Pollination by four species of bees on highbush blueberry

by

Margriet Hilda Dogterom

B. Sc., Simon Fraser University, 1977

M. Sc., Simon Fraser University, 1991

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in the Department

of

Biological Sciences

SIMON FRASER UNIVERSITY

August 1999

Abstract

Four bee species of bees were examined to determine their biological and economic suitability as pollinators of one highbush blueberry, *Vaccinium corymbosum* L. (Ericaceae) var. 'Bluecrop'. Pollination effectiveness depends on how well bee behaviour is suited to flower morphology. Successful pollination of a crop by bees also depends on economic factors such as cost, availability and management requirements of bee species. Pollen loading on flowers by bee species plus data from greenhouse experiments on pollen loading on flowers was used to calculate the value of each bee. Bumble bees deposited significantly more blueberry pollen grains and spent 50% less time on flowers than honey bees during 3 years of this study. More bumble bees (62%) and mason bees (86%) than alfalfa leafcutter bees (36%) and honey bees (7%) (both pollen and nectar foragers) had adhering blueberry pollen on their bodies when collected in front of their nests. However, both field observations and cost estimates indicate that honey bees, *Apis mellifera* L., are the most economical bee for pollinating highbush blueberry although not the most biologically suited. Bumble bees (*Bombus* spp.) are the most biologically suited, but not economical. Similar to bumble bees, mason bees, *Osmia lignaria propinqua* Cresson, are effective pollinators but are costly, although less costly than bumble bees. Alfalfa leaf cutter bees, *Megachilidae rotundata* F., were not suitable for blueberry pollination because they required temperatures >18°C for foraging. Greenhouse experiments indicated that blueberry flowers with 106 ovules required 125 tetrads of 50-100% outcross pollen < 3d old for optimal fruit quality. Raising the pollen load to 300 tetrads did not improve fruit quality (fruit weight, fruit set, or time to ripen). A honey bee colony experiment with pollen-rich and pollen-poor stores in large and small colonies indicated that visitation of blueberries was ~~is~~ not enhanced by pollen store manipulations. The increase in proportion of pollen foragers did not benefit blueberry flowers because only 7.6% of pollen foragers carried *Vaccinium* pollen. Manipulations of colonies that promote nectar foragers would enhance visitation because 60.8% of the nectar foragers had *Vaccinium* pollen on their bodies. Mason bees could be a valuable alternative or supplementary pollinator for highbush blueberry, although not biologically or economically the best bee species. In addition, mason bees can be collected from the wild, although with significant effort. These results have elucidated why some bees are better pollinators than others of a crop and why cost is a critical factor in determining which bee provides the most cost-effective pollination.

Dedication

To my friends

In nature's infinite book of secrecy

A little I can read.

Shakespeare, Antony and Cleopatra I

Acknowledgements

My original thoughts on what I wanted from a Ph.D. thesis was to study in depth, unravel details, and gain a greater understanding of a pollination-related topic. I quickly realized that a Ph.D. is not a solo endeavour. I am very grateful to the many individuals who helped me along the road to my Ph.D. Without their support, I could not have transformed my ideas into reality.

I am indebted to Mark Winston, my senior supervisor, for his unwavering support, his guidance throughout the thesis process and his enthusiastic and well appreciated editing. I also am grateful to my supervisory committee for their diligent and constructive editing: John Borden for his enthusiasm about new ideas and how to present them; Jim Rahe for his questions that challenged me to look at problems from another angle. In addition my sincere thanks to Jim Cane and Zamir Punja for their valuable comments.

I was assisted with the field work by present and former members of the 'Swarm Team' and students of the 'Borden -Winston lab' at Simon Fraser University. In particular, I would like to thank the following: J. A. Bunse, J. Burleigh, S. Devlin, D. Downey, L. Foster, T. Frizzell, J. Gates, T. Geernaert, B. Harris, H. A. Higo, T. Higo, S. Kuhnholz, A. F. Janmaat, P. H. LaFlamme, H. Lin, A. P. Melathopoulos, J. McCarthy, J. Macdonald, J. Pettis, M. Sabo, T. Smith, A. and R. Stone, J. Taylor, C. Watson, R. Welland and J. Whitehead. A very special and warm thanks go to A. Mukai who was invaluable with her skills and talents, including tenaciousness, perseverance and an eye for detail.

I thank J. Pettis T. Pankiw, D. Miller and H. Higo for sharing their ideas on the pollination of highbush blueberry, K. Hodges for her clear thoughts on what a thesis actually is and Doug Talling on his expertise in statistics. I would like to thank J. Gaskin who is a staunch supporter of research and who enthusiastically encouraged the research on his property. Thanks also go to R. Matthews for help with pollen identification, S. P. Vander Kloet for advice on seed germination methodology, M. Makara and J. Gaskin for identification of blueberry varieties; Casino Tropical Plants Ltd. for providing plants in 1996 and 1997 and Gaskin Farms Ltd. in 1997 and the many landowners for permission to carry out mason bee research on their property.

Financial and in-kind support for my research and graduate studies were provided by Simon Fraser University., the Blueberry Research Council of B.C., the B.C. Honey Producers Association, G. Kern, Peace River Apiaries, B. C. Ministry of Agriculture, Fisheries and Foods, Agriculture Canada Applied Research Partnership Program, and the Natural Sciences and Engineering Research Council of Canada.

I also would like to thank my extended family of the dance community who supported me through their warm encouragement and continued interest throughout my tenure because they understand, and in particular Pablo and Andrew.

In particular, I thank Tim for his overwhelming moral support for the road that I chose to follow. His zest for life and his thoughtfulness has had an immense impact on me during my tenure. I also thank my family for their support and in particular to my parents for what they taught me, because this backbone of knowledge has given me the strength and vision to complete the work.

Table of Contents

List of Tables

List of Figures

Chapter I

General Introduction

Pollination is one of the most fascinating of biological interactions, occurring when pollen is transferred from anther to stigma; it is a requirement for ovule fertilization, seed growth and fruit set in most flowering plants. Many plants are bee-pollinated, and pollinators are rewarded with food in the form of pollen and nectar secreted by flowers. At the evolutionary extreme, this mutually beneficial relationship between flowers and their pollinators has co-evolved to form a specific, obligate relationship between some species such as orchids and their long tongued euglossine bee pollinators (Darwin 1876). However, not all pollinators have such a closely linked relationship. Two models of pollination systems are the 'pollination syndrome' where floral characteristics are associated with a certain pollinator group (Faegri and Van der Pijl 1979), and where flowers are pollinated by diverse pollinator species (Waser et al. 1996).

My thesis determines the biological and economic suitability of four species of bees as pollinators of highbush blueberry, *Vaccinium corymbosum* L. (Ericaceae), var. 'Bluecrop'. Highbush blueberry is an economically important crop in North America, with a worldwide yield of 85 million-kg (Eck 1988). It depends on insect pollination for fruit set (Free 1993). Historically, the honey bee has been the major pollinator of blueberry, but I address the question: "Is the honey bee the best pollinator for highbush blueberry?"

The foraging behaviour of insects and morphology of flowers and bee determines the efficiency of transfer of pollen from stamens to stigma and therefore the effectiveness of bees as pollinators (Thorp 1979). In open type flowers, there are landing platforms for pollinators to collect nectar and scrabble over the anthers to collect pollen, resulting in pollination. In blueberry, the fused petals form a partially open umbrella (Appendix). Insect pollinators grasp this corolla by hanging upside down and attempt to obtain pollen and nectar from inside the flower. Pollen is released from the anthers through apical pores. These poricidally dehiscent anthers of blueberry can be vibrated by bumble bees but not honey bees. "By rapidly contracting and relaxing their large indirect flight muscles, uncoupled from the axillary sclerites and the regular flight musculature, bumble bees

transmit strong vibrations throughout their bodies" (Buchmann 1983). These vibrations are called "buzz pollination," and induce pollen grains to leave the anther cone in a cloud.

Generally, highbush blueberry flowers are white and consist of petals that are united to form a bell-shaped corolla 6-12 mm long (Appendix). The corolla is the most visible aspect of the flower and conceals the nectaries at the base of the corolla (Knuth 1906). The corolla encloses the single style that has a stigma at the tip and ovules inferior to the base, and is surrounded by 10 male stamens that bear anthers containing pollen grains. The pendent flowers are clustered, increasing their visibility to potential pollinators and their probability of pollination. The ovule is developed from cells that undergo meiosis. Pollen grains are produced by mother-cells within the anthers that undergo reductive division (meiosis), each producing a tetrad of four haploid cells or microspores. Pollen germinates after it lands on the stigma by first absorbing water and producing a pollen tube. The pollen tube grows down the style and enters the ovule. The fertilized ovule then develops into a young embryo and into a seed, accompanied by fruit growth (Eck 1988; Galletta and Balllington 1996). Generally, *Vaccinium* spp. has 5 carpels, and 25 ovules in each carpel for *Vaccinium corymbosum* (Palser 1961).

Bees are the only significant pollinators of blueberry, and many species visit commercial plantings of highbush blueberry. Bees such as honey and bumble bees are social, with colony populations in the hundreds or thousands, and carry pollen back to their nest in their corbiculae (pollen baskets) on their hind legs (Winston 1987). However, the majority of bees are non-social or solitary. Solitary bees carry pollen back to the nest on groups of plumose hairs called scopae, on the legs, thorax or abdomen. Nectar is sometimes added and mixed with the pollen to enable better packing.

Pollination is an important biological and economic component of agriculture and crop production. In the United States 150 crops in 40 plant families require bee pollination (McGregor 1976; Southwick and Southwick 1992). The annual benefit of honey bee pollinated crops to U.S. agricultural consumers was estimated as Can. $2.5 - 12.7 billion

(U.S. $1.6 - $8.3 billion)(Southwick and Southwick 1992); the benefit to Canadian consumers was estimated as Can. $0.5 billion ($U.S. 0.325 billion)(Charest and Hergert 1993).

Crops at the turn of the century were pollinated by unmanaged wild bees. Blooming crops occupied relatively small areas and could easily be reached by bees from their nesting sites. But the advent of extensive monoculture, which involves large tracts of cultivated land, has reduced wild bee visitation of crops since the distance between crops and nests can be large. In addition, large-scale agriculture has diminished bee populations by removing nesting habitat and through the use of insecticides. Thus, it is not surprising that low bee numbers and low wild bee diversity are characteristic of managed fields due to cultivation practices (Winston and Graf 1982; MacKenzie and Winston 1984; MacKenzie and Eickwort 1996).

