occidentalis*) colonies

Introduction

Bumble bees are used extensively to pollinate greenhouse crops, and have joined honey bees, leaf-cutter bees, and mason bees as important managed agricultural pollinators in the last thirty years (Plowright and Jay 1966, van Heemert et al. 1990, Banda and Paxton 1991, Kevan et al. 1991, van Ravestijn and van der Sande 1991, Straver and Plowright 1991, Dogterom et al. 1998, Pressman et al. 1999). In the late 1990's, however, greenhouse growers in western Canada, the United States, and Mexico became concerned about the availability and quality of *Bombus occidentalis* Greene (Hymenoptera: Apidae) colonies. *Nosema bombi* (Microsporidia: Nosematidae) is a bumble bee parasite that has been recognized since early in the 20th century (Fantham and Porter 1914). Bumble bee suppliers suggested that this parasite was a factor in the late 1990's crash in production in rearing facilities, as well as in poor colony growth and premature colony death in commercial tomato (*Lycopersicon esculentum* Miller) greenhouses.

Nosema spp. are present in both honey and bumble bee colonies. *Nosema* spp. reproduces in the bee's mid-gut, and spores are expelled and transferred to a new host when bees defecate (Fantham and Porter 1914, Fries 1993, McIvor and Malone 1995). The disease is transmitted among bees through the ingestion of contaminated honey-comb (Bailey 1991), contaminated water sources, honey stores (Fries 1993) or from trophallaxis between bees (Webster 1993). High *N. apis* infections are observed in honey bee

colonies during winter when there is increased disease transfer among bees that are unable to leave the colony to defecate (Bailey 1991). The presence of *N. apis* is associated with high mortality of colonies during winter, poor spring build-up, and reduced honey yield (Fries 1993). *N. apis* is controlled by treating colonies with the antibiotic fumagillin dicyclohexylammonium, that also has anti-protozoal activity, via the fall feeding of sugar syrup (Woyke 1984, Furgala and Sugden 1985, Szabo and Heikel 1987, Webster 1994). Fumagillin treatments in the presence of *N. apis* result in an increase in brood production by 20%, and honey production by 19% (Woyke 1984), as well as a decreased spore load (Furgala and Sugden 1985, Szabo and Heikel 1987, Webster 1994) compared to untreated colonies.

When bumble bees are infected by *N. bombi*, their abdomens can become distended and paralyzed (MacFarlane et al. 1995), and infected workers often become sluggish and die early (Bailey 1991, Schmid-Hempel and Loosli 1998). *N. bombi* infection is correlated with an increased production of sexuals, particularly males (Imhoof and Schmid-Hempel 1999).

I examined the relationship between *N. bombi* intensity (the number of spores per bee) and colony population size and brood rearing, and tested fumagillin as a potential control for *N. bombi* in *B. occidentalis* colonies. I also examined how many *B. occidentalis* workers need to be sampled to obtain an precise estimate of *N. bombi* intensity in each colony. In honey bee colonies, *N. apis* is monitored by destructively sampling 25 or more bees from each colony (Cantwell 1970), but this is not a viable option with bumble bee colonies because each contains only 50 to 200 bees. As well, I collected frass samples from colonies as a potential alternative to whole bee sampling for determining the intensity of *N. bombi* infections.

Materials and Methods

Forty-nine *B. occidentalis* colonies, approximately 10 weeks old, were received from Biobest Canada Ltd. over a four-week period starting mid-May, 2002. Upon arrival, all colonies were assessed for colony population size by counting the number of bees (workers, queens, and drones) and amount of brood (number of eggs, larvae, and pupae). They were then randomly assigned to one of three treatments: Control (n = 17), 26 mg fumagillin/L (n = 16), or 52 mg fumagillin/L (n = 16).

