

AN ABSTRACT OF THE DISSERTATION OF

Kimberly M. Skyrn for the degree of Doctor of Philosophy in Entomology presented on April 12, 2011.

Title: Environmental Impacts on Native Bumble Bee Pollinators in an Agricultural Landscape of Western Oregon.

Abstract approved:

Sujaya Rao

Bumble bees provide vital pollination services in both native and agricultural landscapes. However, in recent years, bumble bee populations have experienced global population declines. The primary causes of these declines have been attributed to the environmental impacts of pathogens, pesticide use and habitat fragmentation. While research has examined the impacts of pathogens, there is limited information on the effects of pesticides and habitat fragmentation on native bumble bees. Hence, the objectives of my dissertation research were to: 1) assess the toxicological impacts of pesticides used in two important bee-pollinated crops on queens and workers; 2) determine the impacts of forage resource availability on bumble bee colonies; 3) examine pollen foraging behavior of bumble bees in a late season mass-flowering agricultural landscape; and 4) document observations on trends towards bivoltinism in three western North American bumble bees. This research was conducted in the lab using wild and lab reared colonies, and in an agricultural landscape in the Willamette Valley of Western Oregon.

In pesticide bioassays the impacts of residual toxicity of five classes of pesticides used in highbush blueberry and red clover cropping systems, were tested on queen and worker bumble bees, respectively. The results indicated variation in responses to the same insecticide by queens and workers, and to the same class of compounds by workers. Also, toxic effects were documented for both queens and workers to pesticides considered to be “safe” for bees.

The impacts of forage resource availability were evaluated by exposing bumble bee colonies to four quantities of pollen, four quantities of nectar and three feeding frequencies of pollen. The study documented an inverse relationship between larvae and workers to resource type and availability. In addition, the type of larval mortality displayed by colonies, larval ejection or within clump mortality, was dependent on worker mortality.

The individual and colony-level pollen foraging behavior of bumble bees was examined by placing colonies in red clover. Observations in the field on forager abundance, and at the colony-level on the duration and number of pollen trips and weight of stored pollen documented that red clover is an important resource for bumble bees. Pollen analysis revealed that in addition to red clover, Himalayan blackberry was also a key forage resource for bumble bees. Red clover resources at the end of the season may also benefit bumble bees by allowing for the creation of a second generation. Observations on both field and lab-reared queens document a trend towards bivoltinism in three species of western North American bumble bees.

Agricultural habitats are vital for sustaining bumble bee populations. However, given the potential for pesticide impacts and temporal availability of flowering plants, these landscapes must be managed to provide maximum benefit to bumble bees. Results from this research should assist growers and researchers in developing landscape management and production practices geared toward the conservation and enhancement of native *Bombus* spp. populations in western Oregon.

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Environmental Impacts on Native Bumble Bee Pollinators in an Agricultural
Landscape of Western Oregon

by
Kimberly M. Skyrn

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented April 12, 2011
Commencement June 2011

Doctor of Philosophy dissertation of Kimberly M. Skyrn presented on April 12, 2011.

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request:

Kimberly M. Skyrn, Author

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Sujaya Rao, for her endless support, guidance, and for always taking time to help me with anything. I would also like to extend my sincere thanks to Bill Stephen who has offered his time, energy and encouragement to me throughout this process. Together you both have inspired and mentored me over the past five years and I thank you for all your support and council. A special thanks to Glenn Fisher, Andy Moldenke and Ramesh Sagili for their time and help with experimental design, implementation, statistical advice, manuscript reviews, and technical support. I would also like to thank members of the Rao Lab for their feedback over the years. You all have inspired me to be become a better scientist.

I would like to acknowledge and thank Julie Kirby, Toni Taylor and the 2009 OSU REU group for their assistance in the lab rearing bumble bees and in the field collecting data. I am eternally grateful to my family (Momma, Dad, Ms. June, Danny and the kids) and friends (Angie, Nina, Kate, and Jess) for their constant support and for believing in me. Lastly, there are few fitting words to express my thanks to my partner for her love, patience, support, and encouragement that I could accomplish this task. You all continue to inspire me each and every day to be a better person.

I am also appreciative of John Kennel and Steve Hamilton who allowed me to use their red clover fields for this research. Lastly, I am grateful to the following funding agencies for supporting this research: Western Sustainable Agriculture Research and Education Program, OSU Agricultural Research Foundation, Oregon Blueberry Commission and the Oregon Clover Commission.

CONTRIBUTION OF AUTHORS

Dr. Sujaya Rao assisted with study design, data collection, analysis, and writing of research in Chapter II-V. Dr. William P. Stephen assisted with study design, data collection, and writing of research in Chapter III-V. Dr. Glenn C. Fisher provided chemicals and assisted with the study design, analysis and writing of Chapter II.

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Environmental Impacts on Native Bumble Bee Pollinators in an Agricultural
Landscape of Western Oregon

Chapter I

Introduction and literature review

Bumble bees, *Bombus* spp. (Hymenoptera: Apidae) are important generalist pollinators in both natural and agricultural landscapes (Corbet et al. 1991; Free 1993; Delaplane and Mayer 2000; Memmott et al. 2004). Given their polylectic behavior, bumble bee foragers visit a variety of plants in the landscape to obtain floral resources (Heinrich 1979; Eickwort and Ginsberg 1980), and thus they are considered to be suitable alternative pollinators for declining honey bee populations (Goulson et al. 2008). Over the last three decades, bumble bee populations have experienced global population declines due to environmental factors such as pathogens, pesticide exposure and habitat fragmentation (Fitzpatrick et al. 2007; Kosior et al. 2007; Colla and Packer 2008; Goulson et al. 2008; Grixti et al. 2009; Williams and Osborne 2009; Cameron et al. 2011).

Recent research has documented the occurrence of two intestinal protozoans, *Nosema bombi* Fantham and Porter and *Crithidia bombi* Lipa and Triggiani, in bumble bee workers (Whittington and Winston 2003; Colla et al. 2006; Otti and Schmid-Hempel 2008; Cameron et al. 2011) and the parasitic nematode, *Sphaerularia bombi* Dufour in queens (Maxfield-Taylor et al. 2011). In contrast, there has been very little research conducted on bumble bee responses to pesticides as precautions for pesticide use have been based primarily on studies with honey bees (Johansen 1977). Given their different life history traits, data from studies on honey bees are not applicable to bumble bees (Thomson 2001). The research that has been conducted with bumble bees, has either focused on the responses of workers from commercially available species (Mayer et al. 1994; Bortolotti et al. 2001; Besard et al. 2010; Gradish et al.

2010) or native bumble bee exposure to pesticides which are no longer registered for use in cropping systems (Way and Synge 1948; Stevenson and Racey 1966; Johansen 1972). With the exception of a single study by Wu et al. (2010) conducted in China, there is no other information about the impacts of pesticides currently in use on native bumble bees.

Besides potential negative impacts of pesticides in agricultural landscapes, habitat fragmentation resulting from the conversion of native to agricultural landscapes, is considered to have negative impacts on bumble bees due to loss of nesting sites and foraging resources. However, landscapes with mass-flowering bee-pollinated crops can provide abundant floral resources for bumble bee populations. Previous research that has evaluated the effects of these landscapes has focused only on colonies of the commercially available bumble bee, *B. terrestris*, in one early-blooming mass-flowering crop; *Brassica napus* L. (Goulson et al. 2002; Carvell et al. 2008; Westphal et al. 2009) and one late-blooming mass-flowering crop; *Phacelia tanacetifolia* Benth (Westphal et al. 2006). Given the annual life cycle of bumble bees in temperate regions (Alford 1975), one agricultural crop is not adequate for providing the continuous supply of floral resources needed for bumble bee colony development.

The objectives of this research were to: 1) assess the toxicological impacts of pesticides used in two important bee-pollinated crops on queens and workers; 2) determine the impacts of forage resource availability on bumble bee colonies; 3) examine pollen foraging behavior of bumble bees in a late season mass-flowering

agricultural landscape; and 4) document observations on trends towards bivoltinism in three western North American bumble bees.

The Willamette Valley of Western Oregon provides an ideal setting for these studies. This area is dominated by agriculture given the production of over 200 crops, many of which are bee-pollinated. Given this, there is a diverse bumble bee fauna whose life cycles are synchronized with the bloom periods of many crops grown in the region (Rao and Stephen 2010). Of these, highbush blueberry (*Vaccinium corymbosum* L., Ericaceae) and red clover (*Trifolium pratense* L., Fabaceae), are considered to be two important crops for bumble bees because their bloom coincides with two critical growth periods in bumble bee colonies (Rao and Stephen 2010). Given its three-four week bloom period in May, highbush blueberry provides critical floral resources during early spring for spring-emerged queens that are initiating colonies. Red clover bloom extends over six weeks during July and August providing food resources for colonies during the reproductive phase of development when males and new queens are produced. In this region, seven *Bombus* spp. thrive but *B. vosnesenskii* Radoszkowski is dominant (Stephen 1957; Rao and Stephen 2010), hence it was the focus of my studies. The introduction of non-native bumble bees into Oregon is prohibited (http://www.oregon.gov/ODA/PLANT/IPPM/appr_insects.shtml), so studies were conducted using either wild caught or locally reared bumble bee colonies.

Chapter 2 of this dissertation relates to studies focused on determining the impacts of residual toxicity of five classes of pesticides used in highbush blueberry

and red clover production on queen and worker bumble bees, respectively. The research documented the mortality of bees exposed to treated plant material with field-rates of pesticides using lab bioassays. These data showed variation in responses to the same insecticide by queens and workers, and to the same class of compounds by workers. Also, pesticides considered to be “safe” for bees were documented to be toxic to both queens and workers. This chapter highlights the importance of considering the responses of native bumble bees to pesticides applied on crops that provide floral resources to bees.

Chapter 3 describes studies that examined the effects of pollen and nectar resource availability on larvae and workers, the most important life stages in the nest. In separate lab experiments, the mortality of larvae and workers in bumble bee colonies provided with four quantities of pollen, four quantities of nectar and three feeding frequencies of pollen were evaluated. The study documents an inverse relationship between larvae and workers to resource type and availability. In addition, the type of larval mortality displayed by colonies, larval ejection or within clump mortality, was dependent on worker mortality. This chapter includes a discussion on differences in the colony-level response to food resources and the relationship between larval and worker mortality.

Chapter 4 examines the pollen foraging behavior of both individuals and colonies of bumble bees in the late-season mass-flowering agricultural landscape of red clover. In this study, fields were surveyed over two years to determine the abundance of foragers on flowers and the foraging activity of colonies placed adjacent

to a field. Pollen samples collected from colonies were analyzed quantitatively and qualitatively to determine floral resource utilization. The results indicate that red clover serves as an important forage resource for bumble bee colonies during the period when reproductives are produced. This chapter gives suggestions for growers interested in providing landscapes with continuous, abundant floral resources for bumble bees. Chapter 5 describes observations made in the field and lab on the trends towards bivoltinism displayed by three species of western North American bumble bees. In Chapter 6, the major findings from all studies are summarized in context of the current literature and potential areas for future research are outlined.

Chapter II

Toxicity of spring and summer crop pesticide residues to queen and worker bumble bees

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In preparation for submission to *Journal of Economic Entomology*
Entomological Society of America, Lanham, MD

Abstract

Pesticide label precautions protecting pollinators are traditionally based on studies with honey bees which differ in many ways from bumble bees. We evaluated the toxicity of pesticide residues from five classes of chemicals registered for use in the temperate spring crop, blueberry, and the summer crop, red clover, in which queens and workers, respectively, forage for food resources. Pesticides were evaluated at the minimum, maximum, and 2x maximum field rates and at 24, 48 and 72 hour exposure periods. With the reduced-risk neonicotinoid, imidacloprid, queen mortality was only significant at the highest dose tested compared with the control after 72 hours of exposure ($P = 0.04$). The fungicide, pyraclostrobin/boscalid, did not cause significant mortality over the control, despite 50% mortality in queens after 24 hours of exposure to twice maximum dose ($P = 0.01$). With the reduced-risk insecticide, spinosad, we observed a reverse dose response since queen mortality was significant at the minimum dose compared with the control at 72 hours ($P < 0.01$). In studies with workers, the organophosphate chlorpyrifos and the pyrethroid bifenthrin caused significant worker mortality over the controls at all doses and exposure periods ($P < 0.01$). However, no significant mortality was observed with imidacloprid ($P > 0.01$) while the organophosphate oxydemeton-methyl appeared to be repellent. These results highlight variation in responses of queens and workers to pesticides belonging to different classes, underscoring the need for caution when pesticides are applied to crops that provide foraging resources for bumble bees. Even reduced risk insecticides

and fungicides which are believed to be relatively harmless, can have negative impacts, and these need to be considered when decisions are made for pesticide applications.

Introduction

Bumble bees, *Bombus* spp. (Hymenoptera: Apidae) are important pollinators in natural and agricultural ecosystems. They are considered to be more efficient pollinators than the European honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), in some cropping systems and during rainy, windy and cool climatic conditions (Delaplane and Mayer 2000). However, bumble bee populations are experiencing global declines due to habitat fragmentation, diseases and exposure to pesticides (Goulson et al. 2008; Cameron et al. 2011). The impacts of land use changes (McFredrick and LeBuhn 2005; Greenleaf and Kremen 2006; Kremen et al. 2007), and pathogens (Whittington and Winston 2003; Colla et al. 2006; Otte and Schmid-Hempel 2008) have been examined but there is limited information about effects of pesticides on native bumble bee populations. This is critical since bumble bees forage for pollen and nectar resources in cropping systems which are still largely produced conventionally with pesticides. Precautions for pesticide use are based primarily on studies with honey bees (Johansen 1977). However, due to differences in life histories and foraging behaviors, data generated from these studies are not adequate for assessing toxicity of pesticides to native bumble bees (Thompson 2001). In addition, native bumble bees often nest in the ground and their nests are a challenge to locate.

Hence, unlike honey bees and commercial bumble bees, they cannot be relocated from the field prior to application of pesticides to a crop (Thompson 2001).

Bumble bee life cycles can extend over 3-5 months (Alford 1975), and thus a single colony may use foraging resources from multiple crops, and be exposed to a diversity of pesticides. In temperate regions, queens are exposed to pesticides in spring-blooming crops, while workers are at risk to those applied to late spring through summer crops. Typically, pesticides are applied during early morning and late afternoon hours to reduce impacts on honey bees (Johansen 1977), but these can be peak foraging times for bumble bees (Corbet et al. 1993). Also, between 15% (Free 1955) and 50% (K. Skyrn, personal observation) of bumble bee foragers remain on crop foliage during the night rather than return to their nests at the end of the day. Given their small colony size when compared to honey bees, any loss suffered by the worker population can severely impact bumble bee colony growth and reproduction (Heinrich 1979; Thompson 2001).

Due to the difficulty of obtaining adequate number of individuals, commercial colonies are often used for pesticide toxicity studies on bumble bees (Mayer et al. 1994; Bortolotti et al. 2001; Besard et al. 2010; Gradish et al. 2010). These studies have assessed LD₅₀ and LC₅₀ values of insecticides in the laboratory or on greenhouse crops. Traditionally, studies conducted with wild bumble bees have included exposure to several organophosphates, carbamates, and organochlorine chemicals, which were observed to be toxic to queens (Way and Synge 1948; Stevenson and Racey 1966) and workers (Way and Synge 1948; Stevenson and Racey 1966; Johansen 1972).

However, due to environmental concerns, many of the insecticides tested are no longer registered for use, and have been replaced with products with different chemistries. Only a single study conducted in China has evaluated the impacts of two modern pesticides, a neonicotinoid and a demethylation-inhibiting fungicide, on wild worker bumble bees (Wu et al. 2010). Hence, the objective of this study was to evaluate the impacts of residues from additional pesticides that are currently registered for use in agricultural systems. Crops selected for the study included blueberry (*Vaccinium corymbosum* L., Ericaceae) and red clover (*Trifolium pratense* L., Fabaceae) produced for seed which bloom in synchrony with foraging by queens and worker bumble bees, respectively, in Oregon on the west coast of the USA where the study was conducted.

Materials and methods

Plant material

Highbush blueberry plant material was collected from an unsprayed plot at the North Willamette Research and Extension Center (NWREC) of Oregon State University (OSU) in Aurora, OR. Red clover plant material was obtained from an unsprayed commercial field in Monmouth, OR. The upper 15 cm of material (flowers, stems and leaves) from both plants was removed and used in bioassays on the day of collection.

Test bees

The study was conducted with the native bumble bee, *Bombus vosnesenskii* Radoszkowski (Hymenoptera: Apidae), which is the dominant bumble bee species in western Oregon (Rao and Stephen 2010). Queens were collected from unsprayed blueberry and crimson clover plants in May 2009 at NWREC and the OSU Hyslop Experimental Research Farm in Corvallis, OR. Worker bumble bees were collected in July 2009 from unsprayed fields of red clover. The bees were placed in plastic vials, cooled, and transported to the lab. Both queen and worker bees were exposed to test materials on the day of collection.

Pesticides

The following six pesticides registered for foliar application around blueberry and red clover bloom periods were evaluated.

Pesticides applied to blueberry plant material:

1. imidacloprid (Admire® 2 (21.4% a.i.), Bayer CropScience Inc., Research Triangle Park, NC) – neonicotinoid insecticide registered for suppression of aphid pests (DeFrancesco and Bell 2008).

2. spinosad (Success® (22.8% a.i.), Dow AgroSciences, LLC, Indianapolis, IN) – spinosyn registered for management of lepidopteran pests (DeFrancesco and Bell 2008).
3. pyraclostrobin/boscalid (Pristine® (12.8/25.2% a.i.), BASF Corporation, Florham Park, NJ) – fungicide registered for management of botrytis grey mold (*Botrytis cinerea*) and Anthracnose fruit rot (*Colletotrichum* spp.) (Pscheidt 2008).