Growers normally rent honey bee colonies to pollinate blueberries because honey bees improve fruit set and yield (McGregor 1976; Free 1993). However, honey bees do not pollinate all blueberry varieties equally well (Dorr and Martin 1996). Incompatibilities between varieties due to differing ploidy levels and close genetic relationships are reasons pollination does not result in fertilization nor fruit set (El-Agamy et al. 1982; Hancock and Siefker 1982; Hellman and Moore 1983; Krebs and Hancock 1990). Low numbers of pollinators also can result in poor fruit set (Martin 1966; Brewer and Dobson 1969; Brewer et al. 1969). Low bee numbers in blueberries could be the result of poor attractiveness of the target crop (Dorr and Martin 1966), unsuitable flower shape for the bee visitor (Eck and Mainland 1973; Lyrene 1994), but generally not nectar availability (Wood et al. 1967).

Managed pollinators generally are brought into crops to enhance pollination. Honey bees, *Apis mellifera* L., historically have been the only managed bee species that could be rented and moved into a crop quickly and economically. Recently, however, honey bee colony availability has been placed in jeopardy due to parasitic mite and predatory beetle infestations and the impending threat of the Africanized bees in North America (Torchio

1990a; Winston 1992; Moffett 1999). The uncertainty of honey bee colony availability has increased growers' interest in alternative pollinators.

Four taxa of alternative pollinators have been used for commercial pollination of selected crops. These species are biologically suited for their respective crops, and can be managed in large populations. Alfalfa leaf cutter bees, *Megachile rotundata* F. and alkali bees (*Nomia melanderi*), are used extensively to pollinate alfalfa in North America (Peterson et al. 1992), and bumble bees, *Bombus* spp., are the sole pollinator of tomatoes in greenhouses worldwide (Dogterom et al. 1998). Mason bees, *Osmia* spp., are used to pollinate tree fruits in Japan, although they are not yet available in large numbers in North America.

Pollinator qualities vary between species. Bumble bees buzz pollinate whereas honey bees, alfalfa leaf cutter bees and mason bees do not (Buchmann 1983), although they can drum or batter the anthers using their legs (Cane et al 1993). Bumble bee buzzing behaviour is well-suited to pollen collection from blueberry flowers with poricidal dehiscent anthers, and bumble bees fly at the cool temperatures that are common during blueberry bloom. In contrast, honey bees communicate and recruit to competing blooms, but their inability to buzz pollinate would suggest that they may not be the best highbush blueberry pollinator. The shorter foraging distance of many solitary bees suggests that bees such as *M. rotundata* and *Osmia* spp. would stay in the crop. Bumble bees, leafcutter bees and mason bees do visit blueberry flowers (Cane et al. 1985; Cane and Payne 1988), as do honey bees (Free 1993).

Generally, fidelity to flower species by honey bees is high (Free 1963) and tendency to fly in the same direction of their previous flight also has been observed (Thorp 1979). However, the honey bee dance language, used to communicate flower quality, can direct foragers away from the crop when scouts detect and report higher quality forage. In contrast, bumble bees do not have a dance language, with each individual continuously monitoring available flowers (Heinrich 1979). The solitary alfalfa leaf cutter and mason

bees do not communicate alternative forage sources but may monitor like bumble bees. In addition, foraging behaviour for a flower type is learned by individual bees of all species. Over time, bees become increasingly efficient and thus spend less time collecting the same amount of pollen, thereby increasing the number of flowers visited in an hour.

Although pollination of plants by insects represents a major interface between insects and plants, pollination studies rarely examine both the plant's requirements and the bee's ability. Studies are generally focused on either an insect or plant perspective, perhaps depending on whether the researcher is an entomologist or a botanist. Research on insects usually addresses some aspect of bee behaviour and how it affects foraging or colony development. Insect species may be compared by assessing their pollen deposition, speed of pollination, field distribution, foraging distance and flower constancy on a particular plant species. In contrast, research on plant reproduction often examines processes of evolution (pollen competition, pollen limiting systems, selfing *versus* crossing, and genetic transfer to progeny) and sexual selection (male selection and female choice). Crop pollination from an entomological perspective often deals with the result of insect behaviour, whereas from the botanist's perspective, studies focus on genetic incompatibilities, but rarely are both the requirements of the plant and the ability of the pollinator examined together.

My general objective was to examine pollination of highbush blueberry from the perspectives of both plant and pollinator. Specifically, my objectives were to:

1) determine the potential of four bee species for commercial pollination of highbush blueberry var. 'Bluecrop' by examining stigma pollen loads, proportion of bees with blueberry pollen, factors important for bee activity, distribution of bees from their nests and relationship of bee density and berry weight;

2) examine the number and blueberry variety of pollen grains required by individual highbush blueberry flowers for optimal fruit set and weight, seed number and days to ripen, and also progeny fitness based on seed germination;

3) investigate if pollen deprivation in small and large honey bee colonies would increase the proportion of pollen foragers, the proportion of blueberry pollen carried by foragers, and the weight of pollen carried per bee, and also determine if both nectar and pollen foragers carry blueberry pollen and in what proportions to other pollen types; and

4) elucidate locations in southern B. C. where native populations of the mason bee, *Osmia lignaria propinqua*, are concentrated, nest type and height preferences, period of nest building activity, rate of emergence at different temperatures and the sex ratio of wild mason bees.

Chapter II

Comparison of four bee species as pollinators of highbush blueberry, *Vaccinium corymbosum* L. (Ericaceae) 'Bluecrop'

INTRODUCTION

Constraints in behavioural, physiological and morphological characteristics limit foraging behaviour of bees on flowers. These characteristics determine stigma pollen loading, foraging distance from the nest, optimal foraging temperature, and floral preferences that can influence the success or failure of pollination, and thus fertilization and seed set. Crop pollination could be improved if the best pollinators were identified by determining foraging characteristics of commercial available bee species (Torchio 1990a; 1991a, b; 1994).

Highbush blueberry, *V. corymbosum*, is an economically important crop in North America that depends on insect pollination for fruit set (McGregor 1976; Free 1993). Low numbers and diversity of wild bee species typify managed fields due to cultivation practices (Winston and Graf 1982; MacKenzie and Winston 1984; MacKenzie and Eickwort 1996). Therefore, managed pollinators generally are brought into the crop. Until recently, honey bees, *A. mellifera*, have been the only commercially available bee species, but unpredictable yields (Free 1993) and decreased colony availability due to parasitic mite infestations have increased interest in alternative pollinators. Bumble bees, *Bombus occidentalis* Greene, and alfalfa leaf cutter bees, *M. rotundata*, currently are available for managed pollination of some crops, and other pollinators such as mason bees, *Osmia* spp., are used commercially in Japan (Maeta and Kitamura 1964, 65, 1974, 1981). Research

comparing a variety of pollinator species is needed to determine the best selection of pollinators for commercial blueberry production.

Honey bees (10,000 - 40,000 bees per colony) and bumble bees (200 - 1000 bees per colony) are social insects that live in nests, and work together to rear young, feed colony members and reproduce (Michener 1974). Although honey bee colonies can be brought into a field to pollinate a crop, honey bees may find and communicate forage locations of more attractive forage plants than blueberries, causing a decrease in bee activity and inadequate pollination on the desired crop.

Alfalfa leafcutter bees and mason bees (*Osmia lignaria propinqua* Cresson) are both univoltine solitary bees (Megachilidae) that emerge in the spring or summer, mate and lead a solitary life of provisioning a series of nest cells with pollen and nectar, giving each an egg and plugging up the nest to protect the young. Both species can be moved into a field for pollination, and unlike the honey bee, show good fidelity to their respective floral hosts (Michener 1974).

These two social and two solitary bee species are the most significant managed bee species in different parts of the world for crop pollination, but no studies have compared all four species of bees simultaneously on any crop. Generally, one species (and rarely two species) of managed pollinator has been investigated on a crop. Honey bees have been studied on many crops (Free 1993), including highbush blueberry (Danka et al. 1993; Chapter IV), and more recently, commercially reared bumble bees have been investigated for lowbush blueberry pollination (Whidden 1996). Commercially reared alfalfa leaf cutter bees have been investigated for legume forage crops (Richards 1991; Peterson et al. 1992) and native mason bees have been tested for pollination of almonds (Torchio 1982b), blueberry (Torchio 1990b), prunes (Torchio 1976) and apples (Torchio 1984. 1985; Jacob-Remacle 1989). A few studies have compared honey bees and commercially reared or wild bumble bee foragers on clover (Michaelson-Yeates et al. 1997), apple (Kendall and Solomon 1972; Goodell and Thomson 1997), and pear (Mayer and Lunden 1997). Bumble

bees, leafcutter bees and mason bees do visit blueberry (Cane and Payne 1988; Cane et al. 1985), and honey bees are well-known to visit blueberry (Free 1993).

It is a daunting task to investigate all aspects of pollination. Therefore, studies of cultivated crops generally examine selected aspects of pollination by a particular bee species, such as pollen deposition (Danka et al. 1993; Carre et al. 1994; Cane et al. 1996, Chapter II), bee distribution (Gary et al. 1972), foraging distance (Abrol and Kapil 1994; Aras et al. 1996; Dag et al. 1998), flower constancy (Cane and Payne 1988; Cripps and Rust 1989; Freitas 1997) and pollen source (Free and Williams 1974).

My objective was to compare the potential of four bee species for pollination of highbush blueberry var. 'Bluecrop' by examining some important aspects of their foraging behaviour. I compared honey bees, bumble bees, *B. occidentalis*, and alfalfa leafcutter bees, *M. rotundata* which are commercially available and managed in North America, and mason bees, *O. l. propinqua*, which can be managed. My specific objectives were to determine the: 1) mean stigma pollen load deposited per bee visit; 2) proportion of bees with blueberry pollen adhering to their bodies; 3) effect of climate and nectar availability on bee flight to colonies and nests; 4) distribution and density of bees in a field adjacent to nest sites; and 5) relationship between density of bees and weight of berries. A secondary objective was to compare the costs of the pollinators under investigation.

METHODS

Research was conducted during 1995-97 on highbush blueberry variety 'Bluecrop', at a commercial farm in Coquitlam, British Columbia, Canada (Lat. 49° 17', Long. 122° 43'). Approximately 50% of blueberry plantings in western North America are 'Bluecrop' (Moore 1993).