The recommended fumagillin dose for honey bees is 26 mg fumagillin/L (Furgala and Sugden, 1985; Webster, 1994) and was used to determine if this dose also is appropriate for bumble bees. A 52 mg fumagillin/L treatment also was tested to determine if an increased dose would result in greater control of *N. bombi* and/or harm bumble bees. Treatments were applied to colonies via sugar-water (50:50 by weight) which was replaced weekly. The 26 mg fumagillin/L and 52 mg fumagillin/L treatment groups were prepared by dissolving 1.25 g and 2.5 g of Fumagilin-B® (Medivet Pharmaceuticals Ltd. High River, AB, Canada) powder in each liter of sugar-water solution because there is 21 mg of active fumagillin dicyclohexylammonium per gram of Fumagilin-B® powder. After initial assessments, sugar syrup reservoirs were filled, bee and frass samples were taken, and colonies were randomly placed into a 9.2 ha commercial tomato (*Lycopersicon esculentum* Miller) greenhouse in Ladner, British Columbia, Canada. Colonies were again assessed for their population size after they had been in the greenhouse for eight weeks.

Bees and frass were sampled to determine *N. bombi* infection intensity upon colony arrival at the greenhouse and then again after colonies had been in the greenhouse

for 10 weeks and were no longer considered to be viable pollination units. Five bees were sampled from each colony at each collection period, and the number of *N. bombi* spores in each bee was assessed. At the start of the experiment as much frass as possible was taken from below the hive (0.04 ± 0.03 g SD), and an average of 0.17g (± 0.08 g SD) of frass was taken from throughout the bottom of each colony at week 10. At 10 weeks, an additional 25 bees, for a total of 30, were removed from each of eight colonies to determine how many bees are needed to obtain accurate estimates of overall *N. bombi* intensity. Bee and frass samples were kept frozen until analyzed.

Each bee abdomen and frass sample was mixed to a uniform suspension using a mortar and pestle after a known volume of water (1 mL water per bee, 1 mL water per 0.05 g frass) was added. The number of *N. bombi* spores in each sample was determined by counting the number of spores in samples of the suspension with a hemacytometer (Spencer Bright-Line, American Optical Company, Buffalo, NY) and light microscopy (Cantwell 1970). *N. bombi* identification was verified by Norman Pieniazek at the Center for Disease Control and Prevention (Atlanta, GA) by sequencing the 16s small rRNA subunit.

The effect of treatment and colony age on colony size was examined using a repeated measures analysis of variance. The effect of treatment on *N. bombi* density (the number of spores per gram of frass) in frass, intensity in bees was examined by comparing the change in number of spores between weeks 0 and 8 in each treatment with a one-way analysis of variance. The average number of *N. bombi* spores from sub-samples of bees and frass within each colony was used, and colony was considered to be the experimental unit. The effect of treatment on colony mortality was examined with a Pearson chi-square test. The effect of *N. bombi* intensity on colony size was examined

with a simple linear regression using information from eight-week old colonies. The relationship between the number of *N. bombi* spores in frass and whole bees was examined with a simple linear regression. The accuracy of *N. bombi* estimates with different numbers of bees sampled was examined by observing the relationship between the standard error and mean *N. bombi* intensity using the equation (SD/SQRT(n))/mean. All data were analyzed using JMP 4.0.3 (SAS Institute 2000b).

Results

The intensity of *N. bombi* infections increased significantly over time in whole bee samples ($F_{1,46} = 5.99$, $P = 0.02$; Fig. 5.1) and there was a trend towards this increase in frass samples ($F_{1,46} = 3.88$, $P = 0.054$; Fig. 5.1). Colony populations also decreased significantly over time (workers $F_{1,46} = 47.75$, $P < 0.0001$; brood $F_{1,46} = 46.78$, $P < 0.0001$; Fig. 5.2). There was no effect of *N. bombi* on number of workers ($F_{2,46} = 0.04$, $P = 0.83$), amount of brood ($F_{2,46} = 0.47$, $P = 0.50$), or number of reproductives (queens, $F_{2,46} = 0.53$, $P = 0.59$; drones $F_{2,46} = 2.01$, $P = 0.15$) as measured from whole bee samples.