Pesticides applied to red clover plant material:

1. oxydemeton-methyl (Metasystox-R® Spray Concentrate (25.5% a.i.), Gowan Co. Yuma, AZ) – organophosphate registered for aphid management (Fisher and Dreves 2008).
2. chlorpyrifos (Lorsban® Advanced (40.18% a.i.), Dow AgroSciences LLC, Indianapolis, IN) – organophosphate with Special Local Need (SLN) labeling for suppression of aphids (Fisher and Dreves 2008).
3. bifenthrin (Brigade® 2EC (25.1% a.i.), FMC Corporation, Philadelphia, PA) – pyrethroid with SLN labeling for aphid management (Fisher and Dreves 2008).
4. imidacloprid (Admire® 2 (21.4% a.i.), Bayer CropScience Inc., Research Triangle Park, NC) – not registered for crops grown for seed. However, it is applied during red clover bloom periods to blueberry fields in the region for management of the new spotted wing Drosophila pest (Dreves et al. 2009).

Experimental setup

Pesticide residues were generated using a Potter Precision Laboratory Spray Tower (Burkard Manufacturing Co. Ltd, Rickmansworth, UK) (Potter, 1952) calibrated to deposit spray quantities of $2.0 \pm 0.2 \text{ mg/cm}^2$ per 1ml water at 6.8 psi with a 0.275 atomizer. In pesticide bioassays with bumble bees, typically, twice the maximum recommended field rate is used for evaluating the highest potential toxicity (Gretenkord and Drescher 1993). Hence, treatments in the current study consisted of an untreated control, and three pesticide rates: the minimum, maximum and twice the maximum rate approved for field application of each chemical in each crop. For each pesticide, 2 ml was applied in each cage, the equivalent of 100 gallons per acre. After pesticide application, residues were allowed to dry on plant material for a period of 1-2 hours prior to use in the experiments.

Cylindrical cages, modified from Johansen et al. (1983), were used in all bioassays (Figure 2.1). Each cage consisted of a 15.00 cm plastic petri dish top and bottom surrounded with a 45.70 cm x 5.10 cm strip of metal screen (6.70 meshes per cm) that formed a circular insert to provide ample room for bumble bees to fly and defecate. Plant material was condensed to 6.50 cm x 12.50 cm, and 15.50 g were distributed evenly within cages.

Bumble bees were chilled in a refrigerator at 4°C for 20-30 min to facilitate handling, and then randomly assigned to treatments. Each cage received either two queens (blueberry study) or four workers (red clover study). Bees were provided

nectar (Bee Happy, Koppert Biological Systems, Romulus, MI) using a cotton wick feeder attached to the bottom of each cage (Johansen et al. 1983). During the experiment, bees were kept under controlled environmental conditions of humidity (50-60%), temperature ($28^{\circ}\text{C} \pm 2^{\circ}\text{C}$) and photoperiod (D7:L17). Mortality was assessed after 24, 48 and 72 hours by recording the number of dead bees within each cage. The experiment was set up as a randomized block design with 6 replications. Queens and workers that were alive after 72 hours were released at their respective collection sites.

Data analysis

The data were subjected to an analysis of variance (ANOVA) using S-Plus version 8.0 (Mathsoft Inc, Seattle, WA). To meet the assumptions of normal variance, all values were arcsine, square root transformed prior to analysis (Sokal and Rohlf 1981). In separate analyses, the mean percentage of mortality at durations of 24 hrs, 48 hrs and 72 hrs after exposure was used as the response variable, and treatment (untreated control, minimum, maximum and 2X maximum) was the explanatory variable. When significant differences were present, treatment comparisons were made using Tukey's multiple means comparison. All tests were performed at a significance level of $\alpha = 0.05$.

Results

Mortality of queen bumble bees exposed to pesticide residues on blueberry plant material

Responses of queen bumble bees varied based on pesticide, rate and period of exposure (Table 2.1).

Imidacloprid: There were no significant differences in queen mortality among the four treatments 24 hours after exposure ($P = 0.50$). By 48 hours, over twice as many queens were dead compared to the control, but the difference was not statistically significant ($P = 0.30$). However, by 72 hours, queen mortality was three times greater at the 2x maximum rate compared with the control, and this difference was statistically significant ($P = 0.04$).

Spinosad: There was no statistical difference in mortality among the treatments 24 hours after exposure ($P = 0.77$). However, at both 48 and 72 hours after exposure, queen mortality was highest at the minimum dose compared with the other doses and the control but the difference in mortality was significant only at 72 hours ($P < 0.01$).

Pyraclostrobin/boscalid: There were no significant differences in queen mortality over the control, despite that over six times as many queens were killed with the 2x maximum dose after 24 hours. However, this mortality was statistically significant when compared with the minimum and maximum dose ($P = 0.01$).

Similarly, there were no significant differences in mortality of the queens at the three rates tested as well as the control, when examined 48 or 72 hours after initial exposure to pesticide residues ($P = 0.09$; $P = 0.26$).

Mortality of worker bumble bees exposed to pesticide residues on red clover plant material

Responses of worker bumble bees ranged from non-toxic to highly toxic effects, depending on the chemical (Table 2.2).

Imidacloprid: There were no significant differences in mortality of worker bumble bees exposed to the three rates compared with control 24, 48 or 72 hours after exposure ($P = 0.18$; $P = 0.64$; $P = 0.11$, respectively).

Oxydemeton-methyl: As with the imidacloprid, there were no significant differences in mortality of worker bumble bees among the three rates and the untreated control 24, 48 or 72 hours after exposure ($P = 0.09$; $P = 0.84$; $P = 0.53$, respectively).

Chlorpyrifos: Mortality was three to four times greater and statistically significant at all rates and exposure periods compared with the untreated control ($P < 0.01$, for all three exposure times).

Bifenthrin: At all three doses and exposure periods, mortality was significantly greater compared with the control ($P < 0.01$, for all three exposure periods). No bees were killed in the control 24 hours after the experiment was set up, while over 87% were killed at the three rates of bifenthrin.

Given the high control mortalities observed in this experiment, a separate bioassay of worker exposure to the untreated control treatment was conducted. Mean worker mortality increased from 16.66 to 39.58% after 72 hours of exposure which is similar to observations made in untreated controls in the pesticide bioassays during which 0 to 37.50% worker mortality was recorded after 72 hours of exposure across all chemicals. These results highlight the susceptibility of wild bumble bees to laboratory conditions. Queens are likely to respond in the same manner. However, due to their low abundance in the area after the initial bioassays, a similar study was not conducted with queens.

Discussion

This study documented the impacts to wild bumble bees of insecticides belonging to five classes of compounds. It also highlighted the importance of evaluating impacts of fungicides, a group of pesticides that are typically not included in pollinator toxicity studies despite their extensive use in agriculture. The study is the first to document impacts of pesticides that both queens and workers are exposed to during the life cycle of the colony. With the exception of Wu et al. (2010), it is also the only other study documenting toxicity to wild bumble bees of pesticides registered for use in the twenty-first century. Earlier studies with commercially available species only provide information on impacts on workers (Mayer et al. 1994; Bortolotti et al. 2001; Besard et al. 2010; Gradish et al. 2010). Impacts on queens were documented in

two studies conducted with wild bees (Way and Synge 1948; Stevenson and Racey 1966). The single study (Johansen 1972) that evaluated pesticide toxicity to a North American bumble bee species was conducted prior to advances in pesticide chemistry that led to registration of the diverse compounds currently used in crop production including reduced-risk insecticides. The variation in toxicity of pesticides to queens and workers observed in this study underscores the importance of evaluating impacts on bumble bees of chemicals currently used in crop production.

Since the passage of the FQPA (Food Quality Protection Act) in the United States, organophosphates have been under review by EPA due to their harmful impacts on humans, especially children (Van Steenwyk and Zalom 2005). However, several are still registered for crop use. In the current study, variation was observed in responses of worker bumble bees to residues of organophosphates on red clover. Oxydemeton-methyl did not produce negative effects whereas exposure to chlorpyrifos resulted in 83-100% mortality with all doses tested after 24 hours after exposure. Gretenkord and Drescher (1993) found oxydemeton-methyl to be highly toxic to *B. terrestris* workers; oral LD₅₀ value was 0.75 µg a.i. per bee after 7 days of exposure. Wang et al. (2003) observed > 90% mortality for *B. terrestris* workers after 48 hours of exposure to chlorpyrifos. Both chlorpyrifos and oxydemeton-methyl are considered highly toxic to honey bee workers (Stevenson 1978; Suchail et al. 2000). From the current study, it was not possible to determine the toxicity of oxydemeton-methyl as workers remained at the top of the cages and thus were not in contact with the treated plant material located at the bottom of the cage. This behavior was not

observed in the untreated control cages suggesting that oxydemeton-methyl may be repellent to *B. vosnesenskii* workers which could account for the difference in mortality between the two organophosphates evaluated in the study.

Pyrethroids are one of the fastest developing groups of modern insecticides due to their broad spectrum use, quick knockdown ability and high toxicity at low doses (Inglesfield 1989). However, impacts on beneficial insects such as natural enemies have been mixed (Hull and Starner 1983; Theiling and Croft 1988). Our study highlighted the rapid knockdown of bumble bees to bifenthrin which killed over 87% of workers after 24 hours of exposure to the lowest dose tested. Bifenthrin caused 100% mortality in *B. terrestris* in studies by Bortolotti et al. (2001) and Besard et al. (2010) and was also found to be highly toxic to honey bee workers in a study by Estes et al. (1992) which highlight caution when bifenthrin is applied to crops used as foraging sources by bees.

The reduced risk insecticide imidacloprid has been shown to be moderately to highly toxic causing greater than 50% mortality of *B. terrestris* and *B. impatiens* workers (Bortolotti et al. 2001; Sterk et al. 2002; Scott-Dupree et al. 2009; Gradish et al. 2010). In the current study, we observed a difference in susceptibility of queens and workers to its residues on blueberry and red clover plant material, respectively. On average, over 75% mortality was observed in queens 72 hours after exposure but due to high control mortality, the difference was only significant at the 2X maximum dose. In contrast, none of the rates caused significantly greater mortality of worker bumble bees over the control though these results approached significance for the 72

hour exposure periods. Spring foraging queens are larger in size than workers, but typically older in age and it is possible that older bees are more susceptible to imidacloprid than young bees (Tasei 2002). However, imidacloprid has been shown to be highly toxic to honey bee workers (Suchail et al. 2000) and this has resulted in government restrictions to its use in France (Schmuck et al. 2001; Bonmatin et al. 2005) and Germany (BVL).

The second reduced risk insecticide included in our study, spinosad, is also considered to be toxic, causing greater than 75% mortality in workers of *B. terrestris* (Sterk et al. 2002). In our study, we observed a reverse dose response in queens after both 48 and 72 hours of exposure. One possible explanation for these unexpected results is the potential enhancement of detoxification mechanisms in the bees at the higher doses as suggested by Suchail et al. (2000) for similar observations made in a study on honey bee exposure to imidacloprid. The higher mortality at low doses compared to high doses is believed to be due to a trigger of detoxifying enzymes which increases the metabolism of pesticides, reducing mortality at high doses. Contrary to these results, spinosad is considered to be relatively non-toxic to honey bees (Mayes et al. 2003).

There has been limited attention given to examination of fungicide impacts on bumble bees which could be due to their low toxicity to honey bees (Mayer and Lunden 1986; Johansen et al. 1983; Ladurner et al. 2005). However, Wang et al. (2003) and Wu et al. (2010) observed >40% mortality after 7 to 14 days of exposure to dithiocarbamate, dicarboximide, guanidine, and demethylation inhibitors while studies

by Bortolotti et al. (2001) and Gradish et al. (2010) found <20% toxicity to dicarboximide, phenyl amide, anilinopyrimidine, phenylpyrrole, and potassium bicarbonate fungicides. These studies were conducted with workers exposed to fungicide residues in the laboratory and on greenhouse crops, and there is no data on fungicide effects of queen bumble bees. In our study, 50% mortality of queens resulted from exposure to the 2X maximum dose within 24 hours of treatment. This indicates a rapid knockdown as mortality rates stayed relatively constant during the other exposure periods. However, these values were not different from the untreated control given the high mortality of queens, elucidating the negative effects of laboratory conditions used in pesticide bioassays on wild bumble bees. Despite this, these results highlight the difference in responses of honey bees and bumble bees to pesticides, and underscore the need for toxicity studies conducted on bumble bees prior to registration of new compounds.

Most pesticide studies with bumble bees are conducted with workers due to concerns related to removing queens from wild populations. Information related to workers may be more critical as they spend a lot more time than queens foraging on diverse crops. However, information is needed for impacts of pesticides on queens as negative impacts can be of considerable value as their mortality directly affects colony development and population size. Also, pesticides that queens are exposed to in cropping systems may differ from those of workers due to the difference in periods when they forage. The larger size of queens may enable them to be less susceptible to pesticides. Stevenson and Racey (1966) showed that queens of *B. lucorum* and *B.*

agrorum could tolerate 2 to 10 times the pesticide dose (μg per bee) of organophosphate insecticides when compared to workers and males. Similarly, Way and Synge (1948) found that *B. terrestris* queens were 2 to 5 times less susceptible to pesticides when compared to workers and males. However, as speculated earlier, due to their older age, queen mortality could be higher than worker mortality as was observed in the current study with imidacloprid residues.

Pesticides are still the dominant tactic for pest management in agricultural crops. Producers are aware of potential toxic impacts on pollinators and pesticide applications are avoided during crop bloom. However, there is variation in bloom time across a landscape based on cultivar and production practices. In addition, pesticides applied pre- or post-bloom can drift to neighboring crops in bloom or weeds around fields, and these could have negative impacts on bees foraging on flowers. Despite the considerable amount of data available for honey bee exposure, impacts on native bumble bees also need to be taken into consideration in establishment of pesticide label precautions and restrictions. Even reduced-risk insecticides and fungicides which are believed to be relatively harmless can have negative impacts, and these need to be considered when decisions are made on application of pesticides.

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Figure 2.1. The cylindrical cage design that was used in the pesticide bioassays of *Bombus vosnesenskii* queens and workers.

Table 2.1. Mean (%) mortality of queen *Bombus vosnesenskii* after exposure to pesticide-treated blueberry plant material.

Pesticide / Time of exposure	Mean % mortality (\pm SEM)				Analysis <i>F</i> , <i>P</i> *
	Pesticide concentration ^a				
	untreated control	minimum	maximum	2X maximum	
imidacloprid					
24 hrs	0.00 \pm 0.00a	8.33 \pm 8.33a	16.67 \pm 10.54a	16.67 \pm 10.54a	0.82, 0.50
48 hrs	25.00 \pm 11.18a	66.67 \pm 16.67a	66.67 \pm 16.67a	58.33 \pm 15.37a	1.33, 0.30
72 hrs	25.00 \pm 11.18a	75.00 \pm 11.18ab	75.00 \pm 17.08ab	83.33 \pm 10.54b	3.59, 0.04
spinosad					
24 hrs	0.00 \pm 0.00a	8.33 \pm 8.33a	8.33 \pm 8.33a	8.33 \pm 8.33a	0.38, 0.77
48 hrs	8.33 \pm 8.33a	50.00 \pm 18.26a	25.00 \pm 17.08a	16.67 \pm 10.54a	1.89, 0.17
72 hrs	16.67 \pm 10.54a	75.00 \pm 17.08b	41.67 \pm 15.37ab	16.67 \pm 10.54a	7.86, <0.01

Table 2.1. Mean (%) mortality of queen *Bombus vosnesenskii* after exposure to pesticide-treated blueberry plant material.

Pesticide / Time of exposure	Mean % mortality (\pm SEM)				Analysis <i>F</i> , <i>P</i> *
	Pesticide concentration ^a				
	untreated control	minimum	maximum	2X maximum	
pyraclostrobin/boscalid					
24 hrs	8.33 \pm 8.33ab	0.00 \pm 0.00a	0.00 \pm 0.00a	50.00 \pm 18.26b	5.00, 0.01
48 hrs	8.33 \pm 8.33a	8.33 \pm 8.33a	8.33 \pm 8.33a	50.00 \pm 18.26a	2.55, 0.09
72 hrs	33.33 \pm 16.67a	16.67 \pm 10.54a	16.67 \pm 10.54a	58.33 \pm 20.07a	1.48, 0.26

^aThe minimum, maximum and 2X maximum field dose applied (mg/ml):

imidacloprid: (0.36), (0.48), (0.96)

spinosad: (0.37), (1.20), (2.40)

pyraclostrobin/boscalid: (13.96), (17.36), (34.72)

*Values within each horizontal row followed by different letters are significantly different (Tukey multiple means comparison) at $\alpha = 0.05$.

Table 2.2. Mean (%) mortality of worker *Bombus vosnesenskii* after exposure to pesticide-treated red clover plant material.