Honey bee colonies, commercially produced colonies of *B. occidentalis*, and nests with newly emerged *M. rotundata* and *O. l. propinqua* were placed around a 110 x 518 m blueberry field (5.7 ha) consisting of 36 rows of 400, 20-year-old blueberry bushes (Fig. 1). Nine honey bee colonies plus 50 colonies in an adjacent field were located on the west side of the field, 220 m from the NW corner and 300 m from the SW corner. Five commercially produced (Koppert Biological Systems, Michigan) bumble bee colonies were placed 62 m from the NE corner of the field. Alfalfa leaf cutter bees (16,000 cocoons) were obtained from International Pollination Systems, Manitoba, Canada, and incubated at 30°C for 26 days. Adults were released in front of wooden nest tunnels located in a four-sided shelter placed at the NE corner, 62 m from the bumble bee colonies. Mason bees were trap-nested from Vancouver Island and the Lower Mainland of British Columbia in 1994 (6,000 cells), stored over winter and incubated in a cage at 30°C for 3 days until emerged. Half were released in front of nest tunnels (15 cm long, 7.5 mm diam. cardboard straws with blind ends) in small shelters 40 m from the NW corner of the field (30 m from bumble bee colonies), and the other half released 133 m from the SE corner of the field. Alfalfa leaf cutter, mason bee and bumble bee nests and colonies were located at one end of the field to maximize the number of bee species in one area for sampling. Location of honey bee colonies was less critical for ease of sampling because honey bees are more numerous and their foraging range extends to the whole field. Thus, honey bees were located adjacent to the field and away from other bee nests and colonies.

Stigma Deposition of Blueberry Pollen

One branch with flowers in bud was selected from each of 150 plants within 50 m of the north end of the field. Plants were selected from more than 10 of the 42 rows of plants. Each branch was bagged with polyester bridal veil material and carefully unbagged one at a time when bloom was > 50%. Visits by bee foragers to individual flowers were

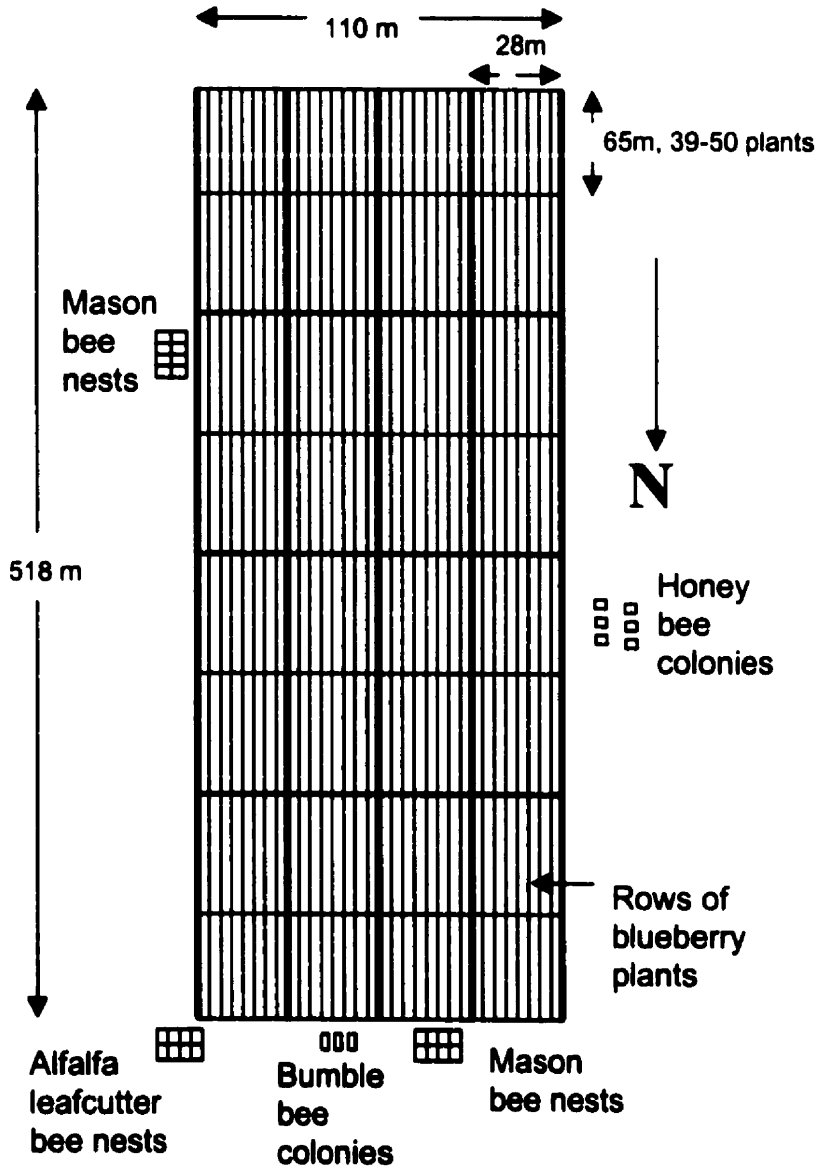

Figure 1. Nest and colony placement in research field.

timed, forager type identified and up to three visited flowers marked with a pen when the forager left. Each visited stigma was removed with a fine-tipped tweezers, pressed three times into fuchsin gelatin previously melted on a glass slide, and left in the gelatin (Kearns and Inouye 1993). A glass coverslip was placed over the gelatin and the slide was heated over an alcohol burner to fix the coverslip onto the melted gelatin. Slides were stored and at a later date, all blueberry pollen tetrads on each slide were counted.

Percent Bees with Blueberry Pollen

Samples of all four types of bees were collected into 142 mm diameter petri dishes during blueberry bloom at nest entrances in 1995 (2, 7, 8, 12, 13 May), and 1996 (21, 24, 26, and 28 May, and 1, 3, 4, 5, and 6 June), fast frozen in the field, placed into individual vials, and stored frozen until processed. Each bee was placed into a centrifuge tube and covered with 90% ethanol and sonicated ("Tru-Sweep" ultrasonic cleaner, Crest Ultrasonic Corp., Trenton, NJ.) for 20 min to loosen any adhering pollen. The bee was removed, the remaining ethanol centrifuged for 20 min, the ethanol decanted and the pollen pellet placed within the boundaries of a wax pencil circle marked on a glass slide. The slide was warmed on a hot plate until the residual ethanol evaporated. A small piece of fuchsin-gelatin (ca. 5 x 5 x 5 mm) was placed over the dried sediment and a glass coverslip added when the fuchsin gelatin had partially melted (Kearns and Inouye 1993). Slides were then cooled and stored. For each sample, along adjacent perpendicular transects, 300 pollen tetrads were examined and identified as blueberry (tetrad) or non-blueberry (non-tetrad). Additional foraging honey bees and bumble bees were collected in 1997 from the field of blooming blueberry bushes (11-17 May 1997) and processed similarly.

Climatic Factors, Nectar Availability and Bee Activity

Observations of bee activity (bee flight to colonies and nests), temperature, and relative humidity were made every 2 h from 0800 - 1800 h on 8, 13, 16, 24 and 28 May 1996 at six different honey bee and five bumble bee colonies and nesting sites for alfalfa leafcutter bees and mason bees. In addition 10 reps of 10 flowers were sampled for sugar availability every 2 h from randomly selected bushes. Bee activity is defined as the number of bees entering a nest or colony per unit time. Nests for alfalfa leaf cutter bees consisted of boxes with grooved boards made from wood (Richards 1984); 320 nesting holes were monitored at any one time. Mason bees were monitored in front of bundles of cardboard straws, 360 straws for each observation period. Honey bee, alfalfa leafcutter bee and mason bee activity was recorded for 1 min; 2 min counts were made for bumble bees, because of less entrance traffic. Four observers were randomly assigned to colonies or nests every 2 h. Counts were standardized as bee activity per min. Sugar produced by blueberry flowers and available as nectar to bees was sampled by collecting 10 flowers from a randomly selected blueberry plant every 2 h. Ten flowers were shaken in 10 mL of de-ionised water (10 blossoms per 10 mL of water) for 5 sec, removed and the remaining liquid stored frozen. Sugar was analysed by a phenol-acid test (Chaplin and Kennedy 1994).

Bee Surveys

The field was surveyed for honey bees, mason bees, leaf cutter bees, wild bumble bees and commercially-produced bumble bees along rows of blueberry bushes on 6, 13, 14, and 15 May 1996 between 1100-1510 h. The commercially produced bumble bees from California were distinguishable from wild *B. occidentalis* by the presence of yellow on the abdomen, and the absence of white at the distal end of the abdomen (Thorp et al. 1983). Field width was divided into four sections (28 x 518 m), with

8-9 rows per section. Each section was divided into eight blocks, 65 m long for a total of 32 blocks, each block 65 x 28 m. Each block was surveyed six times on each date along randomly chosen rows by six rotated observers. Ninety-two rows were surveyed over the study period. At any one time, one row was surveyed in each of four sections over a mean time (± SE) of 24.6 ± 0.6 min (range = 17 - 41 min). The data were pooled across days and the sum of bees observed and mean bee density were calculated for each block.

Density of Bees and Berry Weight

Berries were sampled from all blocks of the field on 23 and 26 July and 6, 12, 14 and 19 August 1996. One hundred berries were sampled from 32 randomly selected bushes evenly spread through each block. The branch closest to an approaching observer was selected, and berries were picked progressively from the end to the base of this branch, stored at 4-7°C, and weighed within 24 h of picking. The data were pooled across days and the mean berry weight calculated for each block.

Statistics

Unless noted otherwise, SAS software was used for GLM procedures (SAS Institute 1990). Deposition of blueberry pollen on individual flowers, and repeated in each of three years, was analysed using the GLM procedure after transformation to meet assumptions of ANOVA, followed by Ryan's Q test for differences between means at $\alpha = 0.05$ (Day and Quinn 1989). Linear regression using the GLM procedure was used to examine the relationships between activity of bee species *versus* climatic variables and sugar availability. In addition I tested the dependent 'bee activity' variable against the independent variables since I suspected that one or more would improve the prediction of the dependent variable. Bee activity was first tested against the full model which consisted of temperature, time of day, humidity and sugar availability. I used the manual step-wise GLM procedure to test whether more than one variable improved the model, by removing one variable with the highest probability at each

stage, until remaining variable(s) were significant. Variability between bee species for percent bees with blueberry pollen, and for distance and density from nest locations were analysed with the non-parametric Wilcoxon 2-sample test or Kruskal-Wallis test when more than two treatments were tested because distributions were non-normal, followed by Kruskall-Wallis Multiple Comparison test for differences between bee species (Conover 1980). The strength of the relationships between bee densities and fruit weight was examined using Pearson correlation coefficients. In all cases, $\alpha = 0.05$.

RESULTS

Stigma Deposition of Blueberry Pollen

There was an overall significant difference for blueberry pollen deposition by year ($F_{2, 319} = 22.26$, $P < 0.0001$) and species ($F_{2, 319} = 14.45$, $P < 0.0001$) (Fig. 2) with no interaction between these two variables ($F_{2, 315} = 0.89$, $P = 0.4677$). Bumble bees (wild and domesticated) deposited significantly more blueberry pollen grains than honey bees in all years. Mason bees were not sampled in 1996, but deposited similar numbers of blueberry pollen grains as honey bees in 1995 and 1997, and bumble bees in 1995, but fewer than bumble bees in 1997. Alfalfa leaf cutter bees were not observed on blueberry flowers.