There was no effect of fumagillin on *N. bombi* infections when either whole bee ($F_{2,46} = 0.16$, $P = 0.85$) or frass ($F_{2,46} = 0.95$, $P = 0.39$) samples were analyzed (Fig. 5.1). Fumagillin also did not affect colony populations (workers $F_{2,46} = 0.19$, $P = 0.83$; brood $F_{2,46} = 0.45$, $P = 0.64$; Fig. 5.2; queens $F_{2,46} = 0.53$, $P = 0.59$; drones $F_{2,46} = 2.01$, $P = 0.15$). One control, three 26 mg fumagillin/L and three 52 mg fumagillin/L colonies were dead or had fewer than ten bees after being in the greenhouse for ten weeks. This difference in colony mortality was not significant ($\chi^2_{2,46} = 1.50$, $P = 0.47$)

There was a significant ($F_{1,94} = 5.07$, $P = 0.03$) but weakly correlated ($r^2 = 0.05$) relationship between *N. bombi* density in frass and intensity in bee samples (Fig. 5.3).

The standard error for the number of *N. bombi* spores found in each colony was large across all sample sizes, equal to the mean even with a sample size of 30 (Fig. 5.4). For a significant difference between means to be detected there must be more than a two-fold difference between the mean *N. bombi* intensities with a sample size of 30. The magnitude of difference between means necessary to detect a significant difference increased greatly as sample size decreased.

Discussion

The intensity of *N. bombi* infections were on average 6 X 10^6 spores/bee at the end of the experiment and were similar to *N. apis* intensities considered harmful in honey bee colonies (Szabo and Heikel 1987, Malone et al. 2001). This number of spores per bee also would be considered a heavy infection in bumble bees, rated as a level three on a four-point scale (Schmid-Hempel and Loosli 1998). However, I found no relationship between *N. bombi* intensity and colony adult population or brood rearing and conclude that the *N. bombi* infections experienced by these colonies were not detrimental. Negative effects of *N. bombi* may have emerged if colonies had been observed to the end of their lifetimes. Fumagillin did not affect queen and drone production, contrary to the findings of Imhoof and Schmid-Hempel (1999). Research investigating effects of *N. bombi* intensity on bumble bees is needed to determine the threshold above which *N. bombi* is detrimental to bumble bee health. However, based on our data *N. bombi* is not a causative factor for poor colony population growth in greenhouse *B. occidentalis* colonies.

Fumagillin did not decrease the *N. bombi* intensity in *B. occidentalis* colonies, as opposed to its effectiveness against *N. apis*. However, there is a large amount of drift of

bees from one colony to another in greenhouses (Birmingham pers. comm.), and this drift may have partially obscured any effects of fumagillin. Fumagillin did not affect colony attributes, although both brood and worker levels in fumagillin treatment groups were slightly lower than control colonies after being exposed for eight weeks. Webster (1994) also found a non-significant trend towards negative effects of fumagillin on brood rearing and queen survival in honey bee colonies.

The density of *N. bombi* in frass was correlated with the intensity of infection in bees, but the relationship was weak and so can not be relied upon as an accurate method for determining the intensity of colony *N. bombi* infections. There was large variation in number of *N. bombi* spores among bees within each colony. Therefore, only the presence of *N. bombi* can be determined unless a large proportion of bees in each colony is sampled. Bee or frass samples can be used to determine the presence or absence of *N. bombi.*

In conclusion, *N. bombi* had no detrimental effect on *B. occidentalis* colonies. Also, fumagillin was as not effective against *N. bombi* as it is against *N. apis*, but research in a non-drift setting would be useful to state this conclusively. Further research investigating the detrimental effects of more intense *N. bombi* infections on bumble bees, and the threshold number of *N. bombi* spores for these effects might be useful, but from my study I conclude that *N. bombi* is not a significant factor in the decline of bumble bee worker populations in greenhouses.