Pesticide / Time of exposure	Mean % mortality (\pm SEM)				Analysis <i>F</i> , <i>P</i> *
	Pesticide concentration ^a				
	untreated control	minimum	maximum	2X maximum	
imidacloprid					
24 hrs	8.33 \pm 5.27a	0.00 \pm 0.00a	0.00 \pm 0.00a	12.50 \pm 8.54a	1.86, 0.18
48 hrs	20.83 \pm 11.93a	33.33 \pm 5.27a	33.33 \pm 12.36a	33.33 \pm 10.54a	0.57, 0.64
72 hrs	20.83 \pm 11.93a	41.67 \pm 5.27a	54.17 \pm 15.02a	62.50 \pm 10.70a	2.37, 0.11
oxydemeton-methyl					
24 hrs	16.67 \pm 8.33a	0.00 \pm 0.00a	0.00 \pm 0.00a	16.67 \pm 8.33a	2.66, 0.09
48 hrs	25.00 \pm 9.13a	37.50 \pm 10.70a	25.00 \pm 6.45a	33.33 \pm 12.36a	0.28, 0.84
72 hrs	37.50 \pm 14.07a	41.67 \pm 8.33a	45.83 \pm 11.93a	58.33 \pm 10.54a	0.76, 0.53

Table 2.2. Mean (%) mortality of worker *Bombus vosnesenskii* after exposure to pesticide-treated red clover plant material.

Pesticide / Time of exposure	Mean % mortality (\pm SEM)				Analysis <i>F</i> , <i>P</i> *
	Pesticide concentration ^a				
	untreated control	minimum	maximum	2X maximum	
chlorpyrifos					
24 hrs	20.83 \pm 4.17a	83.33 \pm 8.33b	100.00 \pm 0.00b	100.00 \pm 0.00b	45.50, <0.01
48 hrs	20.83 \pm 4.17a	95.83 \pm 4.17b	100.00 \pm 0.00b	100.00 \pm 0.00b	75.63, <0.01
72 hrs	29.17 \pm 7.68a	100.00 \pm 0.00b	100.00 \pm 0.00b	100.00 \pm 0.00b	80.00, <0.01
bifenthrin					
24 hrs	0.00 \pm 0.00a	87.50 \pm 8.54b	91.67 \pm 8.33b	95.83 \pm 4.17b	43.75, <0.01
48 hrs	8.33 \pm 5.27a	95.83 \pm 4.17b	91.67 \pm 8.33b	100.00 \pm 0.00b	44.68, <0.01
72 hrs	33.33 \pm 10.54a	95.83 \pm 4.17b	91.67 \pm 8.33b	100.00 \pm 0.00b	17.13, <0.01

^aThe minimum, maximum and 2X maximum field dose applied (mg/ml):

imidaclopid: (0.36), (0.48), (0.96)
oxydemeton-methyl: (4.49), (5.99), (11.98)
chlorpyrifos: (5.99), (11.98), (23.96)
bifenthrin: (0.72), (1.20), (2.40)

*Values within each horizontal row followed by different letters are significantly different (Tukey multiple means comparison) at $\alpha = 0.05$.

Chapter III

Mortality of larval and worker bumble bees in response to pollen and nectar availability

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In preparation for submission to *Behavioral Ecology and Sociobiology*
Springer, New York, NY

Abstract

The role of resource availability on bumble bee worker and larval vitality is unknown, despite the inherent need of colonies to acquire adequate amounts of pollen and nectar for growth, development, and reproduction. Hence, the objective of this study was to evaluate the impacts of resource availability on larval and worker mortality. In separate experiments, bumble bee colonies were provided with four quantities of pollen, four quantities of nectar and three feeding frequencies of pollen. There was an inverse relationship between larval and worker mortality to both pollen and nectar resources. When nectar was completely withheld from colonies, larval and worker mortality was 100% after 7 days. In contrast, these effects were prolonged when pollen was withheld from colonies because larval and worker mortality was 100% after 24 days. In the frequency study, larvae and workers experienced similar mortality for both the third-day and no pollen treatments. There was also a relationship between the type of larval mortality, larval ejection or within-clump mortality, displayed by colonies and worker mortality. Larval ejection occurred in all treatments across all studies, while within-clump mortality only occurred in the lowest treatments. Colonies which received the highest quantity of resources exhibited less worker mortality and subsequently, tended to eject larvae from the nest as opposed to leaving individuals to expire within larval clumps. The results of this study highlight the importance of continuous, abundant food resources for the growth and survival of bumble bee colonies. To ensure that bumble bees are able to access adequate floral

resources, conservation efforts should be directed at managing both natural and agricultural landscapes.

Introduction

Bumble bees, *Bombus* spp. (Hymenoptera: Apidae) are eusocial insects which have an annual colony life cycle that typically lasts 3-5 months in temperate regions (Alford 1975). Colonies store limited quantities of pollen and nectar, which on average, last only 1 (Plowright and Pendrel 1977) to 6 days (K. Skyrms unpublished data), depending on the species and developmental stage of the colony. Hence, a continuous supply of both pollen and nectar resources are vital for ensuring optimal colony growth, survival, and development (Alford 1975). Pollen is the protein source for the colony, while nectar provides carbohydrates (Heinrich 1979; Roulston et al. 2000). Adult bees consume little pollen. Bumble bees primarily collect pollen to feed developing larvae (Alford 1975), whereas nectar is necessary for the performance of daily activities within and outside the nest, and is consumed by both adult and immature bees (Heinrich 1979). Food availability in bumble bee colonies is both a factor of worker number and the abundance of flowering plants in the vicinity of nests (Shykoff and Muller 1995).

Recent reports from Europe (Kosior et al. 2007; Goulson et al. 2008; Williams and Osborne 2009) and North America (Grixti et al. 2009; Cameron et al. 2011) attribute habitat loss and the reduction of the availability and abundance of flowering

plants as two of the primary causes for bumble bee population declines. Studies which have examined the effects of habitat loss have focused primarily on evaluating the density and diversity of bumble bee foragers through surveys of natural populations (Bowers 1985; 1986; Banaszak 1992; Pywell et al. 2005; Westphal et al. 2003; 2006; Carvell et al. 2007; Heard et al. 2007; Elliot 2009), genetic analysis (Chapman et al. 2003; Darvill et al. 2004; Herrmann et al. 2007; Goulson et al. 2010) and museum records (Grixti et al. 2009; Cameron et al. 2011). This research provides no information on the colony-level response to resource availability. The few studies which have analyzed colonies have focused on the commercially available species, *B. terrestris* (L.) (Goulson et al. 2002; Smeets and Duchateau 2003; Westphal et al. 2006, 2009) and *B. impatiens* Cresson (Pelletier and McNeil 2003). Only two studies have examined resource utilization at the colony-level by wild bumble bees, *B. appositus* Cresson and *B. ternarius* Say, in isolated meadow habitats (Pelletier and McNeil 2003; Elliott 2009). However, these studies failed to consider the response of larvae, which are considered to be the most sensitive developmental stage within the nest (Sutcliffe and Plowright 1990).

Unlike the typical single-cell development of larvae in other eusocial Hymenoptera (Michener 1974), the majority of bumble bee larvae develop communally within flexible, waxen envelopes (Alford 1975). Because of this unique development, *Bombus* spp. larvae are fed progressively by workers (Katayama 1973; Alford 1975). The rate at which larvae are fed correlates with the overall biomass of the colony as a relatively large number of workers are needed to nourish developing

larvae (Katayama 1973; Michener 1974; Alford 1975). Bumble bees are categorized as a pocket-maker” or “pollen-storer” based on where larval food is stored within the nest and the way that workers feed developing larvae (Sladen 1912). Pocket makers attach a single, wax covered “pocket” to the bottom of each larval envelope, while pollen-storers “store” larval food apart from larvae in separate receptacles within the nest. Workers of pocket-makers provide a mixture of pollen and nectar in wax pockets situated underneath larvae and feed individuals when these food stores are depleted. In contrast, pollen-storing bumble bees feed larvae individually by placing a mixture of pollen and nectar onto the body of developing larvae several times throughout each day of development (Sladen 1912; Katayama 1973; Alford 1975).

The availability and abundance of pollen and nectar resources are critical for larval development and colony expansion (Alford 1975). Disruptions in resource supplies to larvae result in decreased size (Plowright and Pendrel 1977; Pendrel and Plowright 1981; Sutcliffe and Plowright 1988; Ribeiro et al. 1993), prolonged developmental time (Sutcliffe and Plowright 1990), and deformed wings in adult bees (Free and Butler 1959). It has been suggested that when colonies are faced with inadequate food supplies, colonies reduce the larval population (Plowright 1966). There are two forms of larval mortality in bumble bees: larval ejection from the nest, and mortality remaining within communal larval clumps or “within-clump mortality” (Figure 3.1). Unlike honey bees which cannibalize larvae (Schmickl and Crailsheim 2004), bumble bees and primitively eusocial wasps will eject both live and dead larvae by physically removing individuals from the nest (Miyamoto 1960; Sakagami and

Katayama 1977; Pomeroy 1979; Fisher and Pomeroy 1989; Kukik et al. 1997; Kasuya et al. 2007). Some larvae die within communal larval clumps and are not removed by workers (Pomeroy 1979).

The objective of this study was to evaluate the impacts of both pollen and nectar resource availability at the colony-level on larval and worker mortality.

Materials and methods

Bumble bee colonies

Colonies of *B. vosnesenskii*, a pollen-storer, native to western North America (Stephen 1957), were used in this study. Colonies were either reared by a regional bumble bee propagator (Bee Man Exterminators LLC, Olympia, WA), or in the laboratory from local, spring-collected queens in Benton County, Oregon, using established protocols (Plowright and Jay 1966; Pomeroy and Plowright 1980). Colonies were kept in wooden nest boxes (25.4 cm x 21.6 cm x 19.1 cm) with screened ventilation holes, and an acrylic cover to facilitate observations. Nest boxes were connected via plastic tubing to a foraging chamber (38.7 cm x 25.7 cm x 23.5 cm) (Figure 3.2). Colonies were maintained in the laboratory under controlled environmental conditions of humidity (50-60%), temperature ($28^{\circ}\text{C} \pm 2^{\circ}\text{C}$), and photoperiod (L15:D9).

Pollen and nectar resources

Fresh pollen was collected from local honey bee hives using bottom-mounted pollen traps (Betterbee, Greenwich, NY). Using methods of Tasei and Aupinel (2008), pollen was prepared as balls by mixing fresh, ground pollen with commercial sugar syrup (70:30% w/w respectively). Pollen was placed directly within the nest through a round opening in the acrylic cover to minimize disturbance. Sugar syrup (Koppert Biological Systems, Romulus, MI), herein referred to as “nectar”, was supplied to colonies, using small bird feeders (Living World® Seed and Water Feeder, 70 ml capacity). Nectar was provided to colonies in the foraging chamber. Feeders were replaced daily.

Quantity study design

In separate experiments, the quantity of either pollen or nectar was manipulated. Nectar was provided daily during the pollen experiment, and pollen was provided daily in the nectar experiment. Observations by Allen et al. (1978) on a wild colony in the reproductive phase of development found that under normal foraging conditions, a single *B. vosnesenskii* worker collected on average 0.21g of pollen and 1.18g of nectar, per day. These values were used as the "standard" treatments. The following treatments were tested: zero pollen, zero nectar; ½ standard (low); standard; and 2X standard (high). A total of 40 colonies were used in experiments: 20 for pollen

quantity, and 20 for nectar quantity. The experimental colonies varied widely in their size. Hence, initial colony size was estimated, and colonies were separated into five size classes using the larval/worker ratio (Shykoff and Muller 1995). These size classes were used as blocks and a randomized block design was used for both experiments.

Frequency study design

In this experiment, the frequency of when pollen was provided to colonies was manipulated while nectar was provided daily *ad libitum* throughout the duration of the study. Treatments consisted of the following feeding frequencies: zero pollen, daily pollen, and pollen every third day. Pollen was provided *ad libitum* and replaced daily in colonies receiving the daily treatment. For colonies receiving the third-day treatment, pollen was provided *ad libitum* to colonies at the end of day three for a period of 24 hours, and then removed until being replaced, three days later. A total of 18 colonies were used in this study. Similar to the quantity study, colonies varied in size, and were blocked by size to account for this variation. A randomized block design was used for this experiment.

Data collection

The quantity study extended for 20 days, while the frequency study lasted 30 days. For each experiment there was an initial two day 'starvation' period, during which colonies depleted existing nectar and pollen stores. Following this time period, daily observations were made for 18 days in the quantity study. This time period was considered adequate for assessing the effects of resource quantity on larval vitality given the typical range in developmental time of 10 to 14 days for *B. vosnesenskii* larvae (Figure 3.3). Because larval development time may be prolonged due to reduced feeding frequencies, the frequency study extended for 28 days. On each day, larval mortality was assessed by counting the number of dead larvae removed from the nest (larval ejection) and the number of dead larval clumps within the nest (within-clump mortality). Dead larval clumps are easily identified by their dark coloration and deformed shape, when compared to live larval clumps (K. Skyrn personal observation). Worker mortality was recorded daily by counting the number of dead workers in each colony.

Data analysis

The following formulae were used to determine the proportion of larval mortality in each colony for the quantity study:

1. larval ejection (day 18) = L_e / L_t

2. within larval clump (day 18) = L_c / L_t

- L_e = final number of ejected larvae (day 18)
- L_c = final number of dead larval clumps X 12.77^a (day 18)
- L_t = (initial number of larval clumps from day 1 X 12.77^a) + (total number of larval clumps created from day 2 to day 18 X 12.77^a)

^a Estimated as the average number of larvae per clump based on previous observations on nests (n = 12) of *B. vosnesenskii* (K. Skyrn, unpublished data).

3. worker mortality (day 18) = $W_d / (W_t)$

- W_d = final number of dead workers (day 18)
- W_t = (initial number of workers from day 1) + (total number of workers that emerged from day 2 to day 18)

Because of the variation in colony size of the replicates used in this experiment, the effects colony size was analyzed prior to data analysis. Using separate linear regression analyses for each treatment, the parameters of larval and worker mortality was used as the response variables and size was used as the explanatory variable. The data were subjected to an analysis of variance (ANOVA) using S-Plus version 8.0.4 (Mathsoft Inc, Seattle, WA). To meet the assumptions of normal variance, all values were arcsine square root transformed prior to analysis (Sokal and Rohlf 1981). The parameters of larval and worker mortality was used as the response variables in

separate analyses and treatment was the explanatory variable. When significant differences were observed, treatment comparisons were made using Tukey's multiple means comparison. All tests were performed at a significance level of $\alpha = 0.05$.

Results

Colony size analysis

There was no significant relationship between colony size and the percentage of larval ejection, within-clump mortality or worker mortality for any of the treatments used in the experiments (Table 3.1).

Pollen quantity study

Despite the fact that the zero pollen treatment value was twice as high as the values for the low, standard, and high treatments, there were no significant differences in the percentage of larval ejection among the four treatments ($F_{3,12} = 1.71$; $P = 0.22$) (Figure 3.4) Within-clump mortality was over three times greater in the zero pollen treatment than in the standard and high pollen treatments, and this difference was statistically significant ($F_{3,12} = 6.58$; $P < 0.01$). Within-clump mortality did not occur in either the standard or high pollen treatments. There were no statistical differences in worker mortality among the treatments ($F_{3,12} = 0.40$; $P = 0.75$), even though the

zero pollen treatment had almost twice the worker mortality compared to the low, standard, and high treatments.

Nectar quantity study

There were no statistical differences in larval ejection among the nectar treatments ($F_{3,12} = 0.26$; $P = 0.85$) (Figure 3.5). However, there was a significant difference in within-clump larval mortality, since the zero nectar and low nectar treatments resulted in over four times greater mortality than the standard and high nectar treatments ($F_{3,12} = 102.40$; $P < 0.01$). Within-clump larval mortality did not occur in the standard or high nectar treatments. All larvae in colonies exposed to the zero nectar treatment expired by day 7 of the trial. There were significant differences in worker mortality in response to nectar quantity; the zero nectar treatment was two times greater than the low and standard treatments, and ten times greater than the high nectar treatment ($F_{3,12} = 14.20$; $P < 0.01$). Worker mortality was 100% for all colonies exposed to the zero nectar treatment, after 7 days.

Pollen frequency study

Similar to pollen quantity, there were no significant differences in larval ejection among the three feeding frequencies ($F_{2,14} = 0.34$; $P = 0.72$). The zero pollen and third-day treatments caused twice the amount of larval ejection compared to the

daily addition treatment (Figure 3.6). There were significant differences in within-clump larval mortality where the zero pollen and third-day treatments had over seven times greater mortality than the daily-addition treatment ($F_{2,14} = 51.77$; $P < 0.01$). Within-clump larval mortality did not occur in the daily addition pollen treatment. Unlike pollen quantity, there were significant differences in worker mortality rates among the three feeding frequencies; the zero pollen and third-day treatments caused three times the amount of mortality compared to the daily addition treatment ($F_{2,14} = 37.95$; $P < 0.01$).

Discussion

This study documented that a continuous supply of both pollen and nectar resources is necessary for the growth and survival of bumble bee colonies. With the exception of Pelletier and McNeil (2003) and Elliott (2009), this is the only other study to document the effects of resource availability on colonies of a North American bumble bee species and the first to document the impacts on both developing larvae and workers. Earlier studies only monitored changes in the numbers of adults, or determined final nest contents (Goulson et al. 2002; Pelletier and McNeil 2003; Westphal 2006, 2009; Elliott 2009). The debilitating role of low resource quantities on larval ejection in bumble bees has been alluded to (Plowright 1966), but to our knowledge has never been quantified. The results of this research also provide the

first empirical evidence for both forms of larval mortality, larval ejection and within-clump mortality, in bumble bee colonies.

There was an inverse relationship between larval and worker mortality to both pollen and nectar resources. When nectar was completely withheld from colonies, larval and worker mortality was 100% after 7 days. In contrast, these effects were prolonged when pollen was withheld from colonies because larval and worker mortality was 100% after 24 days. Smeets and Duchateau (2003) obtained similar results in a five month study on workers from *B. terrestris* micro-colonies, since survivorship of bees declined when deprived of pollen.