Bumble bees spent less time on flowers in 1995 than both honey bees and mason bees, and less than honey bees in 1996 (Fig. 2). 'Time' was not a significant predictor of pollen load deposition ($F_{1,307} = 1.09$, $P = 0.296$).

Percent Bees with Blueberry Pollen

Data from 1995 and 1996 were pooled, because there were no significant differences between years for percentage of bees with blueberry pollen (leaf cutter bee, $n = 82$, $\chi 2 = 2.82$, $df = 1$, $P = 0.0932$; honey bees, $n = 129$, $\chi 2 = 1.985$, $df = 1$, $P = 0.159$; mason bees, $n = 36$, $\chi 2 = 0.016$, $df = 1$, $P = 0.89$;

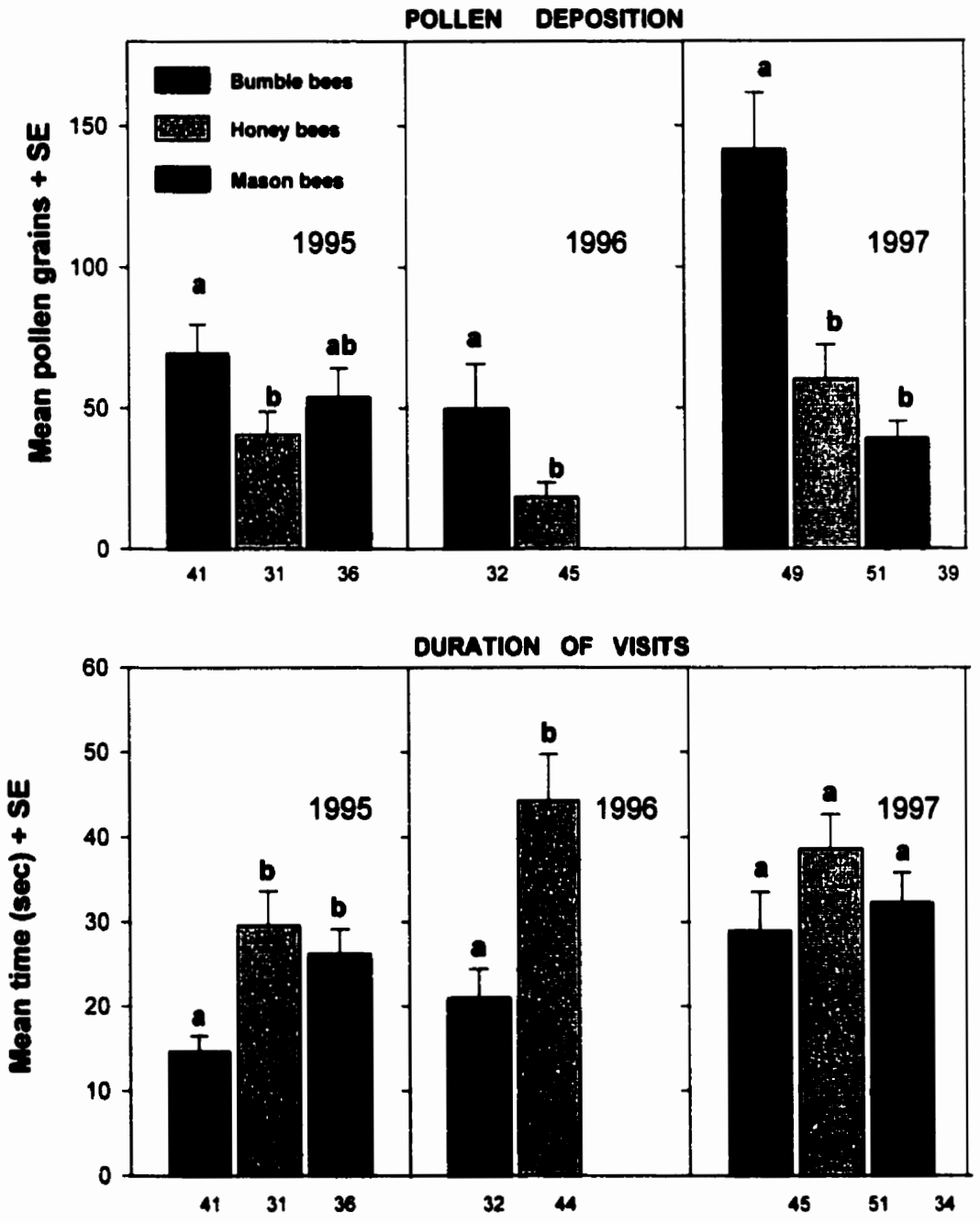

Figure 2. Stigma pollen deposition onto previously bagged flowers visited once by a bumble bee, honey bee or mason bee, and the duration of visits by each type of bee. Means in the same year with same letter did not differ significantly, Ryans' Q test $P < 0.05$. N values are given below bars.

bumble bees, n = 47, $\chi 2 = 2.406$, df = 1, $P = 0.121$). At the nest entrance 86% of mason bees had blueberry pollen, followed by bumble bees (61.7%), alfalfa leaf cutter bees (36.1%) and honey bees (7.0%)(Fig. 3). In contrast, within the blueberry field, almost all honey bees and bumble bees carried blueberry pollen (Fig. 3). Mason bees and alfalfa leaf cutter bees were not collected in the field.

Climatic Factors, Nectar Availability and Bee Activity

In all cases day was not significant, and data were pooled for all sample days. During monitoring of bee activity the ambient temperature range was 7.7- 22°C. Maximum daily temperatures reached 11, 17, 18, 19 and 22°C during the 5-day monitoring period. Ambient temperature was a highly predictive variable for honey bee activity, leafcutter bee activity, and mason bee activity but not for bumble bees (Fig. 4). Time of day was predictive for bumble bee, honey bee, mason bees, activity but not for alfalfa leaf cutter bees (Fig. 4). Relative humidity varied between 40-100% over the 5-day monitoring period, decreasing on a daily basis from >90% in the morning to ≥40% by late afternoon. Increasing humidity was correlated to decreasing activity of bumble bees, honey and mason bees, but not for alfalfa leafcutter bees (Fig. 4). Sugar concentration varied between 137 - 592 *ug* per flower, but was not significantly related to the activity of any of the four bee species examined. The least nectar sugar occurred on the first and coldest day, and the greatest nectar sugar on the day with the warmest temperatures. Sugar generally was higher at the beginning and at the end of each day.

I also examined whether factors in combination would increase the predictive level of the model. Multiple step wise regression indicated that temperature alone was the best predictor of honey bee ($r^2 = 0.76$, $F_1 = 87.27$, $P \leq 0.0001$) and mason bee activity ($r^2 = 0.53$, $F_1 = 30.01$, $P \leq 0.0001$). Temperature in combination with humidity, were better predictors of alfalfa leafcutter bee activity ($r^2 = 0.61$, $F_1 = 10.87$, $P = 0.0014$) than temperature or humidity alone, although temperature effects ($F_1 = 21.09$, $P = 0.0004$) had a greater impact than humidity ($F_1 = 6.21$, $P = 0.03$) on alfalfa leaf cutter bee activity. Bumble bee activity was best predicted by time-of-day and nectar sugar content ($r^2 = 0.85$, $F_3 = 25.71$, $P \leq 0.0001$), with a sugar-time interaction ($F_1 = 10.87$, $P = 0.0053$).

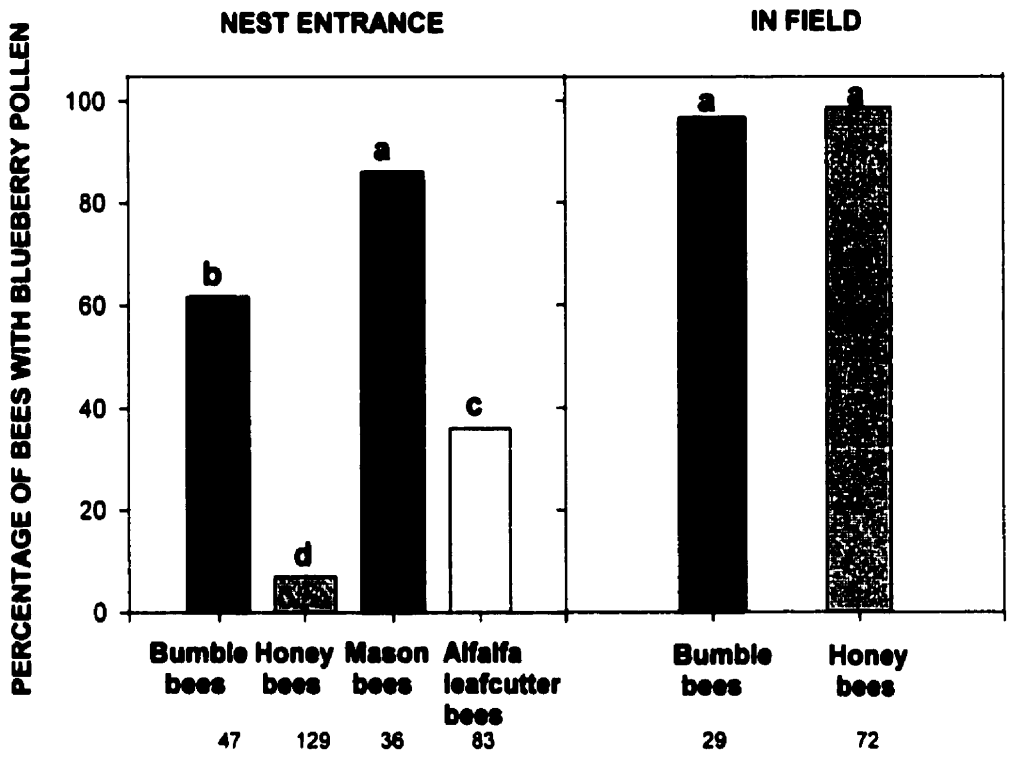

Figure 3. Percentages of bees at nest entrance and in field with blueberry pollen. Percentages within a sub-graph with the same letter were nignificantly different, X^2 test for multiple proportions, $P < 0.05$. N values are given below bars.