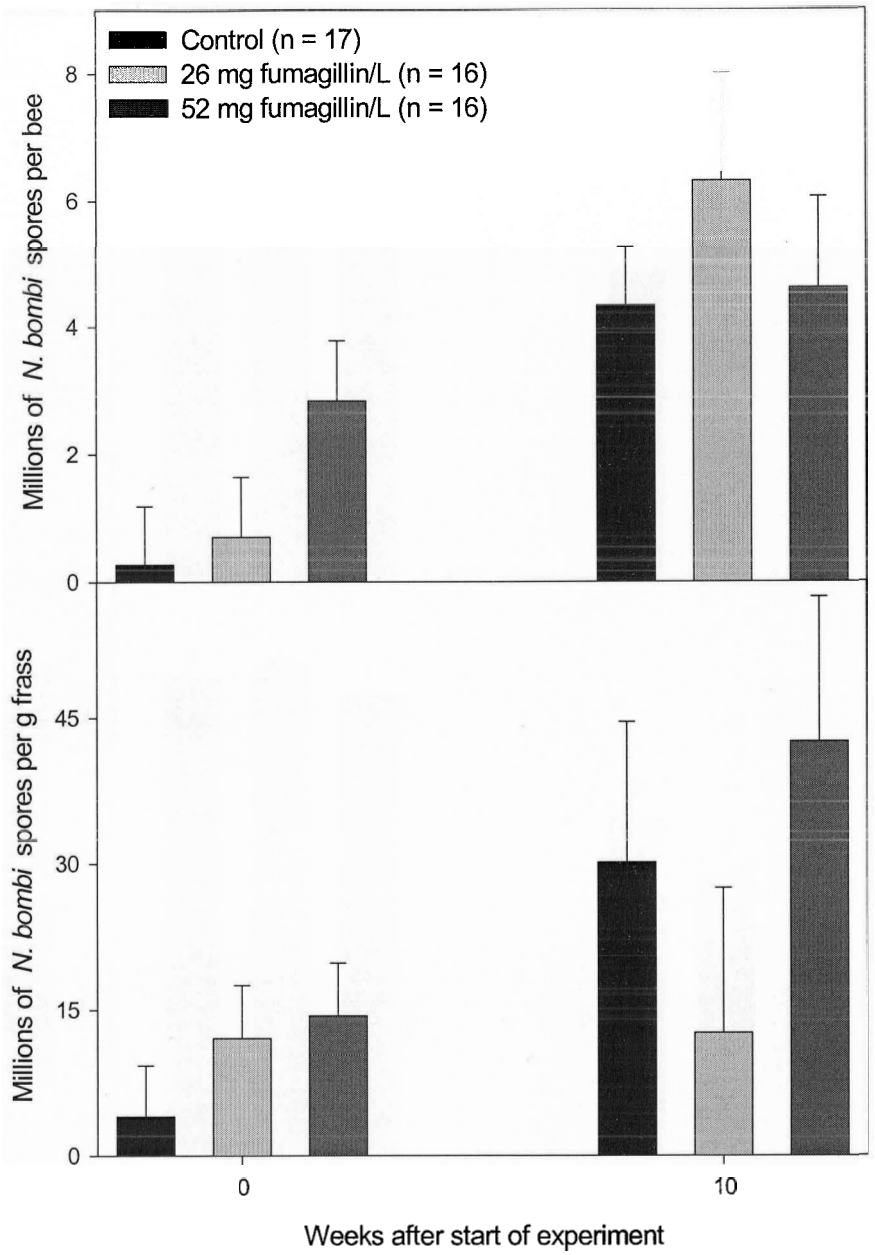

Figure 5.1. Effect of fumagillin on the intensity of *B. occidentalis* colony *Nosema bombi* infections over time as determined from bee and frass sampling. Error bars = $\pm$ 1 SE.