In the frequency study, regardless whether pollen was completely withheld from colonies, or administered every third day, larval and worker mortality was the same. In the only other study to document larval mortality, Plowright and Pendrel (1977) found that *B. terricola* Kirby larvae were able to survive after the colony was inadvertently exposed to a single three day period of pollen shortages. Based on our results, bumble bee larvae may be better able to survive times of low pollen quantities but not prolonged periods of low pollen availability. These results also highlight the importance of the need for continuity in presence of pollen for bumble bee colonies. Thus, colony growth could be severely affected during times of low pollen availability.

In this study, an average of 14% of larvae was ejected even under “standard” feeding conditions. Similarly, Pomeroy (1979) found that 16-36% of developing larvae in *B. ruderatus* colonies were ejected during the normal colony life cycle. These results are contrary to the observations of Plowright (1966) who observed an

increase in the quantity of larvae ejected in captive colonies of *B. terricola* and *B. perplexus* Cresson after they were deprived of pollen. Larval ejection has also been documented to be an infrequent occurrence in colonies of *B. diversus* Smith (Miyamoto 1960; Sakagami and Katayama 1977), *B. ardens* Smith, *B. beaticola* (Tkalcu) (Sakagami and Katayama 1977), *B. terrestris* (Fisher and Pomeroy 1989), and *B. ruderatus* (Fabricius) (Pomeroy 1979). Larval ejection could be beneficial to colonies if it allowed for the production of larger, healthier individuals (Tasei et al. 2000). Larval ejection could also reduce the metabolic mass of a colony, allowing for better gas exchange (Kukuk et al. 1997) and maintenance of the temperature gradient (K. Skyrn, personal observation), which is vital for developing larvae.

In contrast to larval ejection, a high percentage of larvae died within communal larval clumps. Within-clump mortality did not occur in the standard, high, or daily addition treatments. Pomeroy (1979) observed 1.3 to 9.9% within-clump mortality of last instar larvae in colonies of *B. ruderatus* in the laboratory. In a pocket-maker bumble bee like *B. ruderatus*, workers spend less time feeding and interacting with individual larvae, as opposed to pollen-storers. Therefore, pocket-makers may be prone to exhibit within-clump mortality regardless of resource availability, whereas pollen-storers only resort to within-clump mortality during times of low food availability. Given this, we hypothesize that within-clump mortality may vary depending on resource availability and larval feeding strategy.

Larval survivorship within the nest is not only associated with food resources, but also worker abundance. A relatively large number of workers are needed to gather

resources and nourish developing larvae (Michener 1974; Pomeroy and Plowright 1982). Based on our results, there is a relationship between the type of larval mortality displayed by bumble bee colonies, and their worker mortality. Colonies which had a high abundance of resources, and thus exhibited less worker mortality, tended to eject larvae from nests as opposed to leaving individuals to expire within larval clumps. Because of the compact nature of bumble bee colonies, the presence of expired larvae within communal larval clumps could exacerbate the incidence and spread of parasites and disease. Larval ejection could be a sanitation mechanism exhibited by colonies to maintain hygienic conditions within the nest.

For the two quantity studies, calculations of the standard pollen and nectar treatments were taken from estimates of a wild *B. vosnesenskii* colony in the reproductive phase of development (Allen et al. 1978). During this time, bumble bee colonies are known to collect greater quantities of pollen and nectar resources due to the needs of new gynes and males for larger resource supplies (Alford 1975). Hence, we hypothesize that because we used these higher estimates for the treatment values in our studies, our results provide lower estimates of the extent of deleterious effects of low resource availability on larval and worker mortality.

Bumble bee colonies can be subjected to periods of food shortages given the temporal and spatial availability of pollen and nectar resources in the landscape. Results from this study provide support for efforts which seek to enhance the continuity of available forage resources in the landscape, for providing colonies with a continuous supply of floral resources (Ribeiro et al. 1993; Williams and Osborne

2009). Agricultural landscapes consisting of mass-flowering bee-pollinated crops such as red clover and oilseed rape are considered to be important floral resources for bumble bees, but they are only temporally available (Goulson et al. 2008; Westphal et al. 2009; Rao and Stephen 2010). However, agricultural landscapes can be supplemented with additional floral resources through the use of flower-rich hedgerows or uncropped field margins (Kells et al. 2001; Mand et al. 2002; Carvell et al. 2007; Hannon and Sisk 2009). In natural landscapes, increased efforts should be directed at preserving existing vegetation and supplementing landscapes with additional plantings of wildflowers (Goulson et al. 2008). Together both, agricultural and native habitats must be managed to ensure adequate resource supplies for bumble bees (Bowers 1985; Williams and Osborne 2009; Rao and Stephen 2010).

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Larval ejection



“Within-clump” mortality



Figure 3.1. The two forms of larval mortality displayed in bumble bee colonies, larval ejection and within-clump mortality.



Figure 3.2. The nest box and foraging chamber setup used for *Bombus vosnesenskii* colonies in the quantity and frequency experiments.

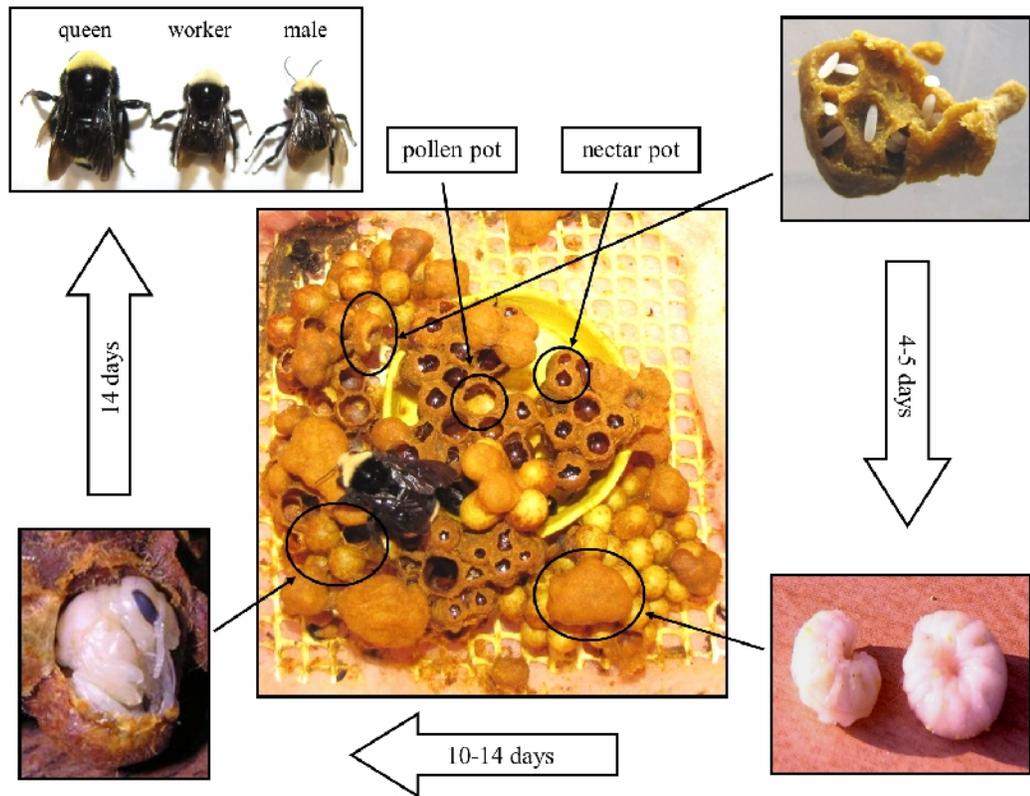


Figure 3.3. The life cycle of *B. vosnesenskii*. The total development time from egg to adult is approximately 28-33 days.

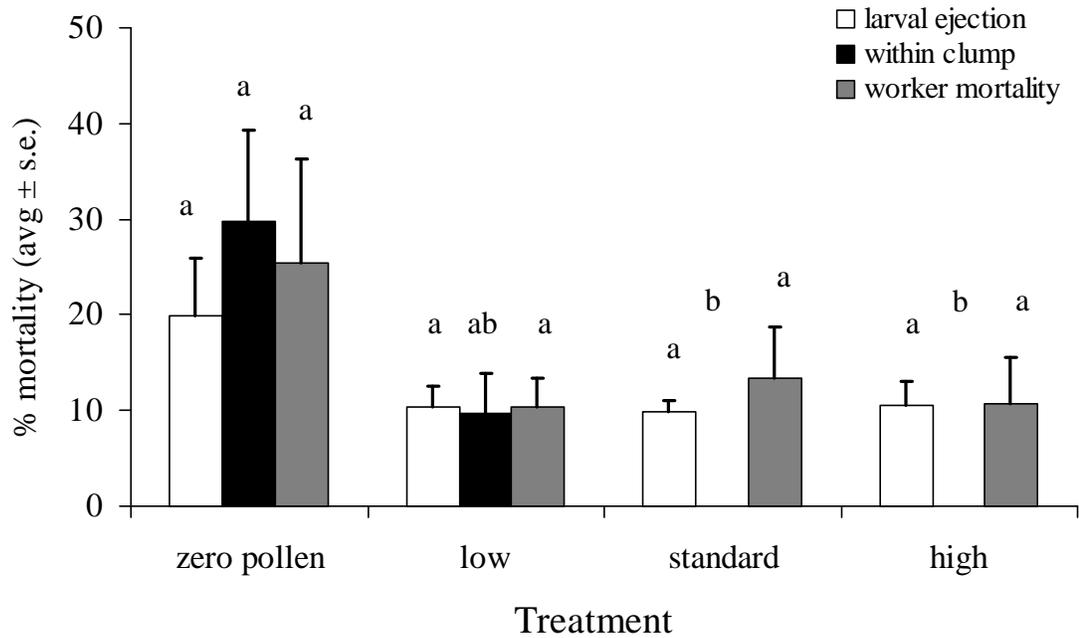


Figure 3.4. Mean (%) mortality of larvae and workers in *Bombus vosnesenskii* colonies after exposure to four quantities of pollen. Values with different letters are significantly different at $\alpha = 0.05$ (Tukey multiple means comparison).

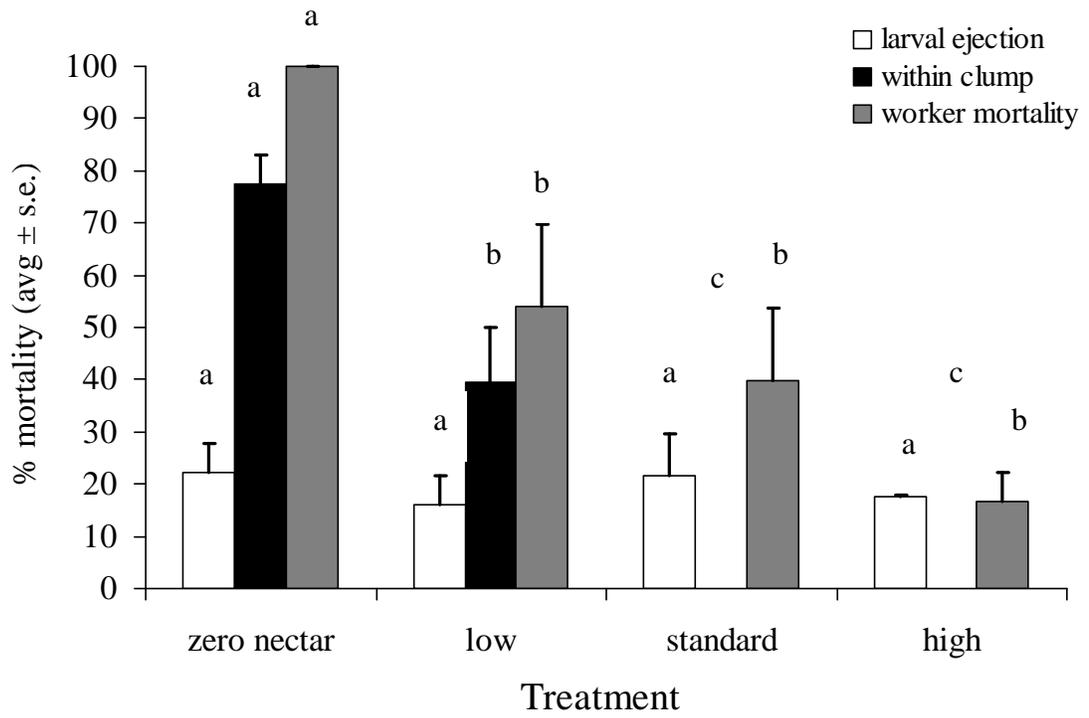


Figure 3.5. Mean (%) mortality of larvae and workers in *Bombus vosnesenskii* colonies after exposure to four quantities of nectar. Values with different letters are significantly different at $\alpha = 0.05$ (Tukey multiple means comparison).

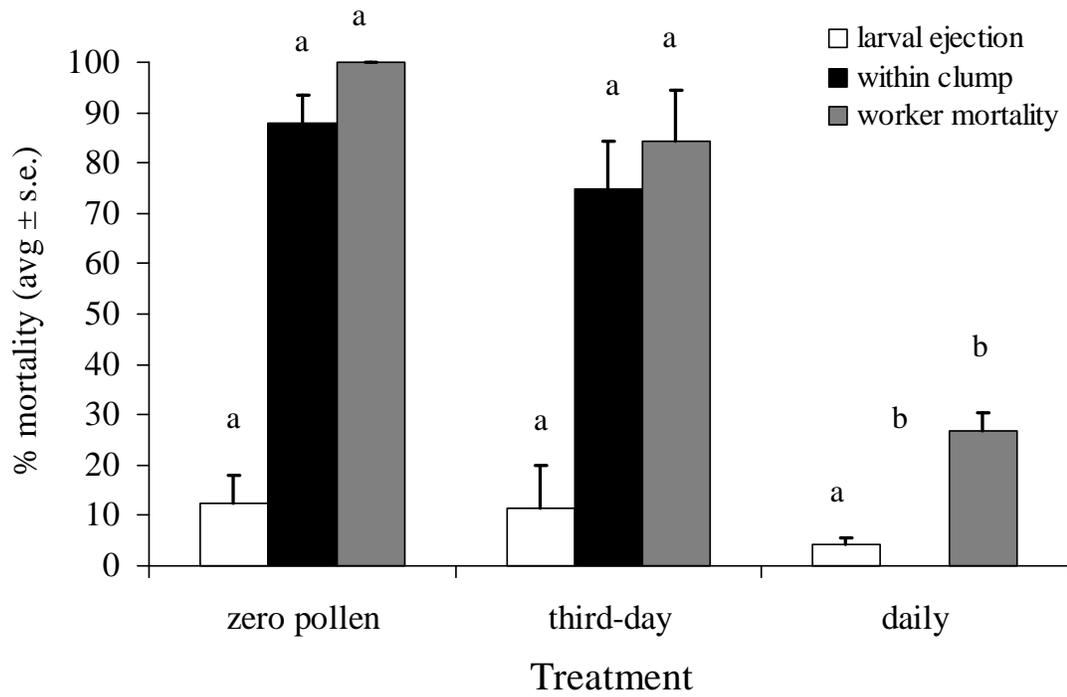


Figure 3.6. Mean (%) mortality of larvae and workers in *Bombus vosnesenskii* colonies after exposure to three feeding frequencies of pollen. Values with different letters are significantly different at $\alpha = 0.05$ (Tukey multiple means comparison).

Table 3.1. Linear regression analysis by treatment for the size variation of *Bombus vosnesenskii* colonies used in the quantity and frequency experiments.

Experiment / Type of mortality	Analysis by treatment (F, <i>P</i> *, adjusted R ²)			
	zero	low	standard	high
Pollen quantity				
larval ejection	0.34, 0.59, <0.01	2.86, 0.17, 0.27	2.13, 0.24, 0.22	0.23, 0.66, <0.01
within-clump	0.20, 0.68, <0.01	<0.01, 0.94, <0.01	N/A	N/A
worker	0.11, 0.76, <0.01	0.22, 0.67, <0.01	0.17, 0.71, <0.01	0.47, 0.53, <0.01
Nectar quantity				
larval ejection	0.12, 0.75, <0.01	1.08, 0.38, 0.02	0.10, 0.77, <0.01	0.52, 0.52, <0.01
within-clump	0.98, 0.39, <0.01	0.10, 0.77, <0.01	N/A	N/A
worker	N/A	0.75, 0.45, <0.01	0.06, 0.82, <0.01	1.68, 0.29, 0.15

Table 3.1. Linear regression analysis by treatment for the size variation of *Bombus vosnesenskii* colonies used in the quantity and frequency experiments.

Experiment / Type of mortality	Analysis by treatment (F, P*, adjusted R ²)			
	zero	third-day	daily	
Pollen frequency				
larval ejection	3.03, 0.16, 0.29	1.53, 0.28, 0.10	1.37, 0.31, 0.07	
within-clump	0.16, 0.71, <0.01	2.25, 0.21, 0.20	N/A	
worker	N/A	2.07, 0.22, 0.18	1.14, 0.35, 0.03	

*Values within each treatment are significantly different (Tukey multiple means comparison) at $\alpha = 0.05$.

Chapter IV

Late season pollen foraging behavior of native bumble bees in a mass-flowering agricultural landscape

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In preparation for submission to *Agriculture, Ecosystems, and Environment*
Elsevier, New York, NY.