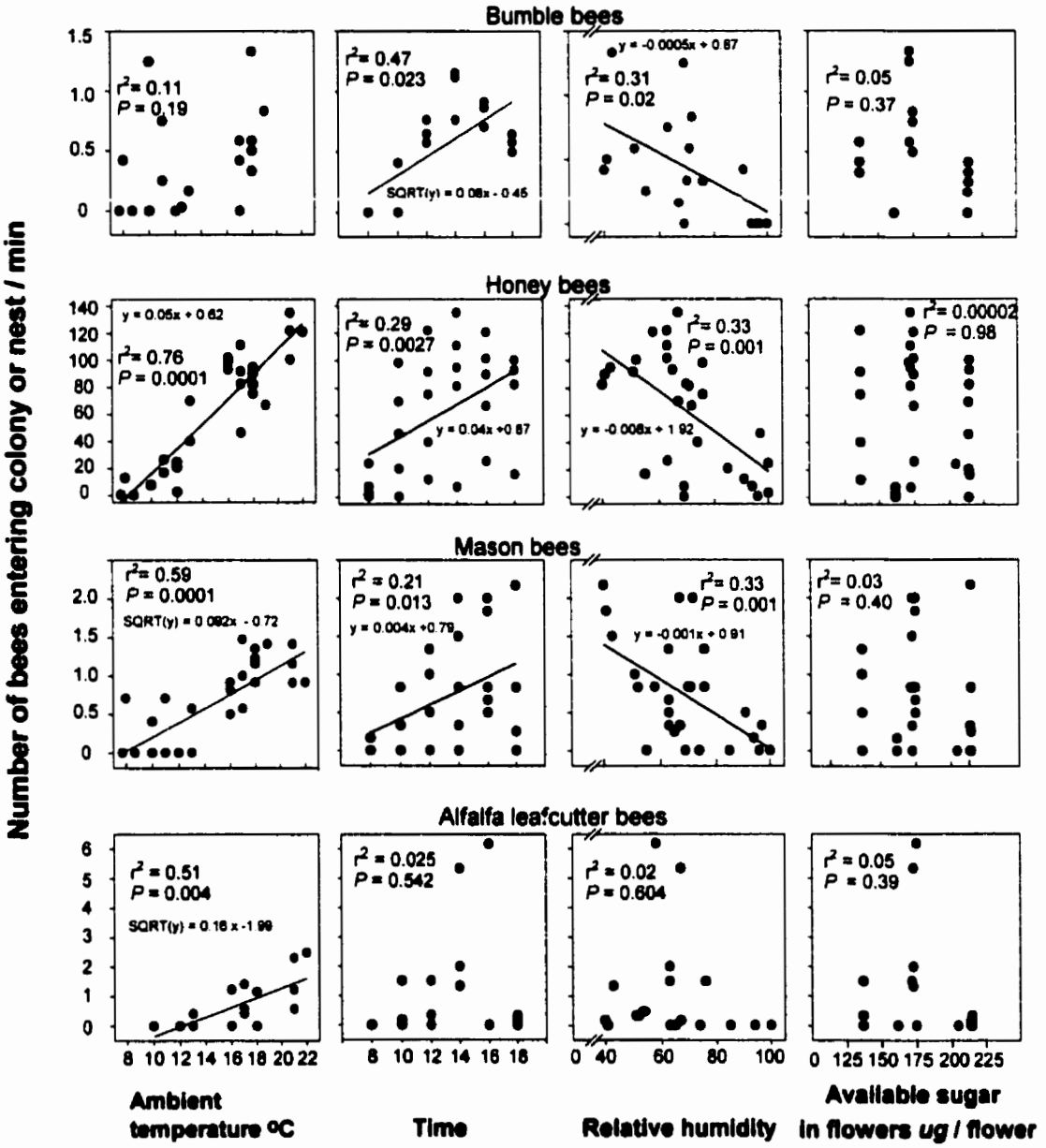

Figure 4. Relationships between bee activity (per min) and ambient temperature, time, percent humidity and available sugar in flowers ug/flower measured every 2 h as compared by linear regression. Square root transformation was used for mason bee and alfalfa leaf cutter bee activity *versus* temperature, and bumble bee activity *versus* temperature.

In this case the time variable was a stronger predictor of bumble bee activity ($F_1 = 18.47$, $P = 0.0007$) than sugar ($F_1 = 4.54$, $P = 0.0512$).

Bee Surveys

Temperatures during surveys ranged between 12 - 17°C and percent open bloom was 9% (n = 859) on 6 May and 42% (n = 606) on 15 May. Frequency distributions of distances of bees from nest sites were significantly different between species ($\chi 2 = 34.3$, $P \leq 0.0001$) (Fig. 5). Mean distances flown from nest sites were 138.9 m for honey bees, 133.8 m for bumble bees and 70.7 m for mason bees (Fig. 5). Distance flown from nest sites by alfalfa leaf cutter bees is not known since these bees were not observed on blueberry flowers. Since bee-distance distributions were highly skewed, median values are better than means as indicators of central tendency. Median distances flown from nest sites were shorter for mason bees (58 m), than bumble bees (68.5 m), and longest for honey bees (130 m). Most mason bees were observed <150 m from the nest and the furthest observation was 234 m from the nest. The furthest observations of commercial bumble bees and honey bees foraging from the nest were 495 m. My observations were limited to the 110 x 518 m field of blueberries and thus did not observe the maximum distance of honey bee and possibly bumble bee foraging.

Bee density was significantly different between species ($\chi 2 = 59.657$, $P \leq 0.0001$). Mean bee density was significantly higher for honey bees (1.32 bees/100 m^2), followed by commercial bumble bees (0.164 bees/100 m^2) and mason bees (0.08 bees/100 m^2). No difference was found between bumble bee and mason bee density. The strength of the relationship between bee densities was significant for honey bees and wild bumble bees ($r = 0.05$, $P = 0.0016$), and for commercial bumble bees and mason bees ($r = 0.54$ and $P = 0.0015$).

Honey bee, bumble bee and mason bees were more abundant close to their nest locations (Fig. 6). Honey bee density dropped more (62-84%) in a short distance (110 m)

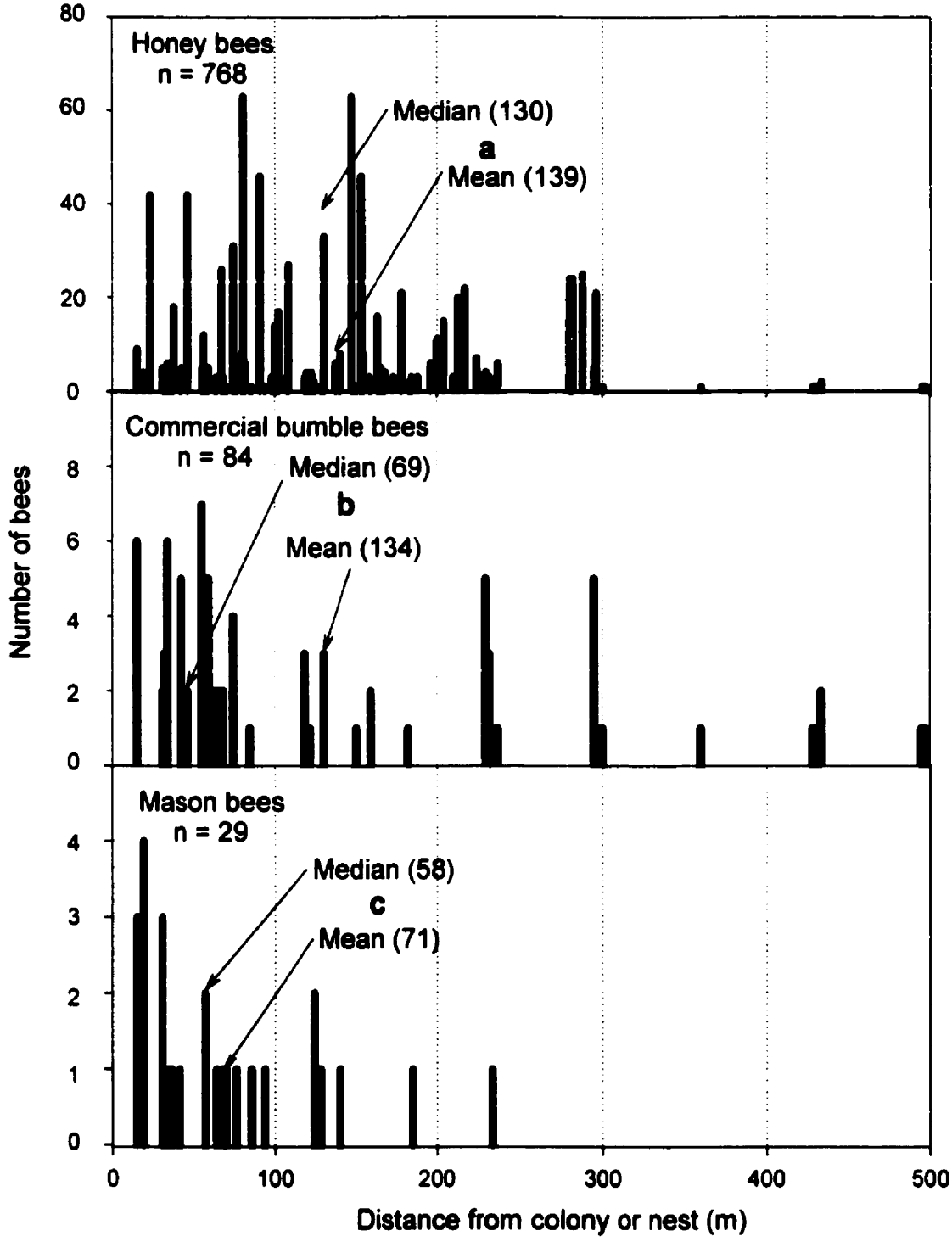

Figure 5. Distribution of observations of bees at distances from nests or colonies. Data for each bee species are significantly different from the other two, Kruskal-Wallis test, $P < 0.05$.