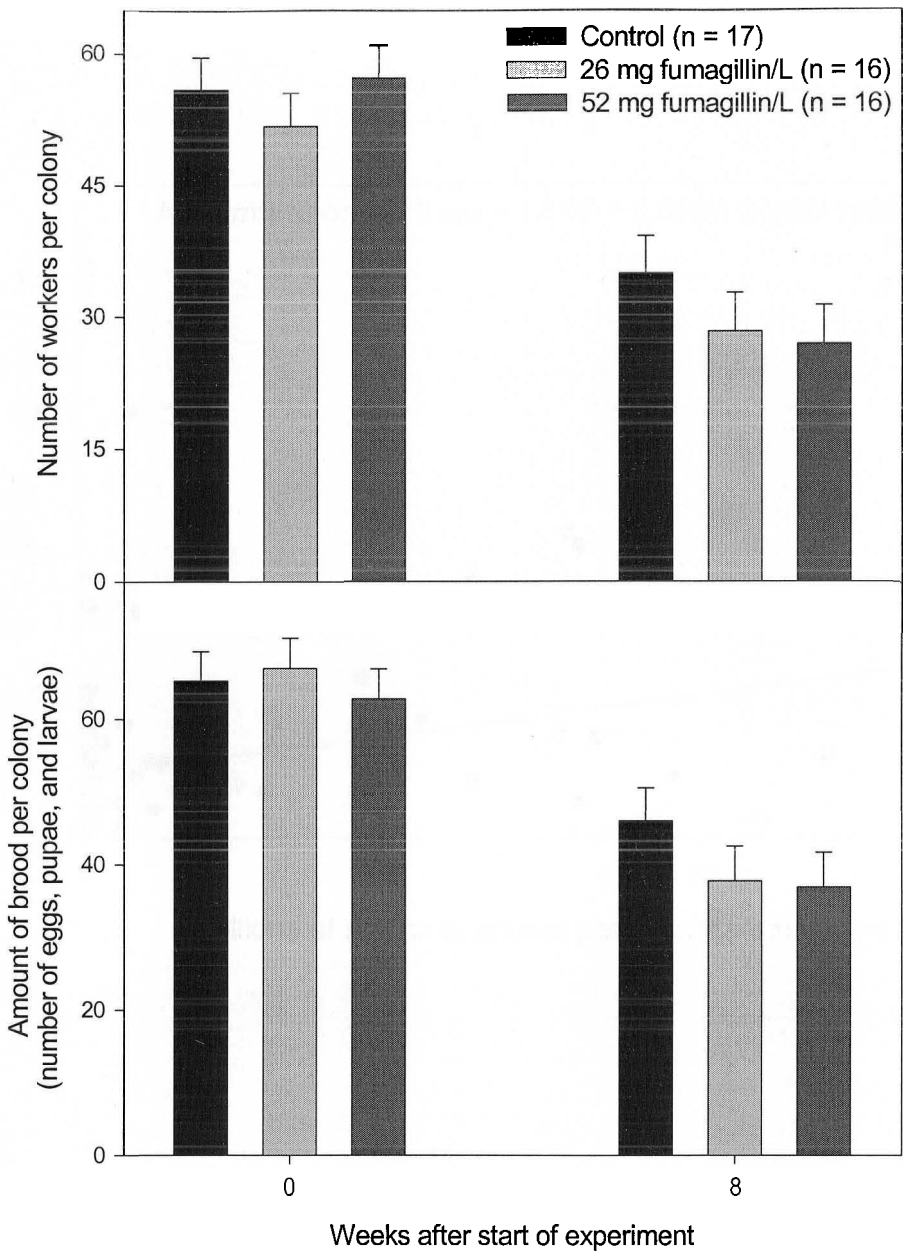

Figure 5.2. Effect of fumagillin on number of workers and amount of brood over time.

Error bars = $\pm$ 1 SE.