Abstract

Bumble bees are important pollinators for both native and agricultural plants. However, their populations are currently in decline. This is primarily due to habitat loss which is speculated to have reduced the availability and abundance of food resources and nesting sites. Mass-flowering agricultural landscapes are considered to be important floral resources for bumble bees, but bloom time can be a limiting factor given the extended annual colony life cycle of bumble bees in temperate regions. Floral resources are especially critical during late summer/early fall when colonies are in the reproductive phase of development, but there is limited information on the effects of late season, mass-flowering crops as food resources for bumble bees. Hence, the objective of this study was to examine the individual and colony-level pollen foraging behavior of bumble bees in a late season, mass-flowering agricultural crop. Fields were surveyed in 2008 and 2009 to determine the abundance of foragers on flowers and the foraging activity of colonies placed adjacent to red clover. In 2008, there were no significant differences in July ($P = 0.30$), but in August there were over four times more *Bombus* spp. observed foraging on red clover flowers compared to *Apis mellifera* ($P < 0.01$). However, during July and August, the percentage of *Bombus* spp. workers observed carrying pollen in the corbicula was over twice that of *A. mellifera* ($P < 0.01$; $P = 0.04$). Of the six *Bombus* spp. observed foraging on red clover flowers, *B. vosnesenskii* workers predominated during both July and August ($P < 0.01$; $P < 0.01$). There was no difference between the abundance of queens and

males during July ($P = 0.68$) and August ($P = 0.09$), despite a three-fold increase in abundance of queens during the last week of observations. At the colony level, there were no significant differences in the average duration of pollen foraging trips during both 2008 and 2009 ($P = 0.90$; $P = 0.56$). Despite this, there was a weekly increase in the percentage of foraging trips made for pollen during both years ($P = 0.04$; $P = 0.03$). The amount of stored pollen in *B. vosnesenskii* colonies increased each week during July, but there were statistical differences only between the first and third sampling periods ($P = 0.02$). In addition, there was a positive linear relationship between colony size (i.e. number of workers) and the amount of stored pollen in colonies ($P = 0.01$). Of the seven different types of pollen stored within colonies, overall a higher proportion of red clover pollen was collected, compared to the other pollen types ($P < 0.01$). When these results were analyzed by week, red clover and Himalayan blackberry dominated pollen samples during the first two weeks ($P < 0.01$; $P < 0.01$), while red clover became the most dominant pollen type during the last two weeks of sampling ($P < 0.01$; $P < 0.01$). Mass-flowering agricultural landscapes of red clover are important floral resources for the late-season growth pattern of bumble bee colonies. Given this, conservation schemes should incorporate red clover plantings in land management schemes used to enhance and sustain bumble bee populations.

Introduction

Bumble bees, *Bombus* spp. (Hymenoptera: Apidae), are social insects which serve as vital generalist pollinators in natural and agricultural plant communities (Corbet et al. 1991; Delaplane and Mayer 2000; Memmott et al. 2004). However, both the diversity and abundance of bumble bees have declined in Europe (Fitzpatrick et al. 2007; Kosior et al. 2007; Goulson et al. 2008; Williams and Osborne 2009) and North America (Colla and Packer 2008; Grixti et al. 2009; Cameron et al. 2011) over the last three decades. The primary causes of these declines have been attributed to habitat destruction and agricultural intensification, both of which are speculated to reduce the availability and abundance of two important floral resources: pollen and nectar (Goulson et al. 2008).

Pollen is the main protein source for the colony, and is crucial for egg production and larval development (Alford 1975; Heinrich 1979; Roulston et al. 2000). Nectar serves as the carbohydrate source which fuels all tasks within the nest (Alford, 1975; Heinrich 1979). Unlike honey bees, bumble bee colonies store limited quantities of pollen and nectar food reserves within the nest (Alford 1975; Plowright and Pendrel 1977; Goulson et al. 2008). Hence, bumble bees in temperate regions rely on an abundant, continuous supply of floral resources from early spring to late summer for successful completion of their annual colony cycle and production of sexuals (Alford 1975). Bumble bee colonies are established in early spring by mated, over-wintered queens, and gradually increase in size with the production of increasing

numbers of workers as the season progresses (Alford 1975). During mid- to late-summer, colonies exhibit exponential growth and switch to producing males and new queens (Alford 1975; Heinrich 1979). Given the substantial increase in the workforce and relatively greater resource needs of male and queen larvae, colonies require significantly more food resources during this period (Michener 1974; Pomeroy and Plowright 1982; Duchateau and Velthuis 1988). The population size of bumble bees is primarily determined by the number of newly mated queens produced at the end of the season (Alford 1975).

Agricultural landscapes are considered to be important forage for bumble bees since they often provide abundant floral resources (Banaszak 1992; Goulson et al. 2002; Herrmann et al. 2007; Morandin et al. 2007; Westphal et al. 2003; 2006; 2009; Rao and Stephen 2009). However, the timing of bloom for the differing crop plants can be a limiting factor. It has been suggested that agricultural landscapes could be managed to provide food resources which bloom in sequence and synchrony with bumble bee colony cycles (Rao and Stephen 2010). Previous research has focused on colonies of the commercially available bumble bee, *B. terrestris*, in one early-blooming mass-flowering crop; *Brassica napus* L. (Goulson et al. 2002; Carvell et al. 2008; Westphal et al. 2009) and one late-blooming mass-flowering crop; *Phacelia tanacetifolia* Bentham (Westphal et al. 2006). Legume crops, such as red clover (*Trifolium pratense* L.), are also considered to be important forage resources, since bloom often coincides with the late season colony growth pattern of bumble bees (Banaszak 1992; Goulson et al. 2008; Rao and Stephen 2009, 2010). Despite

considerable information on individual foraging patterns of bumble bees in agricultural landscapes (Williams and Christian 1991; Banaszak 1992; Fussell and Corbet 1991, 1992; Corbet 1995; Herrmann et al. 2007), there is no information on “colony-level” pollen-foraging patterns.

The objective of this study is to evaluate both individual and colony-level pollen-foraging behavior of bumble bees and pollen resources stored within colonies established in a late season mass-flowering agricultural landscape.

Materials and methods

Study area

The study was conducted in the Willamette Valley of western Oregon in two 40 acre red clover fields located in the Polk County, Oregon (Site A: 44°44'54.42"N, 123°14'35.13"W, elevation 243ft; Site B: 44°45'54.27"N, 123°20'13.33"W, elevation 239ft). Grown as a biennial rotation crop, red clover is typically cut for hay in early spring to minimize pest outbreaks and to synchronize bloom with bee activity. Bloom in red clover occurs over six weeks from July until mid-August in the Willamette Valley. In this study, one field (Site A) was cut for hay during early May, and the other field (Site B) was cut for hay at the end of May. In 2008, data were taken in July at Site A and in August at Site B. During 2009, data were only taken at Site A during July and first week of August. Honey bees were also present in fields during both

years because growers in the region typically incorporate 2.5-5 hives per ha for pollination of red clover (Rao and Stephen 2010).

Bumble bee colonies

In 2008 and 2009, colonies of a native western bumble bee, *B. vosnesenskii* Radoszkowski (Stephen 1957), were utilized in experiments. Colonies were reared by a regional bumble bee propagator (Bee Man Exterminators LLC, Olympia, WA) using spring queens from Benton County, Oregon, and established protocols (Plowright and Jay 1966; Pomeroy and Plowright 1980). A total of 6 colonies of a similar size (3 in 2008 and 3 in 2009) were used in experiments and consisted of 34-52 workers. Colonies were maintained in wooden nest boxes (25.4 cm x 21.6 cm x 19.1 cm) with screened ventilation holes, and an acrylic cover to facilitate observations. The entrance to each nest box was modified with clear tubing (10.2 cm x 2.5 cm) (Tygon® R3603 Laboratory and Vacuum Tubing) to aid visual observations. Each nest box was situated on a three-tiered shelf and established 1 m from the field margin (Figure 4.1). Colonies were placed in the same red clover field (Site A) one week prior to data collection, each year.

Bee abundance

To compare the abundance of honey bees and native bumble bees foraging in red clover fields, transect surveys (Banaszak 1980) were taken in July and August of 2008, during red clover bloom. Observations were made in two separate red clover seed production fields (Site A and Site B). A series of timed visual counts of 2 minute durations (Rao and Stephen 2009) were made along a 5 m x 2 m longitudinal grid during sunny, dry weather. A total of 50 counts were made during morning (08:00) and afternoon (18:00) time periods, two days per week. Previous observations taken on *Bombus* spp. and *A. mellifera* in red clover indicated that these were the peak foraging periods of workers in the fields (K. Skyrn, personal observation). All bees observed on red clover flowers were noted, as was their caste and presence of corbicular pollen loads.

Foraging behavior

To determine the amount and duration of pollen foraging trips, colonies were observed two days per week during morning (09:00 – 12:00) and afternoon (14:00 – 17:00). Bees were individually marked on the thorax with distinct colored tags (Betterbee, Greenwich, NY) (Figure 4.2). The entrance of each colony was monitored to determine the number of foragers entering and leaving the nest box, and the presence of visible pollen loads within the corbicula. Due to production practices, in

2008, observations of three colonies were made only during three weeks in July. In 2009, observations were made on three colonies during three weeks in July, and one week in August. Each week, colonies were also surveyed to determine their size by counting the number of workers within nests.

Pollen collection

To examine pollen reserves in nests, weekly samples of stored pollen were taken during 2009 from three pollen storage pots within each of the three colonies observed. To determine the total amount of pollen stored in each colony, counts were made of the total number of pollen pots in each colony. The following formula was used to determine the total amount of pollen stored within colonies: total weight of stored pollen (weekly) = (total weight of 3 pots / 3 pots) x (total number of pots). After pollen samples were extracted from colonies, they were placed in separate storage containers and transported to the lab for processing. Plants located in the vicinity of the red clover field were also surveyed. Pollen was collected from each plant species in bloom, each week, and processed for use as a reference. Pollen samples were then dehydrated in a drying oven at 60°C for 24 hrs to remove moisture, prior to being weighed. Each sample was then diluted using methods of Telleria (1998), and 1 ml was extracted for analysis. To determine floral source, both colony and reference pollen samples were processed using acetolysis (Erdtman 1954; 1960) and light microscopy. A total of 900 grains per sample were identified. Identification

of pollens was made using reference to Moore and Webb (1978) and Sawyer (1981) as well as the reference collections. Some pollen types were only identified to the family level, due to the difficulty of identifying pollen of certain plant species.

Data analysis

Separate Student's t-test analysis were used to compare the abundance of *Apis mellifera* and *Bombus* spp, in the two red clover fields surveyed in this study. The number and percentage of bees per count with corbicular pollen were the response variables and bee species was the explanatory variable. To compare the abundance of *Bombus* spp. and the castes of *B. vosnesenskii*, analysis of variance (ANOVA) was used in separate analyses for each field. The parameters of percentage and number of bees per count were used as the response variables, while bee species and week were used as explanatory variables.

Analysis of pollen foraging behavior at the colony-level was conducted separately for each year using ANOVA. The average duration and percentage of trips made for pollen by all bees in each colony was used as response variables while week and colony size were included as explanatory variables. Similar to pollen foraging, ANOVA was used to compare the weekly weight of stored pollen collected by colonies. In these analyses, average weight of stored pollen was the response variable and week was the explanatory variable. Simple linear regression was used to evaluate the relationship between colony size and the weight of stored pollen. The percentage

of pollen from each plant type in the pollen samples were analyzed separately each week using ANOVA. Percent pollen was used as the explanatory variable and plant type was the response variable.

S-Plus version 8.0.4 (Mathsoft Inc, Seattle, WA) was used for all statistical analyses. To meet the assumptions of normal variance, counts were square root transformed and proportions were arcsine square root transformed prior to analysis (Sokal and Rohlf 1981). When significant differences were observed, treatment comparisons were made using Bonferroni's multiple means comparison. All tests were performed at a significance level of $\alpha = 0.05$.

Results

Bee abundance

There were no significant differences in bee abundance during July ($t = 1.24$; $P = 0.30$) (Figure 4.3). However, in August, the number of *Bombus* spp. workers observed foraging on red clover flowers was over four times greater than *A. mellifera*, and this difference was statistically significant ($t = -16.43$; $P < 0.01$). The percentage of *Bombus* spp. workers observed carrying pollen was over twice that of *A. mellifera*; these differences were statistically significant throughout the observational period ($t = -6.43$; $P < 0.01$; $t = -3.96$; $P = 0.04$) (Figure 4.4). Six *Bombus* spp. workers were observed foraging on red clover including *B. vosnesenskii*, *B. californicus* F. Smith, *B.*

appositus Cresson, *B. nevadensis* Cresson, *B. griseocollis* (Degeer) and *B. mixtus* Cresson (Figure 4.5). *Bombus vosnesenskii* workers were over three times more abundant than the other *Bombus* spp. during both July and August ($F_{5,15} = 28.11$; $P < 0.01$; $F_{5,10} = 435.57$; $P < 0.01$). Given the low numbers of queens and absence of males observed on red clover flowers in July, there was no significant difference between castes ($t = 0.45$; $P = 0.68$) (Figure 4.6). Similarly in August, there was no statistical difference between the number of queens and males on red clover flowers, despite a three-fold increase in abundance of queens during the last week of observations ($t = -2.53$; $P = 0.09$).

Pollen foraging

A total of 1267 foraging trips (717 in 2008 and 550 in 2009) consisting of 576 pollen trips (280 in 2008 and 296 in 2009) were recorded over 168 total hours of observation. There were no significant differences in the average duration of time for pollen foraging trips in 2008 and 2009, respectively ($F_{2,5} = 0.11$; $P = 0.90$; $F_{3,7} = 0.52$; $P = 0.68$) (Figure 4.7). The minimum pollen foraging trip time over both years was 5 minutes, and the maximum trip time was 116 minutes. In 2008, the percentage of pollen foraging trips increased each week, but there was only a significant difference between the first and third observational periods ($F_{2,5} = 6.16$; $P = 0.04$) (Figure 4.8). Similarly in 2009, there was an increase in the percentage of pollen trips made by

colonies but there was only a significant difference between the first two weeks and the last week of observations ($F_{3,7} = 4.71$; $P = 0.04$).

Pollen collection

The weight of stored pollen in colonies increased each week, and there were statistical differences between the first two and last two weeks of sampling ($F_{3,7} = 14.50$; $P < 0.01$) (Figure 4.9). As colony size increased, the weight of stored pollen within colonies also increased in a positive linear relationship ($F_{1,10} = 20.18$; $P < 0.01$; $R^2 = 0.67$) (Figure 4.10). Bumble bee colonies collected and stored pollen from a total of seven different plant families: *Trifolium pratense* L., (Fabaceae), *Rubus armeniacus* Focke, (Rosaceae) *Daucus carota* L. (Apiaceae), *Convolvulus arvensis* L. (Convolvulaceae), *Allium* sp. (Liliaceae), Asteraceae and Brassicaceae (Figure 4.11). Overall all pollen samples, a higher proportion of *T. pratense* pollen was collected compared to the other pollen types ($F_{6,18} = 13.09$; $P < 0.01$) (Figure 4.12). When these results were analyzed by week, both *T. pratense* and *R. armeniacus* dominated pollen samples during the first two weeks and were significantly different from the other plant types ($F_{1,6} = 13.48$; $P < 0.01$; $F_{1,6} = 17.11$; $P < 0.01$). (Figure 4.13). Despite an increase in other plant pollens in the samples, *T. pratense* pollen was the most dominant pollen type during the last two weeks of samples and this difference was statistically significant ($F_{1,6} = 15.21$; $P < 0.01$; $F_{1,6} = 16.43$; $P < 0.01$).

Discussion

This study demonstrated that an agricultural landscape with a mass-flowering legume crop served as an important forage resource for native bumble bees. A complex of six native bumble bees was observed on red clover flowers, of which *B. vosnesenskii* was the most dominant species. There was a diversity of pollen collected by bumble bee colonies with the presence of seven plant families in stored pollen reserves. This is the first study to document the colony-level pollen foraging habits for a North American bumble bee in an agricultural landscape. An earlier study conducted by Goulson et al. (2002) evaluated only the pollen collected by individuals returning to the nest of a commercially available bumble bee in a European agricultural landscape. With the exception of Westphal et al. (2006), it is also the only other study to evaluate the effects of late-season mass-flowering crops on bumble bee colonies. Colony level impacts have only been documented in the United Kingdom and Germany for the commercially available bumble bee, *B. terrestris*, in the early-blooming crop, *Brassica napus* (Goulson et al. 2002; Carvell et al. 2008; Westphal et al. 2009).

Populations of *Bombus* spp. workers in red clover fields was higher in August than in July, at which time *A. mellifera* populations were lower in the field. Rao and Stephen (2009) observed a similar trend at other red clover field sites in Western Oregon. Unlike bumble bees, *A. mellifera* colonies are perennial, have large population sizes consisting of 10,000 to 50,000 workers, and communicate the

location of floral resources in the landscape (Seeley 1985, 1997). Honey bees are known to forage up to 14 km away from the hive, but generally they concentrate most of their foraging efforts within 1 km radius of the colony (Eickwort and Ginsberg 1980; Seeley 1985). Hives of *A. mellifera* are typically brought into red clover fields at the time of 5% bloom, (early July) and removed just prior to harvest. However, after a few weeks, *A. mellifera* workers leave red clover fields and forage on other flowering plants in the vicinity, which offer more easily accessible pollen and nectar rewards, compared to red clover (Westgate and Coe 1915; Peterson et al. 1960; Rao and Stephen 2010). This suggests that the low numbers of *Bombus* spp. during July may be the result of competition with *A. mellifera*. In Europe, Wermuth and Dupont (2010) found an overall lower abundance of *Bombus* spp. in red clover fields that had honey bee hives, compared to fields without hives. Other studies have documented negative affects of *A. mellifera* on the reproductive success of bumble bee colonies (Thomson 2004), worker size (Goulson and Sparrow 2009), and foraging behavior (Forup and Memmott 2005; Thomson 2004, 2006; Walther-Hellwig et al. 2006) in both native and agricultural landscapes.