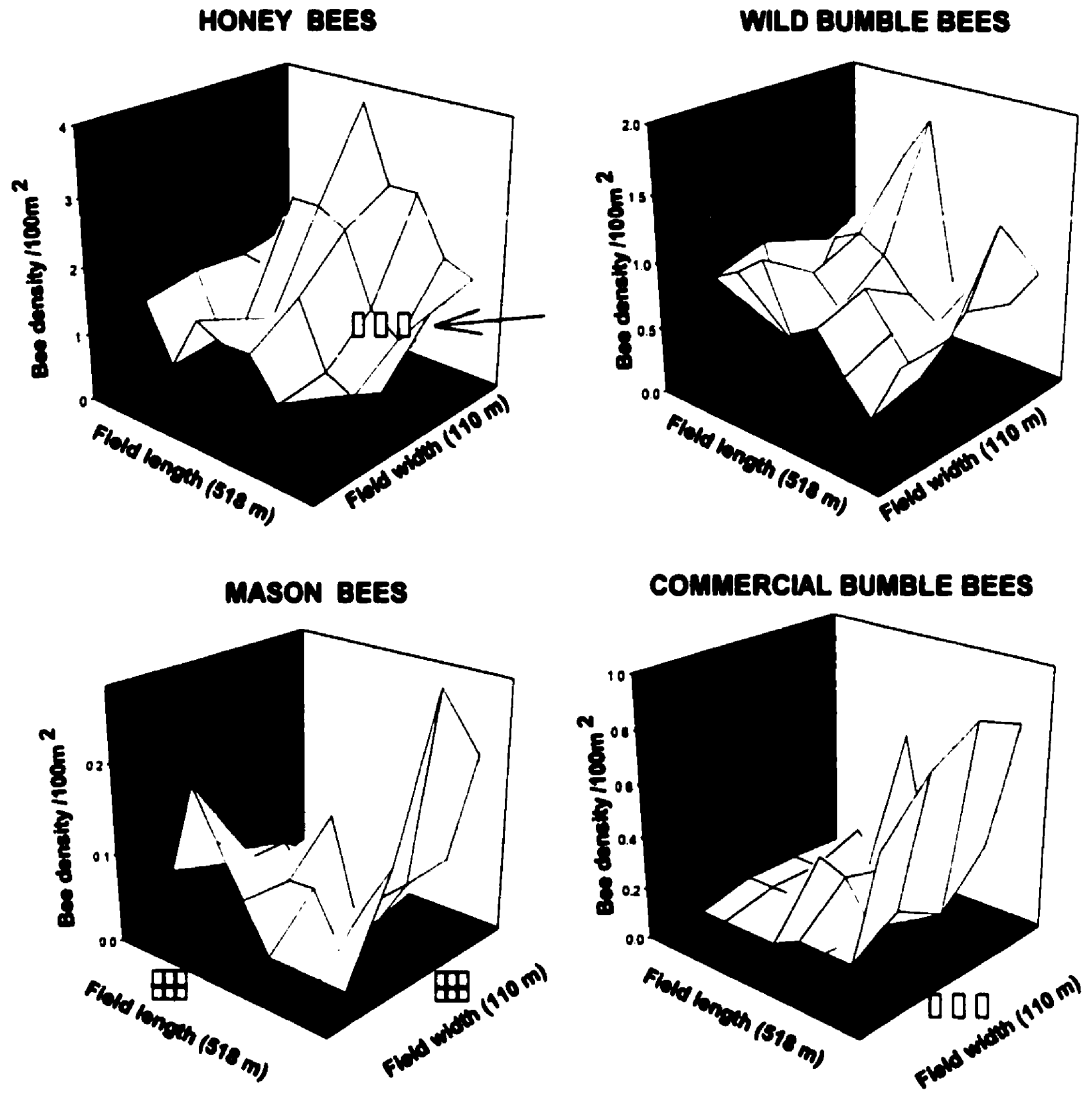

Figure 6. Three-dimensional representation of honey bee, wild bumble bee, mason bee and commercially produced bumble bee density over a field of highbush blueberry with bee colony and bee nest locations depicted.

across rows than in a long distance (170 m) along rows (41-45%). Low bee densities and location of bumble bee colonies and mason bee nests at the narrow end of the field did not provide comparable data for bee densities across rows *versus* along rows for these species.

Density of Bees and Berry Weight

No significant relationship was found between total bee density, or density of individual bee species and mean berry weight (Fig. 7).

DISCUSSION

Evaluation of Pollinators

Bumble bees were the best biological pollinators of highbush blueberry, because a large proportion of bumble bees visited the crop, deposited more pollen onto the stigma than other bees, and foraged independent of temperature. However, honey bees remain superior economically for managed highbush blueberry pollination (see discussion below).

Only in 1997 did the 50 - 141 pollen grains deposited onto stigmas in a single visit by bumble bees meet the standard of 125 pollen grains as necessary for optimal fruit quality (Chapter III). Thus, 1 - 3 bumble bee visits are needed to provide a blueberry flower with adequate fertilization. Similarly, at 18 - 60 pollen grains per visit, 2 - 6 honey bee visits per flower are needed to ensure adequate pollination, and at 39-54 grains per visit, 2-3 mason bee visits per flower are needed to ensure adequate pollination.

The superiority of bumble bees is augmented by their spending less time than honey bees visiting individual flowers, allowing for more floral visitation per forager minute. Bumble bees can sonicate blueberry flowers to obtain pollen and thus could shorten the duration required for both pollen and nectar foraging. Unlike bumble bees,

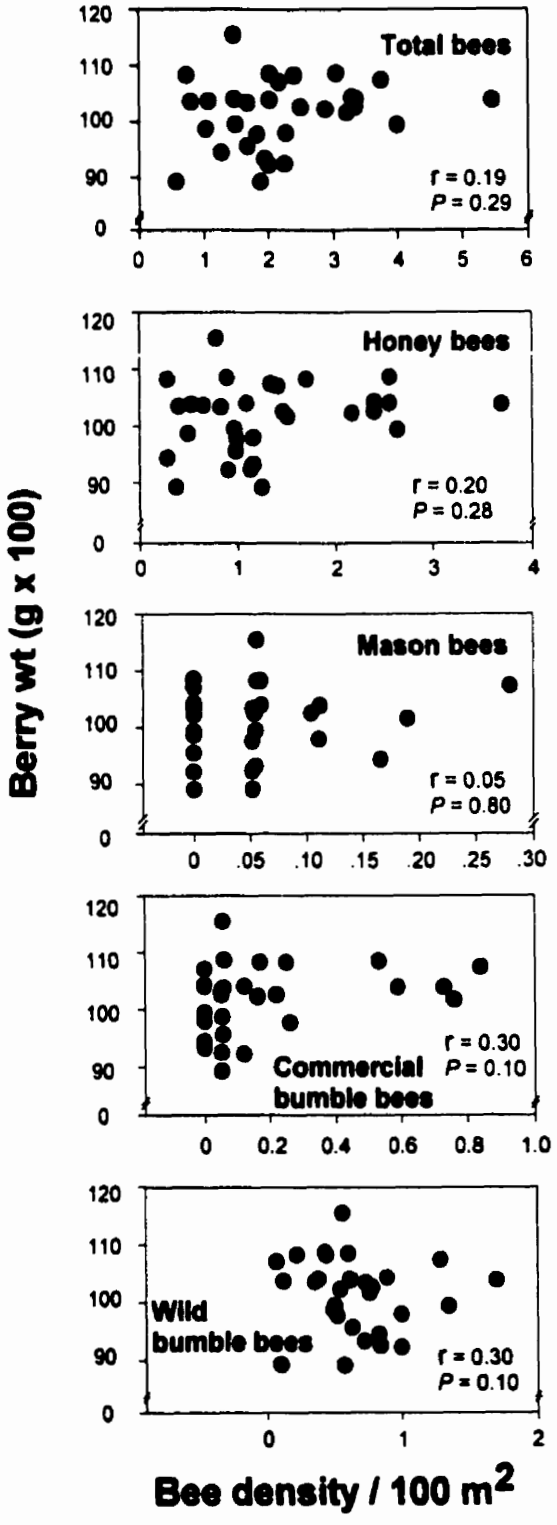

Figure 7. Relationships between berry weight (g x 100) and bee density /100 m^2 for total bees, honey bees, mason bees, commercial bumble bees and wild bumble bees as compared by Pearson correlation coefficients.

honey bees forage primarily for nectar on blueberries (Chapter IV), and nectar gathering by honey bees on blueberry likely takes longer than pollen gathering.

Most mason bees and bumble bees carried blueberry pollen at the nest entrance, whereas only 38% of alfalfa leafcutter bees and < 10% of honey bees carried blueberry pollen at nest entrances. Blueberry pollen on bees is primarily a result of floral visits and thus accurately indicates the percentage of bees visiting blueberry. However, blueberry pollen on honey bees and bumble bees could be over-estimated at some level because of within-hive transfer of pollen (DeGrandi-Hoffman 1986). Within-nest transfer in mason bees and alfalfa leafcutter bees is not likely because each bee has its own individual tunnel nest. Thus, most mason bees and bumble bees observed at nest entrances visit blueberry, whereas honey bees and alfalfa leafcutter bees do not.

This study and a related study (Chapter IV) demonstrate the importance of identifying forager type to determine if foragers carry certain pollen. In this study, in which forager type was not identified, >10% of all honey bees carried blueberry pollen. In contrast, it was found that 60% of returning nectar foragers and 7% returning pollen foragers were found to carry blueberry pollen on their bodies, indicating that while nectar foragers visited blueberry, pollen foragers had been visiting other flowering species.

Temperature was the most predictive climatic variable measured for the activity of all but bumble bees, accounting for 76% of the variation in honey bee activity. As temperature warmed, bee activity increased and pollination intensity also would be expected to increase. Both mason bee and alfalfa leafcutter bee activity accelerated exponentially above 14° and 18°C, respectively, and temperatures above these levels are necessary for any appreciable activity of either of these bees to begin. These bees increase their body temperature prior to flight by basking, explaining why foraging activity increases exponentially with increasing temperatures (Heinrich 1979). Honey bees increase their flight activity with increasing temperatures because they are able to convert sugars to heat enabling flight (Winston 1987; Seeley 1995). However, their small size limits flight to

warmer temperatures since heat loss is extensive at colder temperatures. The fact that bumble bee flight was affected by time of day, but not temperature, indicates that bumble bees forage under both warm and cool weather conditions. Bumble bees warm-up their bodies prior to flight by vibrating their thoracic muscles, enabling these bees to fly under both cool and warm conditions. Surprisingly, available sugar in blueberry flowers had no relation to bee activity, although sugar together with time improved the predictive value of the model for bumble bees.

Bee foraging density generally decreases with distance from colonies and nests (Free 1993), as I found. The more rapid decrease in honey bee density across rows compared to along rows with distance from the nest was similar to that found for honey bee distribution in almonds (Gary et al. 1976). Colonies in highbush blueberry fields should be placed at the end of rows to increase foraging range and increase the possibility of cross pollination, which is known to improve yield and fruit set in certain varieties (Free 1993; MacKenzie 1997).

Foraging distances from colonies or nests vary according to the availability of suitable forage (Free 1993). Honey bees foraged further on alfalfa, onions and carrots (198 - 240 m)(Gary et al. 1972; Gary et al. 1973), melons (305 m)(Gary et al. 1975) and almonds (80 - 261 m)(Gary et al. 1976) than on highbush blueberry (139 m). Perhaps abundant nectar in close proximity to colonies reduced the need to forage further afield.