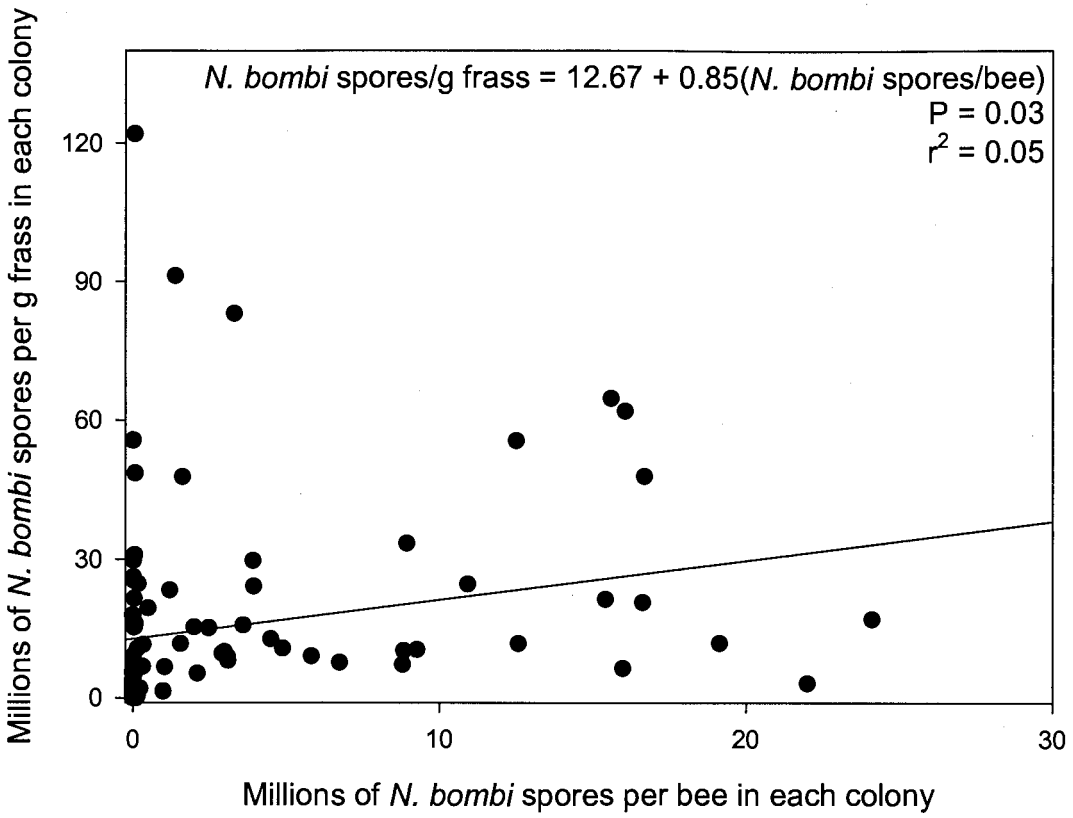

Figure 5.3. Relationship between *Nosema bombi* spore density in frass and the intensity of infections in bee samples.