Alternatively, these results could be due the colony life cycle and growth pattern of *Bombus* spp. in the region. In addition to forager abundance on red clover flowers, the highest percentage of *Bombus* spp. workers observed with corbiculate pollen occurred during the later part of red clover bloom in August. During this time period, we observed increased numbers of *B. vosnesenskii* workers, males, and queens in red clover fields. At the colony level, we also observed an increase in the

percentage of pollen trips, quantity of pollen stores, and the number of workers within colonies. These results are indicative of the typical late-season growth pattern of bumble bee colonies in temperate regions. These data highlight the benefits of providing a late season forage crop such as red clover for bumble bee colonies. Westphal et al. (2006) observed shorter foraging trips, increased weight gain, and larger amounts of brood in *B. terrestris* colonies established in late-flowering *P. tanacetifolia*. Colonies established in early-blooming, mass-flowering crops also increased in workers and overall colony weight (Goulson et al. 2002; Carvell et al. 2008; Westphal et al. 2009). Yet, the beneficial effect of abundant resources during the early phase of colony development does not automatically translate into greater reproductive success, if pollen and nectar resources are limited during the later part of the season. Agricultural landscapes, which provide both abundant early-season and late-season resources, would improve colony growth and sexual production (Bowers 1985, 1986; Pelletier and McNeil 2003).

The percentage of red clover pollen collected by *B. vosnesenskii* colonies was similar across all weeks of this study. However, colonies did not rely solely on red clover for their pollen needs, as indicated by the presence of *R. armeniacus* and five other weedy plants in pollen stores. Since the majority of *Bombus* spp. are generalist foragers, they tend to be opportunistic in their foraging behavior by visiting a variety of plants in the landscape. Our results indicated that colonies majored on red clover and *R. armeniacus* pollen during the first two weeks of July. Because of the ease of access to both pollen and nectar resources on *R. armeniacus* compared to red clover

flowers, bumble bees may have gained greater energetic benefits by foraging on this plant (Heinrich, 1979). Alternately, these results could be due to the presence of competition from the high numbers of honey bees present in fields during this time. Given their mass foraging effort, honey bees have the ability to exploit red clover flowers at a faster rate, therefore depleting resources and causing bumble bees to seek alternative plant species for floral resources. Further research is needed to determine whether these differences are due to competition with honey bees, or the foraging preferences of bumble bees themselves.

Bumble bees need a continuous, abundant supply of floral resources for colony growth, survival and reproduction. While mass-flowering agricultural landscapes serve as important forage for bumble bee colonies (Herrman et al. 2007; Carvell et al. 2008; Westphal et al. 2003, 2006, 2009), they are only temporally available. Given this, conservation efforts should be directed at augmenting landscapes to provide floral resources that bloom sequentially and in synchrony with the life cycle of bumble bees, especially during early spring and late summer. Cropping systems such as the Willamette Valley of western Oregon serve as a good model for bumble bee conservation because there is sequential bloom in agricultural crops throughout the season (Rao and Stephen 2010). In areas where a sequence of bloom in bee-pollinated crops is not an option, landscapes can be altered by incorporating hedgerows of native and cultivated plants or planting a series of plants that bloom in sequence (Corbet 1991; Fussell and Corbet 1991; Banaszak 1992; Corbet 1995; Hannon and Sisk 2009; Rao and Stephen 2010). Other methods of enhancing agricultural areas include

augmenting production practices so that they are better suited to the life cycle of bumble bees. For instance, field margins could be left uncropped and unsprayed to serve as nesting sites and forage resources for bumble bees (Kells et al. 2001).

Agricultural producers could also reduce the number of honey bee hives incorporated into fields for pollination, and place hives in fields when native bumble bees are not as abundant.

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Figure 4.1. The shelf setup used for *Bombus vosnesenskii* colonies placed adjacent to a field of flowering red clover (Site A) during bloom.



Figure 4.2. A marked colony of *Bombus vosnesenskii*.

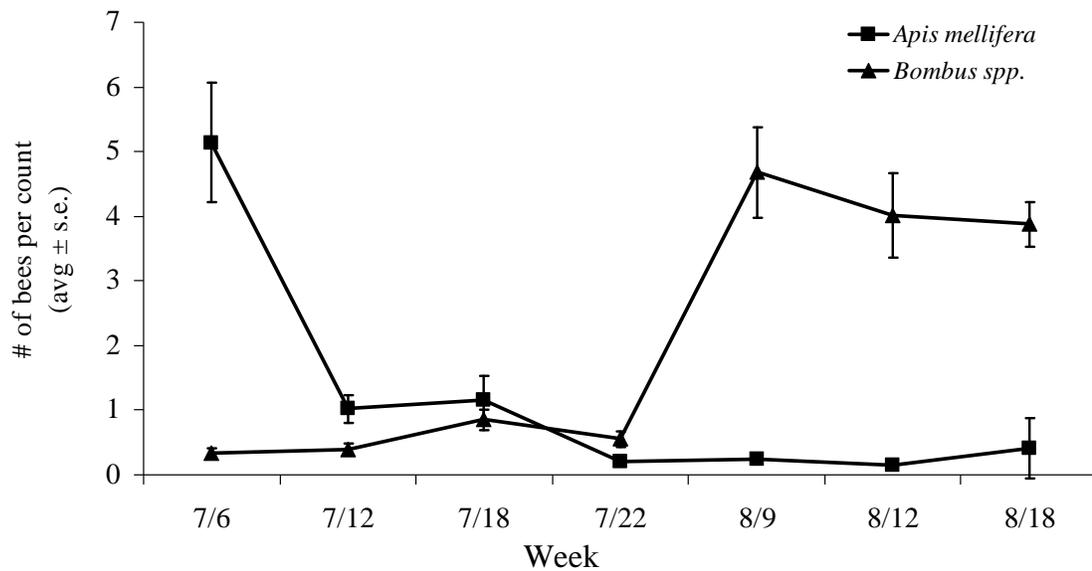


Figure 4.3. Mean number of *Apis mellifera* and *Bombus spp.* workers observed foraging in two red clover fields (Site A and B) during 2008 (n = 1,400 counts).

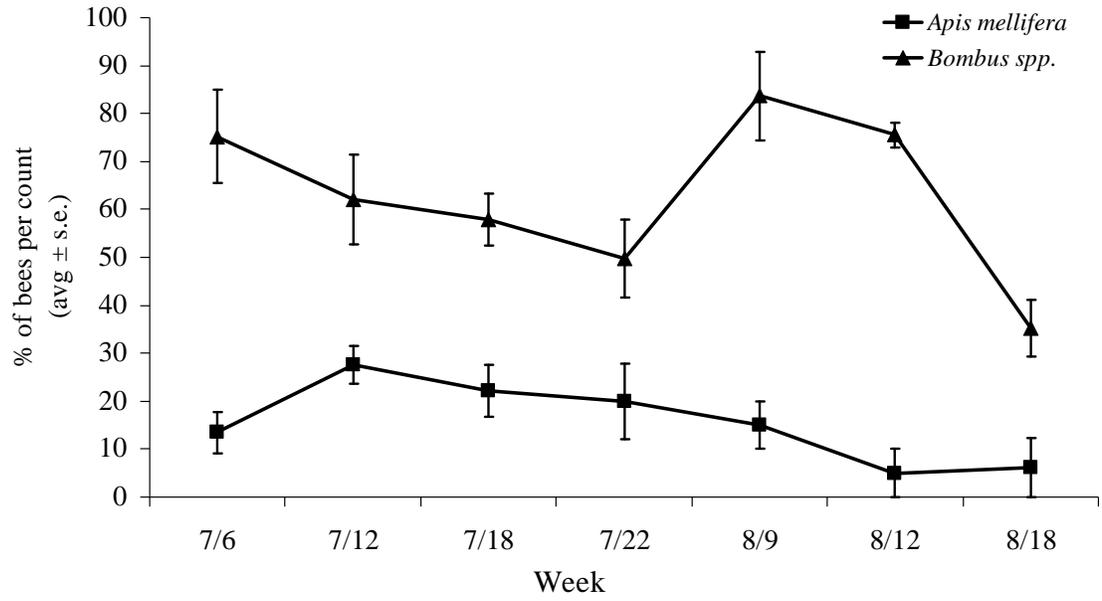


Figure 4.4. Mean percentage of *Apis mellifera* and *Bombus* spp. workers observed foraging in two red clover fields (Site A and B) during 2008 with pollen in their corbicula (n = 1,400 counts).

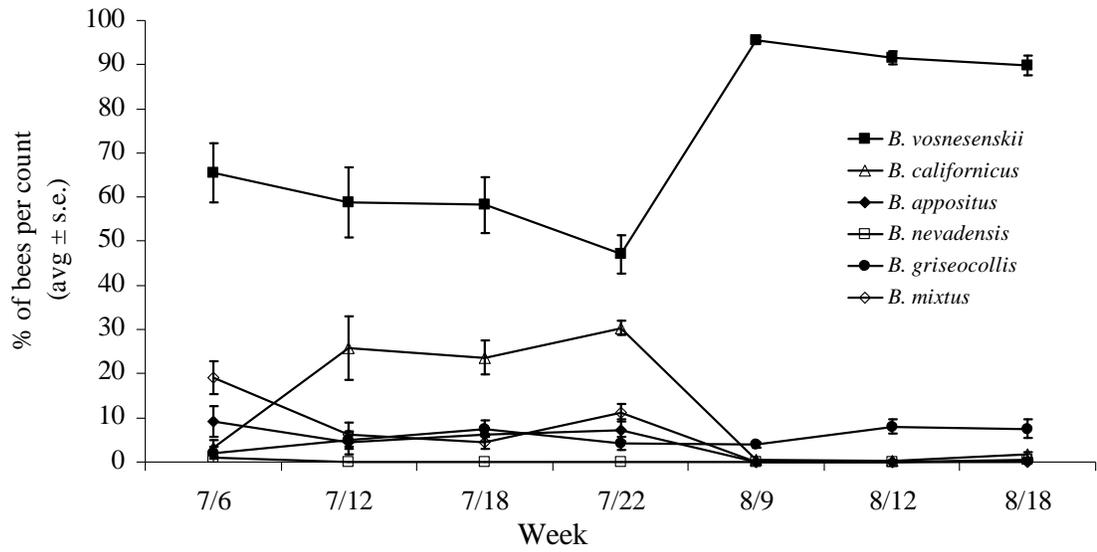


Figure 4.5. Mean percentage of *Bombus* spp. workers observed foraging in two red clover fields (Site A and B) during 2008 (n = 1,400 counts).

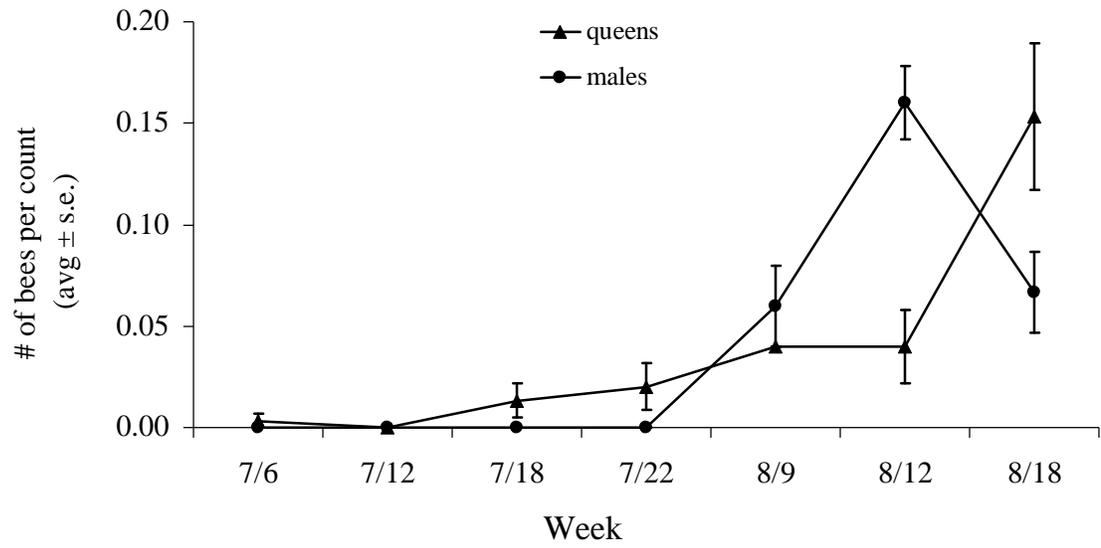


Figure 4.6. Mean numbers of *Bombus vosnesenskii* queens and males observed foraging in two red clover fields (Site A and B) during 2008 (n = 1,400 counts).

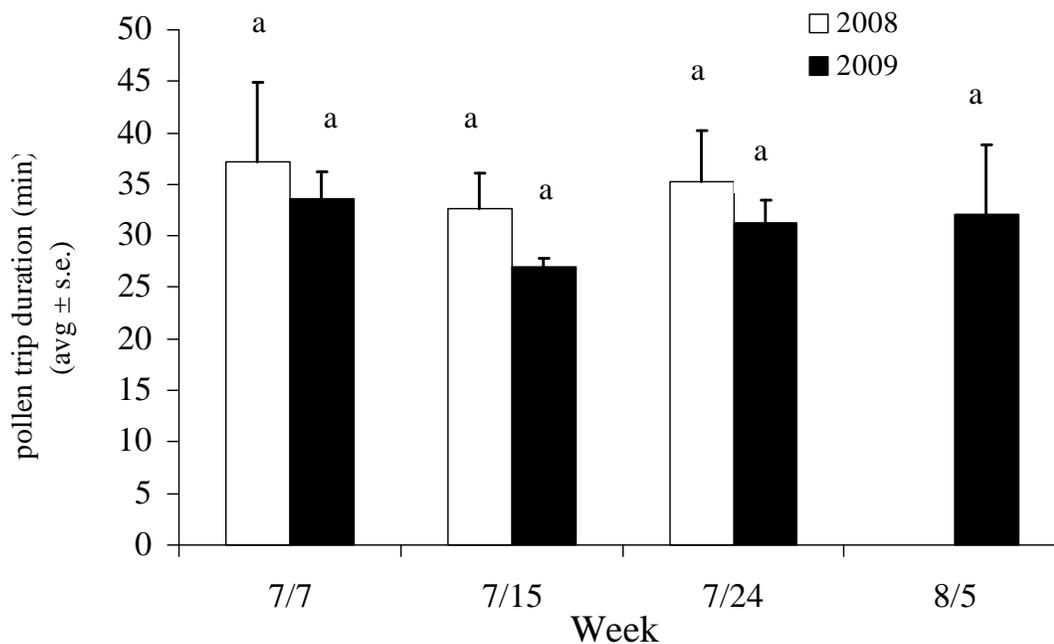


Figure 4.7. Mean duration of time for pollen foraging trips made by workers in three *Bombus vosnesenskii* colonies placed adjacent to a red clover field (Site A) during bloom ($n = 280$ pollen foraging trips for 2008; $n = 296$ pollen foraging trips for 2009). Values with different letters are significantly different at $\alpha = 0.05$ (Bonferroni's multiple means comparison).

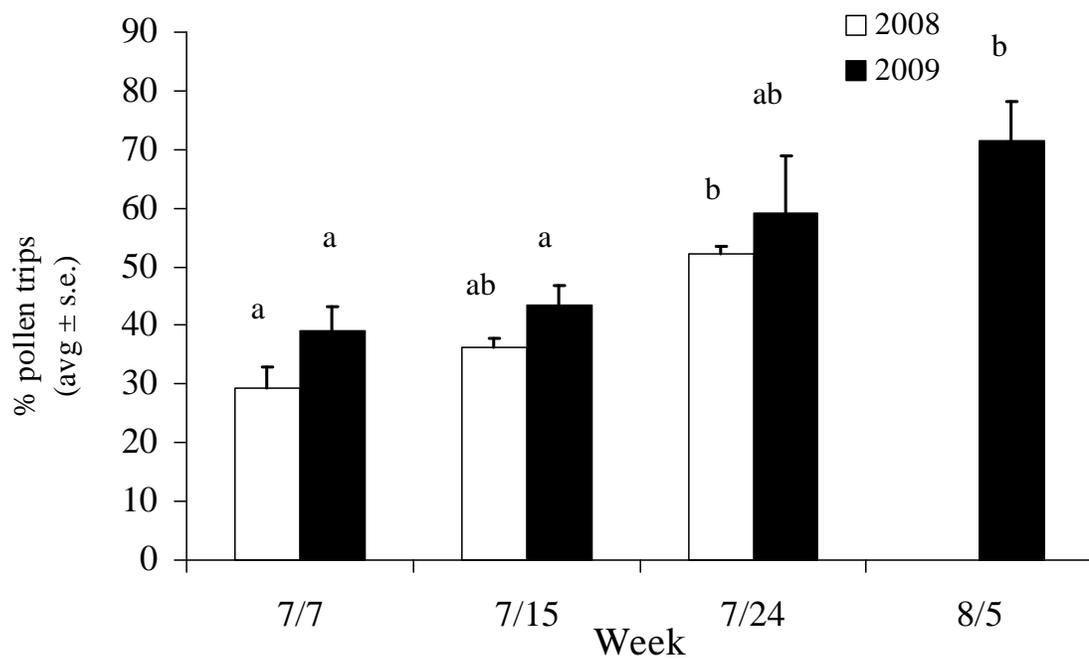


Figure 4.8. Mean percentage of foraging trips made for pollen by workers in three *Bombus vosnesenskii* colonies placed adjacent to a red clover field (Site A) during bloom (n = 717 foraging trips for 2008; n = 550 foraging trips for 2009). Values with different letters are significantly different at $\alpha = 0.05$ (Bonferroni's multiple means comparison).