Median foraging distances from colonies or nests for mason bees and bumble bees were about half that of honey bees. To ensure an even distribution of these bees, nests and colonies would have to be placed every 120 -140 m whereas honey bees can be placed every 260 m (Fig. 5).

The similarity in distributions of wild bumble bees and honey bees (Fig. 6) with the highest densities at the same location may indicate that wild bumble bees were attracted by other bees foraging at that location, although this phenomenon has yet to be documented. An alternative explanation is that foragers are recruited at locations where better rewards

have at least temporarily accumulated. The steep decline in densities of commercial bumble bees and mason bees with distance from their nests indicates that nests would have to be located throughout the field to obtain an even foraging population. Conversely, their localized foraging decreases the likelihood that they would forage beyond the target crop.

The lack of a positive relationship between bee density and berry weight was unexpected. Sampling for 20% of the 25 -day bloom period may not be sufficient to predict fruit weight. On the other hand, colony density for optimal pollination is not known although five honey bee colonies per ha generally are recommended in high bush blueberries (Free 1993). Cage studies with known bee densities could be used to determine the relationship between bee density and berry weight and thus determine colony density required for optimal fruit set and berry weight. Cage studies showing the number of needed visits per flower to maximize fruit production could also improve our understanding of blueberry pollinator density requirements (Danka et al. 1993).

The Economics of Pollinators

Alfalfa leafcutter bees are not suitable for blueberries because cool weather conditions limit their foraging so economic suitability for blueberry pollination was evaluated only for bumble bees, honey bees and mason bees. Factors such as the number of foraging hours per day were not included in these calculations.

Bumble bees deposit twice the number of pollen grains on individual blueberry flowers than do honey bees, and spend about 50% less time per visit per flower than do honey bees. Thus, one bumble bee is equivalent to four honey bee foragers. There are approximately 8,000 honey bee foragers in a honey bee colony, so 2,000 bumble bee foragers are required to equal one honey bee colony. There are approximately 100-200 foragers in a bumble bee colony and the current cost of a bumble bee colony is $250 Cdn. (B. Macadam, Westgro Sales Ltd., Delta, B. C. Canada). Therefore, the cost of 2,000 bumble bee foragers is about $2,500 - 5,000, depending on the number of foragers in a

colony. In comparison, one honey bee colony with about 8,000 foragers costs $50 to rent, and five honey bee colonies per ha are recommended for blueberry, costing $250. The equivalent number of bumble bee colonies would cost $12,500 - $25,000 Can. or a mean cost of $18,750 Cdn.

Mason bees are used to pollinate apples in Japan and currently cost 10 yen ($0.14 Can.) per bee (Y. Maeta, Shimane University, Matsue, Japan). Pollinating 1 ha of blueberries at 740 nesting bees per ha (Torchio 1990b) would cost $414.40 Can. (2,960 bees, 50:50 male female ratio, with 50% mortality of female bees). Thus mason bees based at Japanese costs would be slightly higher than the cost of honey bees per ha ($250 Can.). However, the cost of mason bees in North America is $0.75 - $1.50 Can. since these are produced in small numbers and sold only to home gardeners. Using the higher North American costs of bees, pollinating 1 ha in North America would cost $2,220 Can. (2,960 bees x $0.75) to $4,440 Can. (2,960 bees x $1.50), or a mean of $3,330 Can.

Comparing amounts of pollen deposited and foraging speeds between honey bees and bumble bees may have inflated the cost of bumble bees. For example, bumble bees may forage more hours per day and thus visit more flowers than honey bees. However, even if additional analyses doubled the pollination effectiveness of bumble bees (one bumble bee = eight honey bees) costs would still be a prohibitive $9,375 Can. per ha. Costs of mason bees and bumble bees could be decreased by improving production efficiency, increasing competition between producers, and providing economies of scale in large production facilities. If placement of mason bees into blueberries improved cell production (Torchio 1990b), increased availability could decrease cost of mason bees to a competitive level and decrease or even eliminate the need to buy new stocks annually. In contrast, rental charges for honey bee colonies are likely to increase as management costs increase, because of problems associated with varroa and tracheal mites, but honey bees clearly are and likely will remain economically superior to the three other bee species for highbush blueberry pollination.

Honey bees are the most economical bee pollinator for highbush blueberry, although not the most biologically suitable bee. However, various colony management strategies could improve honey bee pollination efficacy. Foraging distance of honey bees can be increased if colonies are placed at the ends of rows rather than adjacent to rows. Also, the attractant 'Fruit Boost' spray on blooming highbush blueberry increases the number of foragers and yield in the crop (Winston and Slessor 1993), and the percent of nectar foragers can be increased by having large colonies (Harbo 1986) with empty comb for storing nectar (Rinderer et al. 1979), since nectar foragers are the major forager type that visits and therefore pollinates highbush blueberry plants (Chapter IV).

Honey bees, bumble bees, mason bees and alfalfa leaf cutter bees all visited highbush blueberry, but species-specific foraging behaviours and economics showed wide variability between species. Bumble bees are biologically the best blueberry pollinators, but are expensive when compared to honey bees and mason bees. Alfalfa leaf cutter bees visit blueberry, but their high temperature threshold of 18^{o}C makes them unsuitable for blueberry pollination, since days are often cooler than 18^{o}C when highbush blueberry blooms. The costs of mason bees and bumble bees limits their usefulness until production techniques are improved and costs decrease.

Chapter III

Effect of pollen load size and source (self, outcross) on fruit and seed production in highbush blueberry, *Vaccinium corymbosum* L. (Ericaceae) 'Bluecrop'

INTRODUCTION

Pollinators often deposit more pollen than is required to fertilize all ovules and produce optimal fruit set and yield (Mulcahy et al. 1983; Snow 1986; Levin 1990; Spira et al. 1992), but in some cases insufficient pollen is deposited (Bierzychudek 1981; Snow 1982). However, few studies of cultivated crops have investigated pollen deposition by pollinators (Danka et al. 1993; Carre et al. 1994; Cane et al. 1996), and pollen requirements for optimal fruit yield are not well understood. It is important to determine pollen requirements of cultivated plant species, since fruit yield and seed production are affected by the source and quantity of pollen (Ter-Avannesian 1978; Bertin 1990). In general, the number of pollen grains required for optimal fertilization exceeds the number of ovules since not every pollen grain is successful at fertilizing an ovule, but too much surplus pollen may reduce the success of individual pollen grains by pollen tube attrition and physical blockage. Pollination research with known numbers of pollen grains and with a known pollen source is needed to determine the precise amount of pollen and varietal sources required for optimal fruit quality.

Highbush blueberry is an economically important crop in North America that depends on insect pollination for fruit set, but the amount and source of pollen required for adequate pollination of any highbush blueberry variety is not known. Growers commonly rent honey bee colonies, but recently bumble and alfalfa leaf cutter bees have become available for commercial pollination. However, low yields remain common as a result of inadequate pollen transfer (Brewer et al. 1969; McGregor 1976; Free 1993), and because

pollination from the same or closely related varieties results in low fruit set, sterile seeds and small fruit (Hancock and Siefker 1982; Czesnik et al. 1989; Free 1993).

Pollen source is thought to be an important consideration for obtaining optimum blueberry production. Pollen transferred between varieties can increase yields of blueberries compared to varietal selfing (Free 1993) by producing more seeds (Harrison et al. 1994), heavier and larger berries (Lang and Danka 1991; Harrison et al. 1993), greater fruit-set (El-Agamy et al. 1981), and earlier ripening larger berries (Lyrene 1989). Generally, pollen from more distantly related blueberry types produces heavier berries (Gupton 1984) with increased seed number (Hellman and Moore 1983; Gupton and Spiers 1994) than does pollen from more closely related types, but increases in fruit set can be limited by poor crossing of specific genotypes (Vander Kloet 1984; Rabaey and Luby 1988). Thus, the source of pollen is an important variable to consider in order to improve fruit weight and ripening time.

Similarly, sufficient stigmatic pollen deposition is considered important to optimize yield of other fruits. Generally, higher but not excessive numbers of pollen grains are beneficial for optimal fruit production since pollen competition (Mulcahy and Mulcahy 1987), ovule and seed abortion (Stephenson et al. 1986), pollen tube attrition (Smith-Huerta 1997) and physical blockage of pollen grains (Snow 1986) limit the number of successful fertilizations and seeds set. More fruit and seeds and more vigorous seedlings are produced when surplus pollen and pollen from mixed sources causes pollen tube competition to occur in the style (Snow 1986; Bertin 1990; Marshall 1991), but not in every study (Snow 1990). Under these conditions, pollen tubes grow at different rates (Walsh and Charlesworth 1992; Johnston 1993; Snow and Spira 1993) and compete to fertilize ovules, and this pollen competition results in ovule fertilization by the faster pollen tubes. In zucchini squash, this results in better quality seeds and fruit since faster pollen tubes are more successful at fertilizing ovules (Davis et al. 1987).

Few studies of pollen quantity and source have defined pollen loads in precise terms (Ter-Avanesian 1978), but rather compare "large to small" loads instead of the exact number and source of pollen grains transferred. Also, research on pollen transfer either focuses on pollen competition and progeny fitness without examining fruit characteristics important for crop yields, or concentrates on yield parameters but ignores the exact pollen loads deposited and progeny fitness. I examined 90 studies in the botanical literature that investigated a variety of subjects related to pollen load and source, and was unable to find any studies that precisely quantified the number and source of pollen grains transferred in experimental tests, assessed progeny fitness in relationship to pollen competition, and investigated yield parameters for fruits produced under varying treatments of load size and source (unpublished).

The objective of the current research was to determine the number and variety of pollen grains required by individual highbush blueberry flowers for optimal fruit set and weight, seed number and days to ripen, and also to examine progeny fitness based on seed germination. This research was part of a broader study to determine the best managed bee pollinator of highbush blueberry, variety 'Bluecrop'. My specific objectives were to: 1) determine the optimum number of 'Patriot' variety pollen grains for cross-pollinating the variety 'Bluecrop'; 2) examine the effect of outcrossing ('Patriot') and selfing with pollen from 'Bluecrop' and/or 'Patriot'; and 3) determine the size and fertility of 'Bluecrop' seeds.

METHODS

Highbush blueberry variety 'Bluecrop' was chosen as the maternal seed parent and 'Patriot' was chosen as the pollen parent since it is grown by commercial growers and has a different parentage than 'Bluecrop'. Both 'Bluecrop' and 'Patriot' are northern highbush varieties (Levi and Rowland 1997), sharing different proportions of genetic contributions

from five varieties, with 'Patriot' containing the genetic contributions from two additional varieties (Hancock and Siefker 1982).