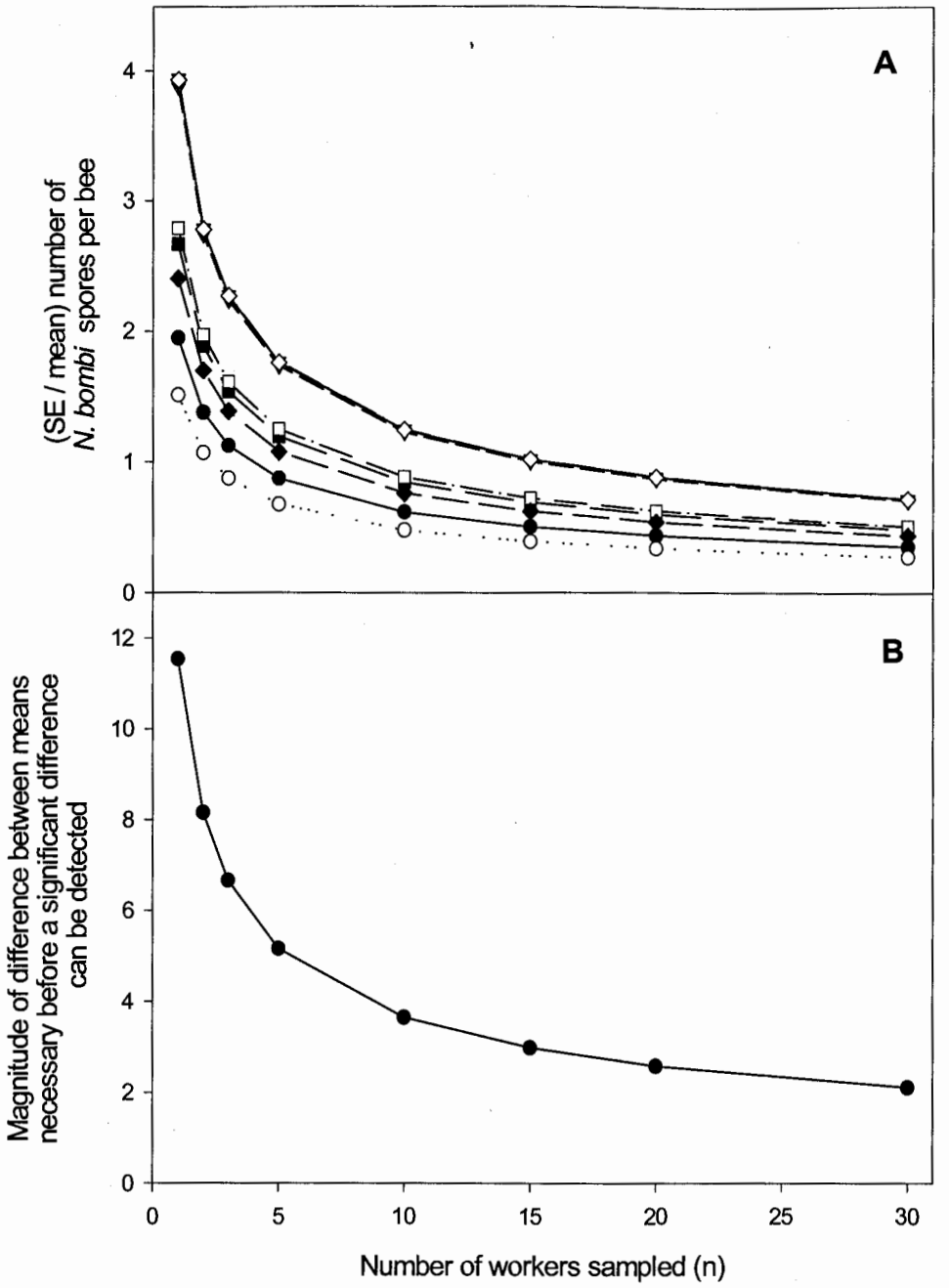

Figure 5.4. Relationship between standard error and the mean number of *Nosema bombi* spores (A), and the magnitude of difference necessary before a significant difference can be detected (B) as number of workers sampled changed for eight *Bombus occidentalis* colonies.

Chapter VI
General Conclusion

B. occidentalis colonies are obtaining adequate nutrition from greenhouse tomato flowers. However, colony populations do not grow while they are in the greenhouse but either maintain or decrease in size over time. There is more worker loss from greenhouse colonies than from completely enclosed colonies or from colonies outside the greenhouse. One reason for this high level of worker loss may be the inability of workers to orient themselves in the uniform greenhouse environment. The potential inability to orient properly may result in workers entering the wrong hive and dying from aggressive interactions with guard bees from other hives.

B. impatiens colonies grew larger and foraged more, but left colonies with pollen loads more frequently than *B. occidentalis*. My data also suggest that inter-specific competition with *B. impatiens* may negatively affect *B. occidentalis* colony growth. When deciding which bumble bee species to use, regulating agencies must weigh the direct economic cost of using the smaller native *B. occidentalis* colonies against the potential indirect environmental cost of accidentally releasing non-native *B. impatiens* into the environment.

B. occidentalis workers left the greenhouse to forage outside an average 14% of all pollen flights between January and September with peaks in foreign pollen collection in May and July. Greenhouse growers may be able to avoid a pollination shortfall in these months by screening their vents to prevent bumble bees from foraging outside of the greenhouse or by monitoring the bloom of plants outside the greenhouse and increasing bumble bee colony density in the greenhouse accordingly.

N. bombi did not affect bumble bee populations, and fumagillin did not affect levels of infection by *N. bombi*. However, further research in a controlled laboratory setting should be conducted before fumagillin is abandoned as a potential treatment. Frass sampling is a poor estimator of colony *N. bombi* intensity in adult workers, although this sampling method would still be useful for determining the prevalence of *N. bombi* in bumble bee colonies. Whole bee sampling shows the number of *N. bombi* spores among bees within each colony to be highly variable and an accurate estimate of *N. bombi* intensity can only be determined by sampling a large proportion of the colony.

In conclusion, bumble bee colony populations, in particular those of *B. occidentalis*, are not as large as they could be in tomato greenhouses. I have ruled out nutrition as a factor limiting colony growth, and demonstrated that the presence of *B. impatiens* may negatively affect *B. occidentalis* colony performance. As well, during summer a large proportion of adult workers forage outside of the greenhouse and hence greenhouse growers are losing part of the pollination force to outside forage. *N. bombi* was not the causative factor for poor colony population growth in greenhouse *B. occidentalis* colonies. However, further research investigating the effects of higher *N. bombi* intensities would be useful to determine the threshold above which *N. bombi* might be detrimental to bumble bee health. If *N. bombi* is found to be a severe bumble bee parasite, further research in a controlled laboratory setting investigating fumagillin as a potential treatment would be useful.

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