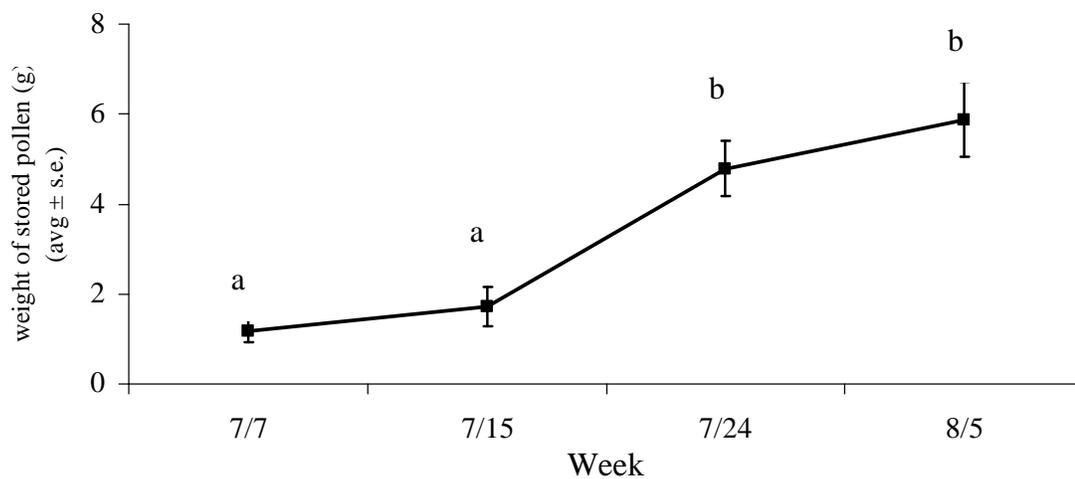


Figure 4.9. Mean weight of stored pollen reserves in three *Bombus vosnesenskii* colonies placed adjacent to a red clover field (Site A) during bloom in 2009. Values with different letters are significantly different at $\alpha = 0.05$ (Bonferroni's multiple means comparison).

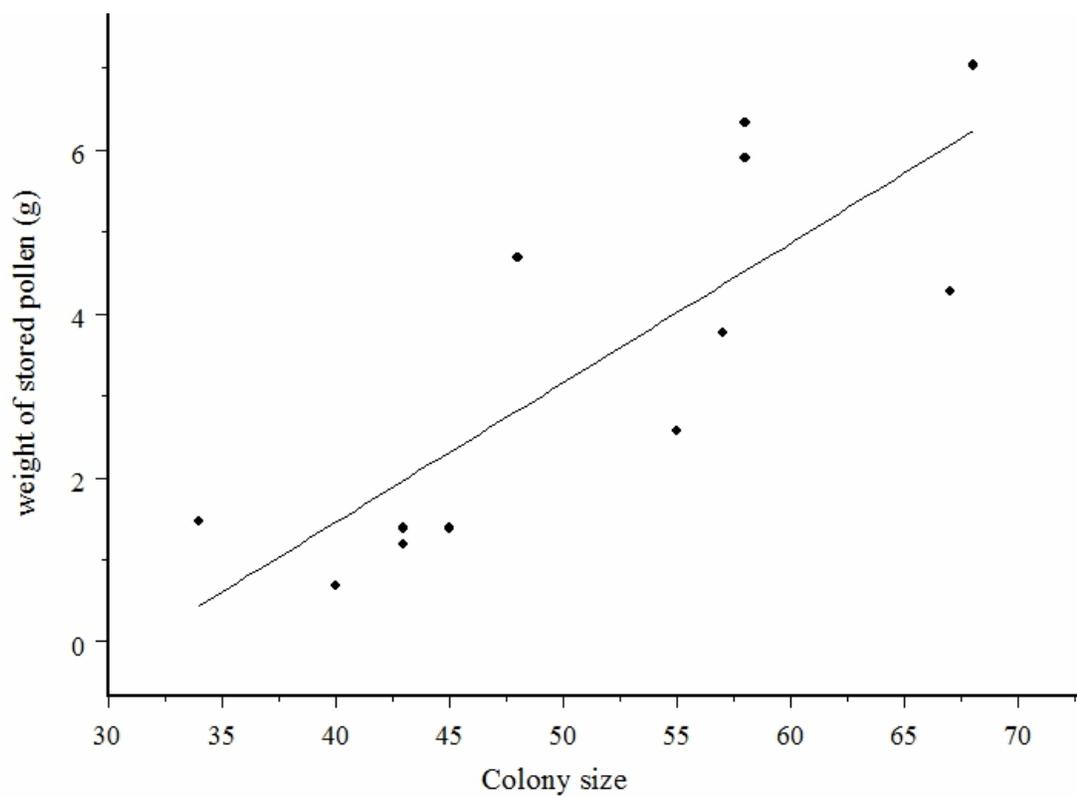


Figure 4.10. Linear regression analysis for the weight of stored pollen reserves ($n = 12$) and colony size (number of workers) of three *Bombus vosnesenskii* colonies placed adjacent to a red clover field (Site A) during bloom in 2009 ($F_{1,10} = 20.18$; $P < 0.01$; $R^2 = 0.67$).

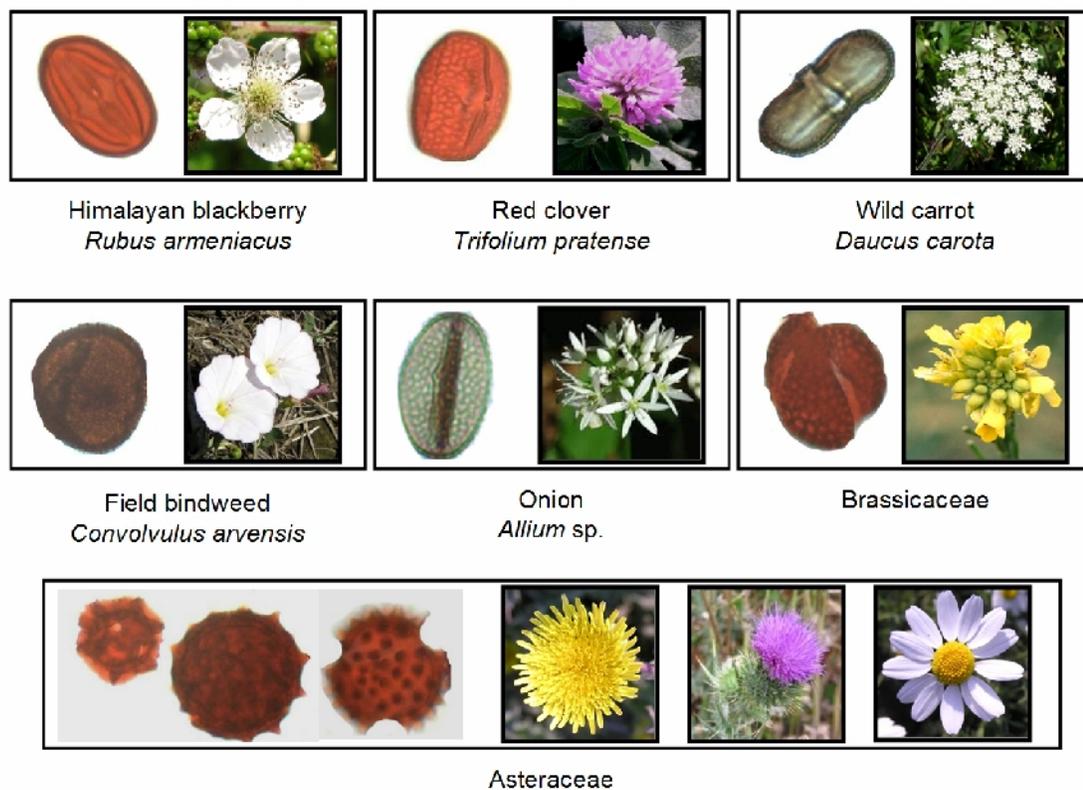


Figure 4.11. The seven different plant types identified from the pollen reserves ($n = 12$) of three *Bombus vosnesenskii* colonies placed adjacent to a red clover field (Site A) during bloom in 2009.

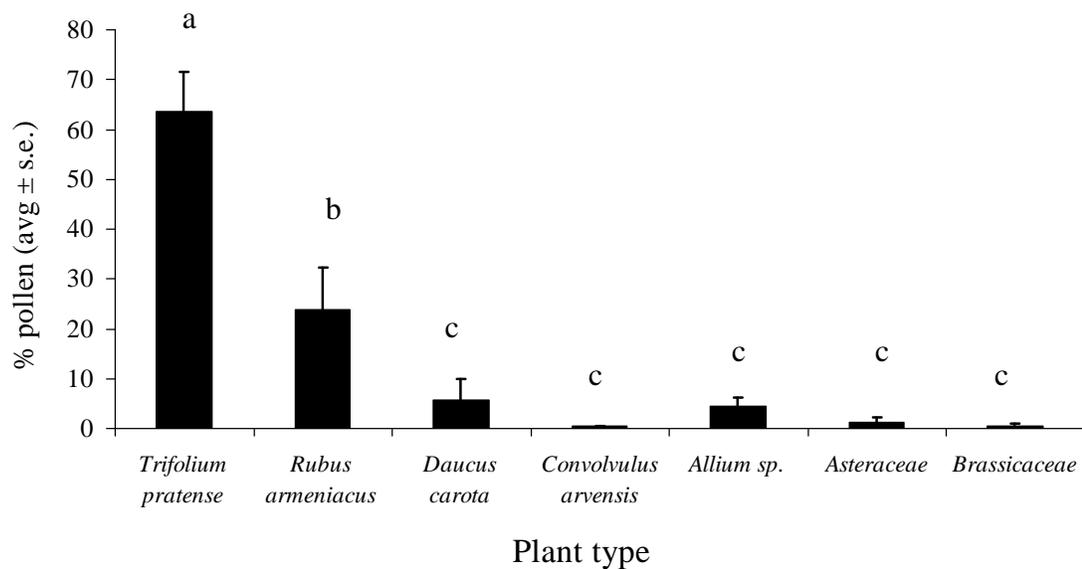


Figure 4.12. Mean percentage of pollen collected from each plant type during 2009. Pollen samples were taken from the pollen reserves ($n = 12$) of three *Bombus vosnesenskii* colonies placed adjacent to a red clover field (Site A) during bloom. Values with different letters are significantly different at $\alpha = 0.05$ (Bonferroni's multiple means comparison).

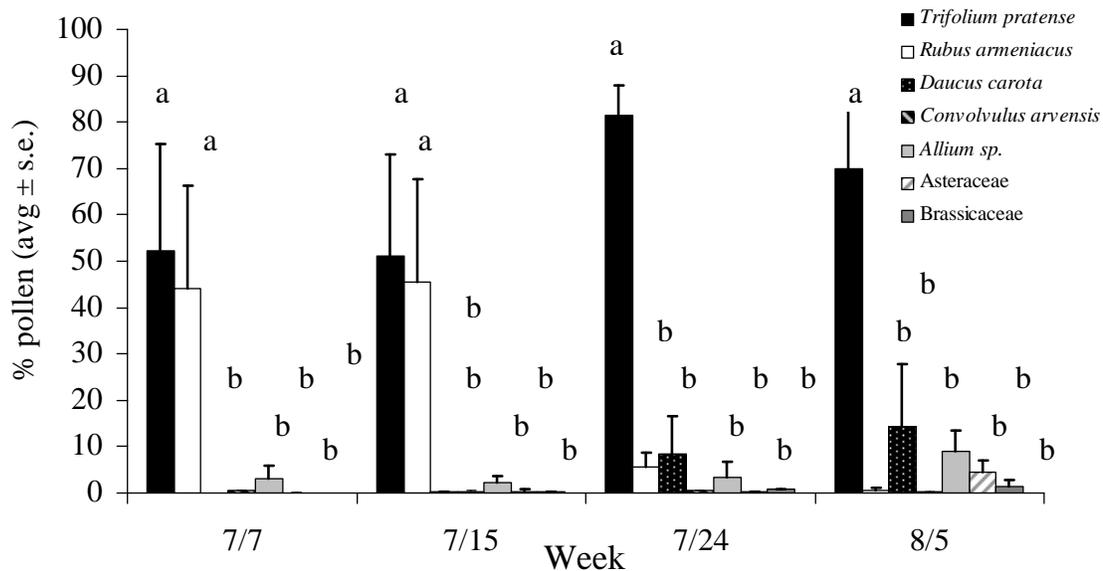


Figure 4.13. Mean percentage of pollen collected from each plant type weekly during 2009. Pollen samples were taken from the pollen reserves ($n = 12$) of three *Bombus vosnesenskii* colonies placed adjacent to a red clover field (Site A) during bloom. Values with different letters are significantly different at $\alpha = 0.05$ (Bonferroni's multiple means comparison)

Chapter V

**A scientific note on a trend towards bivoltinism in Western North American
bumble bees**

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Submitted to *Apidologie*
Springer, New York, NY
Accepted for publication

Bumble bees, *Bombus* spp. (Hymenoptera: Apidae), are eusocial insects that require a continuous supply of floral resources for colony growth and development (Alford, 1975). In temperate regions, mated over-wintered queens emerge in early spring, forage for pollen and nectar, establish nests, and lay a series of eggs. Subsequently, workers emerge, forage, and the colony grows. During late summer, if adequate floral resources are available in the landscape, the colony increases considerably in size. At the end of the colony life cycle, reproductives, namely males and new queens, are produced. These exit the nest, and mate, after which males die and the queens seek over-wintering sites for diapause. Thus, the colony life cycle typically extends for 3-5 months with worker foraging activity coinciding with spring or summer blooming plants (Heinrich, 1979). Consequently, bumble bees in temperate regions have one generation a year while, with continuous availability of foraging resources, tropical species produce several generations a year (Sakagami, 1976). However, some temperate species appear to undergo more than one generation in a year. Workers of *B. terrestris* (L.) have been observed foraging throughout the year in England (Stelzer et al., 2010), Tasmania (Buttermore, 1997), Corsica (Rasmont and Adamski, 1996), New Zealand (Donovan and Weir, 1978), and Sardinia (Krausse, 1910a, 1910b), suggesting that a second generation is produced in the same year. Similar observations were reported for *B. jonellus* (Kirby) in Norway (Meidell, 1968; Douglas, 1973), and for *B. pratorum* (L.) and *B. hortorum* (L.) in England (Sladen, 1912). Here, we present the first report of a trend towards bivoltinism in three

temperate bumble bee species, all in the subgenus *Pratobombus*, in the Willamette Valley of western Oregon in western USA, based on the following studies.

Over the past six years, we have surveyed *Bombus* spp. intensively in this region, and have observed queens of *B. vosnesenskii* Radoszkowski, *B. mixtus* Cresson and *B. melanopygus* Nylander foraging as early as January-February and workers as late as September-October. Given that colony life cycles typically extend less than 5 months in temperate regions (Heinrich, 1979), the presence of workers of these three species over eight months in Oregon suggests the occurrence of a second generation. In 2008 and 2009, we serendipitously observed nest initiation behavior in queens of *B. vosnesenskii* that emerged in summer from colonies placed on a field stand in June of each year adjacent to a field of flowering red clover (*Trifolium pratense* L.; Fabaceae). In both years, all workers and queens in each of the colonies used in the study were marked on the thorax with distinct colored tags (Betterbee, Greenwich, NY) for monitoring colony development and foraging behavior. Colonies were surveyed daily to determine emergence of workers and queens. Queens were distinguished from workers based on their emergence from queen pupae and on their large body size. During July of 2008, unexpectedly, from 3 of the 10 colonies, we observed three newly emerged queens displaying typical nest initiation behaviors in small cavities located near to, but outside of, their natal nest. They collected pollen, constructed both pollen and nectar storage cells, laid eggs and incubated the egg clumps. The queens recruited one to two large-sized workers from their original colony to aid in nest initiation, behavior that is typically observed in tropical species (Sakagami, 1976).

In 2009, a new set of eight colonies were initiated from wild queens collected in spring, and subsequently placed in the field, one colony produced a new queen. This queen also displayed nest initiation behaviors including the formation of pollen and nectar storage cells, egg laying, incubation of brood, and recruitment of a worker from the natal nest, in small cavities located outside of her original nest. During both years, nest founding behavior was, however, disrupted when colonies had to be removed from the field because of red clover seed harvest operations. We do not know if the queens produced in 2008 and 2009 were mated.

During June of 2010, one wild colony each of *B. vosnesenskii*, *B. mixtus* and *B. melanopygus* were transferred to the lab and reared for another study. In each of these colonies we observed similar trends towards bivoltinism. In August, a total of 30 *B. vosnesenskii*, 7 *B. mixtus* and 15 *B. melanopygus* queens emerged from a single colony of each species. After emergence, all queens were placed with males for mating, and subsequently moved to nest boxes after 24 hours. Queens were not forced to forego diapause, nor were they exposed to any of the common lab manipulation techniques used in commercial rearing to mimic diapause in lab-reared queens. However, queens were provided with a worker from their natal nest as is typically done for facilitating nest initiation (Kwon et al., 2006). All of the queens of all three species initiated nests by constructing nectar storage pots, laying eggs and incubating egg clumps. Each of the 7 colonies initiated by *B. mixtus* queens and the 15 colonies by *B. melanopygus* produced workers (ranging from 7 to 25 and from 6 to 23, respectively), and males (ranging from 1 to 5 and from 2 to 4, respectively). The 30 colonies of *B. vosnesenskii*

produced only males (ranging from 5 to 14). We speculate that the absence of workers produced from the *B. vosnesenskii* colonies was due to the lack of mating under laboratory conditions. There are no published reports of successful mating in *B. vosnesenskii* under laboratory conditions; perhaps this species needs a larger space than what we provided for adequate flight prior to and during mating than what the other species required.

We believe that the observations described above provide evidence of a trend towards bivoltinism rather than examples of colonies being usurped by workers from the same or other colonies. Workers can get broody when the original queen dies and no longer produces the pheromones or engages in the behaviors that suppress ovary development in workers (Honk et al., 1980; Roseler et al., 1981). However, in our field study, the original queen was alive when the newly emerged individuals (which we referred to as queens) were broody. Secondly, broody workers typically lay eggs within the natal nest (Duchateau and Velthuis, 1989), while the queens of *B. vosnesenskii* that emerged near the red clover field laid eggs in cavities outside of their natal nest. In addition, when workers get broody, other workers will assist them in nest initiation but subsequently eat a large proportion of the newly laid eggs (Duchateau and Velthuis, 1988). This did not happen in our study. Finally, when we reared wild colonies of *B. mixtus* and *B. melanopygus* in the lab, the queens that emerged mated and initiated new colonies producing both workers and males without first going through diapause. Typically for colony initiation in the laboratory, bumble

bee queens must be exposed to either a period of diapause or be stimulated to forego diapause prior to laying eggs (Horber 1961; Tasei 1994).

For temperate bumble bees to develop a second generation in a year, the presence of floral resources is critical. In two studies conducted on *B. jonellus* in Norway, abnormally mild and dry summers over two years were believed to be responsible for queens producing two brood cycles in each season (Meidell, 1968; Douglas, 1973). We speculate that the trend towards production of a second generation exhibited by *B. vosnesenskii*, *B. mixtus* and *B. melanopygus* in our studies is due to the presence of an abundance of red clover bloom towards the end of summer in the Willamette Valley. Legumes are considered to be important for development of bumble bees, and their recent absence in landscapes in Europe has been attributed as a factor leading to declines in bumble bee species (Goulson et al., 2005). Thus, bumble bee species in the Willamette Valley may benefit from the late-blooming red clover crop not only for colony development during the period that males and new queens are produced (Rao and Stephen, 2010) but also for development of a second generation. Further research is needed to assess the extent of bivoltinism in *B. vosnesenskii*, *B. mixtus* and *B. melanopygus* in the Willamette Valley, and to determine whether other bumble bee species in the region, and in other regions in North America, exhibit the same trend.

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Chapter VI

Summary and conclusions

A common theme in this dissertation is the importance of agricultural habitats in providing both floral resources and nesting sites for bumble bees. However, agricultural landscapes have the potential to negatively impact bumble bee populations, given the use of pesticides and temporal availability of floral resources in these habitats. The intention of the studies in this dissertation was to document the responses of bumble bees to these environmental impacts by increasing the knowledge of toxicological impacts of pesticides on native bumble bees, assessing the colony-level impacts of resource availability, evaluating the effects of a late season, mass-flowering crop, and documenting the occurrence of bivoltinism in western bumble bees. In field and lab studies, the preceding chapters address these topics at both the individual and colony-level for native bumble bees.

This dissertation highlights the difference in toxicity and level of susceptibility for pesticide exposure of queens and workers, given their foraging behavior in multiple crops. The colony life cycle and foraging patterns of native bumble bees should be taken into consideration in the establishment of pesticide label precautions and restrictions for bees. Evaluation of resource availability revealed not only the colony-level need for abundant pollen and nectar resources but also a continuity of resources for the survival of developing larvae and workers. Adequate food resources are especially important for bumble bee colonies during late summer when reproductives are produced. The field study in particular documented the importance of red clover, a mass-flowering legume crop, in supplying the late-season forage resource needs of bumble bee colonies. Observations on a trend towards bivoltinism

in three western *Bombus* spp. further highlighted the importance of such late-season floral resources in stimulating the development of a second generation.

Despite their potential for negative effects, agricultural habitats are important floral resources for bumble bees. The impacts of environmental factors in these landscapes can be mitigated through proper land management techniques and conservation schemes. The Willamette Valley of western Oregon serves as good model system for demonstrating beneficial management practices for bumble bees since this area is dominated by an abundance of bee-pollinated crops which bloom in sequence and in synchrony with the colony life cycles of bumble bees in the region. However, due to the economy, low-value crops such as red clover are increasingly being replaced with wind-pollinated crops which provide no benefit to bees. Increased efforts should be directed at the continuation of providing legume crops such as red clover in the landscape so that they can continue to support local bumble bee populations.

Future studies similar to the ones described in this dissertation have the potential to provide useful insight on environmental factors affecting bumble bees. These data are needed for both land management and conservation practices to form a course of action in sustaining bumble bee populations.

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Appendix

Development of a new bumble bee rearing protocol

In preparation for submission to *Psyche*
Hindawi, New York, NY.

Rearing room

As a method for obtaining bumble bee colonies for my dissertation research, I established a rearing room on the campus of Oregon State University (OSU) in Corvallis, Oregon and investigated the feasibility and success rate of rearing western *Bombus* spp. species colonies in a laboratory setting. The rearing room was created with adjustable shelves, overhead lighting and heating apparatuses for maintaining adequate environmental parameters required by bumble bees. Queens and colonies were kept under controlled environmental conditions of humidity (55-60%), temperature ($28^{\circ}\text{C} \pm 2^{\circ}\text{C}$) and photoperiod (L15:D9). Red lights (250 watt heat lamps) were used for feeding and recording observations. A humidifier (Carefree PermaWick, Hunter Fan Company, Memphis, TN) and portable fans were used for maintaining adequate humidity and air circulation. A temperature recording device (Hobo Temperature Logger, Onset, Bourne, MA) was used to ensure the maintenance of adequate environmental conditions for colony growth and development.

Collection of bumble bees

From January to May 2010, queen bumble bees were collected from private residences in the local area and the OSU Hyslop Farm Research Laboratory in Corvallis, Oregon and used to initiate colonies. A total of 7 species were evaluated for their potential in mass rearing: *B. vosnesenskii*, *B. melanopygus*, *B. mixtus*, *B.*

griseocollis, *B. californicus*, *B. nevadensis*, and *B. appositus*. These species were chosen given their abundance in the area and temporal emergence of queens which coincide with the bloom time of several bee-pollinated crops grown in the region.

Queen and colony boxes

Using modified protocols from Plowright and Jay (1966) and Pomeroy and Plowright (1980) queens were established in plastic starter boxes (24.5 cm x 17.5 cm x 10.0 cm) with screened ventilation holes and acrylic covers to facilitate feeding (Figure A.1). Colonies (i.e. queen, workers and brood) were relocated to larger, wooden nest boxes (25.4 cm x 21.6 cm x 19.1 cm) (Figure A.2) after reaching a minimum size of 20 workers. Similar to queen-starter boxes, nest boxes had screened ventilation holes and acrylic covers to facilitate observations. Nest boxes were connected via plastic tubing to a plastic foraging chamber (38.7 cm x 25.7 cm x 23.5 cm) to facilitate feeding.

Based on preliminary rearing trials conducted in 2008 and 2009, I created a supplemental egg cup (16oz Dart insulated foam cup bottom) containing a wax nectar pot and a foam pupa to stimulate nest initiation and egg production (Figure A.1). Wax nectar pots were constructed from honey bee wax (GloryBee Foods, Eugene, OR) and glued to the bottom of the supplemental egg cups. Foam pupae (Great Stuff Gaps and Cracks, The Dow Chemical Co.) were glued onto the supplemental egg cups in close proximity to the wax nectar pots. Each queen starter box was also supplied with a

piece (10.16 cm x 2.54 cm x 10.16 cm) of fiberglass insulation nesting material (Pink FiberglasTM insulation R-13, Owens Corning, Toledo, OH). These components were incorporated into all queen-starter boxes besides those listed in the experiments described below to better insulation of developing brood and easier transfer of queens, and subsequently colonies, to nest boxes. All queens were left undisturbed for 48 hours after their initial placement in queen-starter boxes. After this time period, queen-starter boxes were checked daily for the presence of eggs. All queens were monitored for a period of 21 days. If they did not lay eggs during this time, then they were released.

Pollen and nectar

Sugar syrup (Pro-Sweet Liquid Feed, Mann Lake, Hackensack, MN), herein referred to as “nectar” was supplied to queens using modified Petri dish feeders (Figure A.3) and to colonies using small bird feeders (Living World[®] Seed and Water Feeder) (Figure A.4). Fresh honey bee collected pollen was purchased from a regional source (Pacific Crest Apiaries, Watsonville, CA). Pollen was prepared as pollen balls using methods of Tasei and Aupinel (2008). Nectar and pollen was provided directly to queens within starter boxes. Nectar was provided to colonies in the foraging chamber while pollen was placed directly within colonies through a round opening in the acrylic cover. Nectar feeders were replaced weekly in both queen starter boxes

and colony nest boxes. Pollen was provided to queens in starter boxes every other day and daily to colonies.

Techniques used to enhance egg-laying and nest initiation

Several methods have been used to stimulate egg laying and nest initiation in the commercially available bumble bee, *B. terrestris*, including adding a male pupa, honey bee worker, bumble bee worker and a bumble bee queen to queen-starter boxes (Ptáček 1985, 1991; Eijnde et al. 1991; Gretenkord and Drescher 1997; Ptáček et al. 2000; Kwon et al. 2003, 2006; Velthuis and van Doorn 2006). However, comparable data for native *Bombus* spp. is limited. Therefore, in addition to documenting a new protocol for rearing bumble bees, I also evaluated the effectiveness of two stimulation methods on the nest initiation of queens: 1) the use of supplemental pupae; and 2) the use of a supplemental queen.

Supplemental pupae

A total of 36 *B. vosnesenskii* queens from a single wild-caught colony were utilized in this experiment. After emergence, queens were placed with a male for mating. Each queen was then put singly into a queen-starter box with one of the following treatments: supplemental egg cup with no pupa (n = 12), supplemental egg cup with a foam pupa (n = 12), and supplemental egg cup with a dead bumble bee

pupa ($n = 12$). Queens were left undisturbed for 48 hours after their initial placement within queen-starter boxes. After this time period, queen-starter boxes were monitored every other day for a period of 21 days by recording the presence of egg clumps on or near pupae and mortality of queens. At the end of the experiment, all queens which did not lay eggs were released.

Supplemental queen

A total of 39 wild-caught *B. vosnesenskii* queens were utilized in this experiment. The treatments consisted of: solitary queen ($N = 10$), two queens (queen-queen) ($N = 10$), and a solitary queen with a single, wild-caught *B. vosnesenskii* worker (queen-worker) ($N = 9$). Similar to the supplemental pupae experiment, queens were left undisturbed for 48 hours after their initial placement within queen-starter boxes. After this time period, queen-starter boxes were monitored every other day for a period of 21 days by recording the presence of egg clumps and mortality of queens. At the end of the experiment, all queens which did not lay eggs were released.

Summary

A total of 432 queens were collected and used for rearing bumble bee colonies (Table A.1). Of these, 191 produced eggs, whereas 169 died prior to egg production

and 72 were released. The average time for queens to lay eggs varied from 1 to 18 days depending on the species. A total of 108 colonies were produced in 2010.

Several interesting observations made during these rearing efforts serve as indicators of broody behavior and subsequently, egg-laying by queens. The first sign of broody behavior in queens involved a change in their behavior. After their initial placement within the nest box, queens spent a considerable amount of time searching. However, queens that were within 1-2 days of laying eggs began to show aggressive behaviors when nest boxes were opened for feeding. These behaviors included loud buzzing and displaying defensive postures of showing their stinger and lifting their middle legs.

The next behavior involved the manipulation of nesting material. Broody queens were observed to mold and shape nesting material with their hind legs prior to egg-laying. These queens usually laid eggs within 1-2 days after this behavior was observed. However, queens that created small tunnels in the nest material never laid eggs. Additionally, the use of wax nectar pots was also an indicator of broody behavior and subsequently egg-laying. Queens were observed to either recycle the wax from the nectar pots and create their own wax nectar pot or augment the existing nectar pot with wax that they secreted and then use it as a storage receptacle for nectar. All queens that augmented or utilized the wax nectar pots laid eggs.

The last indicator of broody behavior occurred when queens were observed to rub their abdomen on the foam pupae provided in the supplemental egg cup in queen-starter boxes. Queens which displayed this behavior either laid eggs on top or in close

proximity to the foam pupae. Egg-laying typically occurred within 2-3 days after observing this behavior. All of the behaviors described above were observed for all *Bombus* spp.

Supplemental pupae

There was no egg production by the queens observed in this study. Queen mortality was 86.11%. One possible explanation for these results could be due to poor mating of queens in captivity. This was the first documented attempt to mate *B. vosnesenskii* queens in captivity, maybe queens need larger areas for mating including a space that would allow for flight.

Supplemental queen

In contrast to the supplemental pupae study, 27.58% of queens laid eggs. Egg production occurred more frequently in the queen-queen (17.24%) compared to the queen-worker (10.34%) and solitary-queen treatments (0%). The average amount of time for egg production varied. Queens in the queen-worker treatment (13.33 days) displayed the shortest amount of time when compared to the queen-queen treatment (18.00 days). Overall, queen mortality was 48.71%. The queen-worker (7.69%) treatment had the lowest amount of mortality when compared to the solitary queen (15.38%) and queen-queen (25.64%) treatments. The results indicate that the queen-

queen and queen-worker treatments were the most effective methods of stimulating egg production. However, given the high mortality of queens in the queen-queen treatment, the queen-worker treatment was a more suitable method of nest initiation. The mortality rate for the queen-queen treatment could be reduced if queen-starter boxes are monitored daily for the presence of broody behavior. Once broody behavior is observed by a queen, then the queens could be put into separate containers, thereby preventing queen death.

Future research

Overall, this research provides insights into methods that can be used to successfully rear western *Bombus* spp. in captivity. The results of this research illustrate the need for large numbers of queens at the start of rearing endeavors given the high mortality rate of queens and low success rate of colony production involved in rearing bumble bees in captivity. Mortality in queens could be due to the incidence of disease and parasites in wild-caught queens prior to their introduction into the rearing facility. Future research should evaluate pathogen loads of queens used in rearing efforts and combine this information with their egg-laying and nest initiation behaviors. Also, additional research should be conducted to examine the mechanisms used in these studies on other *Bombus* spp. as well as other possible mechanisms that are commonly used to stimulate egg-laying and nest initiation in bumble bees.



Figure A.1. Plastic queen-starter box setup used for *Bombus* spp. queens.



Figure A.2. Wooden nest box setup used for *Bombus* spp. colonies.



Figure A.3. Modified petri dish feeder used to supply nectar in queen-starter boxes.

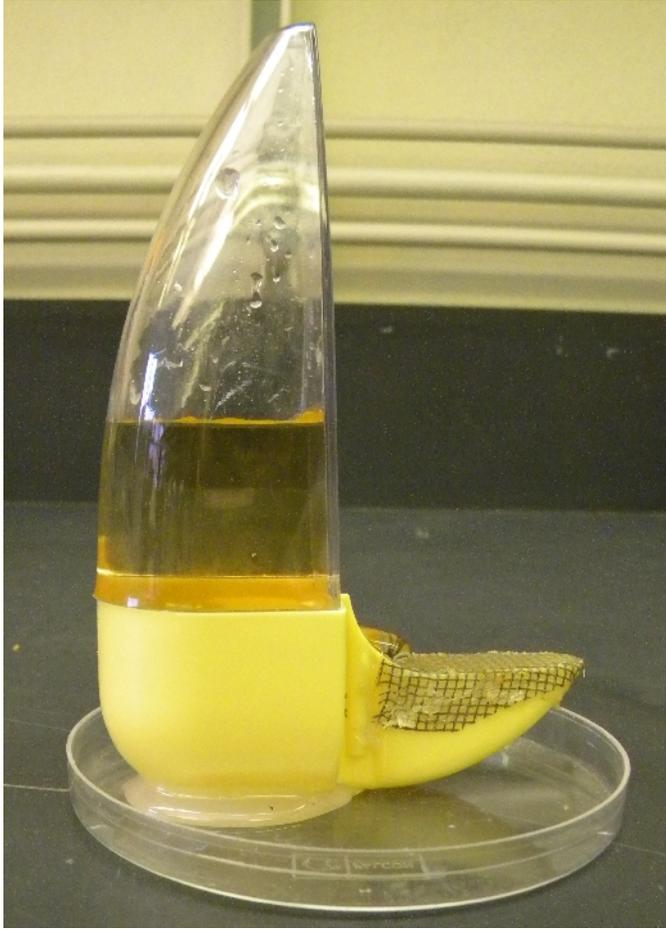


Figure A.4. Modified bird feeder used to supply nectar in the foraging chamber of colony nest boxes.

Table A.1. Western *Bombus* spp. queens used for rearing colonies during 2010.

<i>Bombus</i> spp. queen	Number of queens collected	Number of queens released	Number of queens that died prior to egg-laying	Number of queens that laid eggs	Number of colonies produced
<i>B. vosnesenskii</i>	360	68	147	145	82
<i>B. melanopygus</i>	33	2	13	18	13
<i>B. mixtus</i>	15	0	0	15	6
<i>B. californicus</i>	8	0	4	4	1
<i>B. griseocollis</i>	9	1	2	6	3
<i>B. nevadensis</i>	6	1	3	2	2
<i>B. appositus</i>	1	0	0	1	1
Totals	432	72	169	191	108