Ovule number

The number of ovules and carpels of 'Bluecrop' flowers was determined by dissecting 90 ovaries (9 or 10 from each of 10 'Bluecrop' plants). These were fixed for 24 h in FPA solution (40% formalin, concentrated propionic acid, 50% ethanol; 5:5:90 by volume) and stored in 70% ethanol. Ovaries were dissected in 70% ethanol with two fine forceps on a black-wax dissection dish under 32X magnification. Ovules and carpels were counted.

Pollination with outcross pollen loads

One, 5-year old 'Patriot' and nine 3-year old 'Bluecrop' plants were obtained from Casino Tropical Plants Ltd., Surrey, B. C. on 13 Feb. 1996. They were potted in separate 25 cm diam. pots with a 50:50 peat-sawdust mixture, placed into a greenhouse with diurnal temperatures set at 22°C, and under natural and artificial light (12:12 L:D), fertilized twice monthly with 15-30-15 (N, P, K) flowering plant fertilizer plus slow release fertilizer (16-10-10, 180 day release), and watered twice daily for 30 min. Bloom began after 2 weeks.

In my study, pollen loads were chosen to incorporate both the maximum number of grains (300) that could be loaded onto a blueberry stigma (Parrie and Lang 1992) and the number of microspores expected to be required for optimal seed production (generally between 1-5 pollen grains to one seed; Bertin 1990; Spira et al. 1992). In blueberries there are four microspores per pollen tetrad (Stushnoff and Palser 1969) and the ratio of microspores to seeds produced is approximately 4:1 (Snow 1986). Thus, 100 pollen tetrads or 400 microspores are required for the average blueberry flower with 100 ovules, presuming that all ovules can be fertilized and matured.

Nearly mature closed 'Bluecrop' flowers were emasculated prior to anthesis by first removing the corolla and than the stamens with two fine forceps, leaving the style attached

to the ovary. Emasculated flowers were marked individually with a colored thread and tag around the base of the flower and left to mature for 40-48 h. Treatments consisted of transferring 10, 25, 125 or 300 'Patriot' pollen tetrads to 22 randomly assigned emasculated flowers as they opened on a given day. These numbers fall within the range of pollen loads delivered by bees visiting blueberry flowers in the field (Chapter II). The means $\pm$ SE tetrads actually deposited in the above treatments were: 9.72 $\pm$ 0.5, 25.0 $\pm$ 0.5, 126.5 $\pm$ 1.5, and 302.3 $\pm$ 2.0. Open 'Patriot' flowers were rolled between thumb and forefinger, and the released pollen collected onto a coverslip with a black ink dot marked on the undersurface of the coverslip to improve visibility. The coverslip was glued to one corner of a microscope slide to ease handling of the coverslip. Pollen on each coverslip was removed with the head of an insect pin under a dissecting microscope, leaving pollen over the black dot. This pollen was counted and transferred by gently lowering the flower and touching the stigma onto the pollen tetrads, with the aid of a 3x head held magnifier. Pollen tetrads left on the coverslip were counted under a dissecting microscope, and the process was repeated until the required number of tetrads was loaded onto each stigma. This technique named the black-dot on slide technique is a new innovation. Pollen transfer always took less than 1 h. Each replicate consisting of all four treatments was located on the same or an adjacent branch of a plant. Fruit was picked when blueberries were blue in colour, and weighed and stored at 4°C, until seeds were counted and sorted to type (large, small and flat) by squashing individual berries onto filter paper in a Petri dish.

Pollination with outcross, self and mixed pollen loads

Four-year old 'Bluecrop' (17 plants from Gaskin Farms Ltd., Coquitlam, B. C.) and 6-year old 'Patriot' plants (3 from Casino Tropical Plants Ltd. Surrey, B. C.) were placed into a greenhouse with daytime temperatures set at 22°C (actual range 14.5-34°C). In all 1997 experiments, 'Bluecrop' emasculated flowers were left to mature for 20-24 h

(Parrie and Lang 1992) to shorten experimental protocol, rather than 40 - 48 h as in the 1996 experiments. Treatments were: 1) 25 'Bluecrop' tetrads (SELF-25); 2) 25 'Patriot' tetrads (CROSS-25); 3) 125 'Bluecrop' tetrads (SELF-125); 4) 125 'Patriot' tetrads (CROSS-125); and 5) 63 'Bluecrop' tetrads plus 63 'Patriot' tetrads (SELF-CROSS-125). The means ± SE of tetrads actually deposited were: 24.1 ± 0.3; 25.5 ±. 0.4; 123.8 ± 0.5; 125.5 ± 0.4; 125.2 ± 0.8.

Time interval between pollination events, using outcross pollen

This experiment compared one pollen load of 70 tetrads to two pollen loads of 35 tetrads, applied 3-4 h apart (range = 165 - 225 min, n=14). The means ± SE of tetrads actually deposited in these treatments were 70.5 ± 0.6, and 69.6 ± 0.7. Five maternal 'Bluecrop' plants were pollinated with pollen from one 'Patriot' plant on 3 or 4 April 1996.

1-Day *versus* 3-day outcross pollen loads

This experiment examined the effect of 125 pollen tetrads from 1-day and 3-day old 'Patriot' flowers (one plant) on 'Bluecrop' fruit and seed production (5 plants). 1-day old flowers were flowers that had been open for a minimum of 24 hours. Twenty-five replicates were completed between 14 - 20 April, 1997. The means ± SE tetrads actually deposited in these treatments were: 124.9 ± 0.5 and 124.7 ± 0.5.

Characterization of seed types

Seed size was measured using a calibrated ocular micrometer, and seeds were sorted into three groups: large and dark brown (n = 107), small and golden brown (n = 68) and flat (n = 113). A separate random sample of seed types, large (n = 259), small (n = 302) and flat (n = 123) seeds were placed individually on a well watered

peat pellet adjacent to a colour pin coded for treatment and incubated at 70% relative humidity, 12:12 light regime, day-time temperature of 24 – 30°C and night-time temperature of 3-4°C. Each seed type was represented on each of 10 trays, and location within trays was randomly assigned. Seeds were examined once weekly for germination for 3 months.

Pollen viability on agar plates

Pollen viability was tested between 20 March and 14 April, 1997 using nutrient agar medium in 100 mm Petri dishes (Stushnoff and Feliciano 1968; Lang and Parrie 1992). Pollen from 0- to 5-day-old open flowers was released at a low density onto an agar plate by rolling the flower between the thumb and forefinger. The number of pollen tubes produced by 100 tetrads was counted after incubation at 20°C for one and two days.

Pollen tube growth in the style from 25 outcross tetrads

I examined pollen tube production from 25 pollen tetrads from one 'Patriot' plant placed on the stigmas of five 'Bluecrop' plants on 20 -21 April 1997. Styles were removed from flowers 72 h after pollination when pollen tube growth was complete (Stushnoff and Palser 1969; El-Agamy et al. 1982) and fixed in FPA solution. The KOH-aniline blue fluorescence technique (Martin 1959; Kearns and Inouye 1993; Hood and Shew 1996) was used to count the number of pollen tubes in each style. Styles were fixed in FPA solution for 24 h, stored in 70% ethanol, washed in running water for 30 min, softened in warm 8N NaOH for 3 h on a hot-plate, washed in tap water for 1 h, stained for 30 min in 0.1% aniline blue in 0.1N potassium acetate (pH 6.9), and washed in tap water for 1 h. Preparations were mounted in glycerol on glass slides, the preparation was covered and squashed by pressing down on the coverslip, and tubes observed through an epifluorescence microscope equipped with a filter set (maximum transmission 365 nm). The fluorochrome portion of

the analine blue dye (Colour Index # 42755) binds to plant glucans and polysaccharides that are present in pollen tubes but not in stylar tissue.

Statistics

Except where otherwise stated, data were transformed to obtain normality and analyzed using the GLM procedure (SAS Institute 1990) followed by Ryan's Q test for differences between means (Day and Quinn 1989). Differences between mean percent fruit set were analyzed using Fisher's Exact Test, and differences between mean length and width of the three seed types were analyzed using a Kruskal-Wallis Test with PROC NPAR1WAY (Chi-square approximation)(SAS Institute 1990). *In-vitro* pollen tube germination data were analyzed using the paired-difference t-test (PROC UNIVARIATE Procedure in SAS Institute 1990). Bonferroni corrections were made to P values and are shown as corrected values. In all cases $\alpha = 0.05$ unless noted otherwise.

RESULTS

Ovules per 'Bluecrop' flower

The mean number of ovules per 'Bluecrop' flower was 106.1 ± 1.5, with a range of 94 - 117, with 6.0 ± 0.1 carpels per flower and 17.7 ± 0.2 ovules per carpel (Appendix).

Pollination with outcross pollen loads

Fruit weight increased significantly as pollen load increased from 10 and 25 to 125 pollen tetrads (Fig. 8), but did not increase when tetrad number was increased to 300. Similarly, the number of large seeds increased with pollen load, but unlike fruit weight, seed number continued to increase with 300 tetrads. The percent fruit that developed from

pollinated flowers, or fruit set, was not significantly different between treatments. The number of days to ripen decreased with increased pollen load; it took longer to ripen fruit from the low pollen treatments of 10 and 25 than for the high pollen treatments of 125 and 300. At pollen loads of 10 or 25 tetrads, it took 7 - 13 days longer for fruit to ripen than at 125 or 300 tetrads.

Pollination with outcross, self and mixed pollen loads

Generally, fruit characteristics improved with both large and mixed pollen loads, although increase in pollen load size produced more statistically significant differences than mixed loads (Fig. 9). The heaviest fruits, most seeds, and highest percent fruit set were produced from pollen loads that contained either 125 mixed or 125 outcross pollen tetrads. Loads with 125 crossed or 125 mixed pollen loads were statistically identical in all characteristics. Time to ripen was significantly slower for low pollen loads of 25 selfing tetrads than for any size loads of outcross pollen or larger loads of selfing pollen.

Time interval between pollination events using outcross pollen

There were no significant differences ($P > 0.05$) between mean fruit weight, number of large seeds, mean percent fruit-set, and number of days to ripen for 'Bluecrop' fruit resulting from 70 'Patriot' tetrads in one load *versus* two 35 'Patriot' tetrad loads deposited with a 3.5 h time interval between loads (Fig. 10). No significant plant or plant-treatment interaction was found for any variable.

1-Day versus 3-day old outcross pollen loads

There were no differences ($P > 0.05$) in fruit weight, number of large seeds, mean percent fruit-set and number of days to ripen fruit when pollen was collected from 1 or 3-day old flowers (Fig. 11). A significant plant effect was found only for fruit weight ($F = 5.89$, $df = 5, 42$, $P = 0.0003$), and a borderline significant plant effect for days to ripen

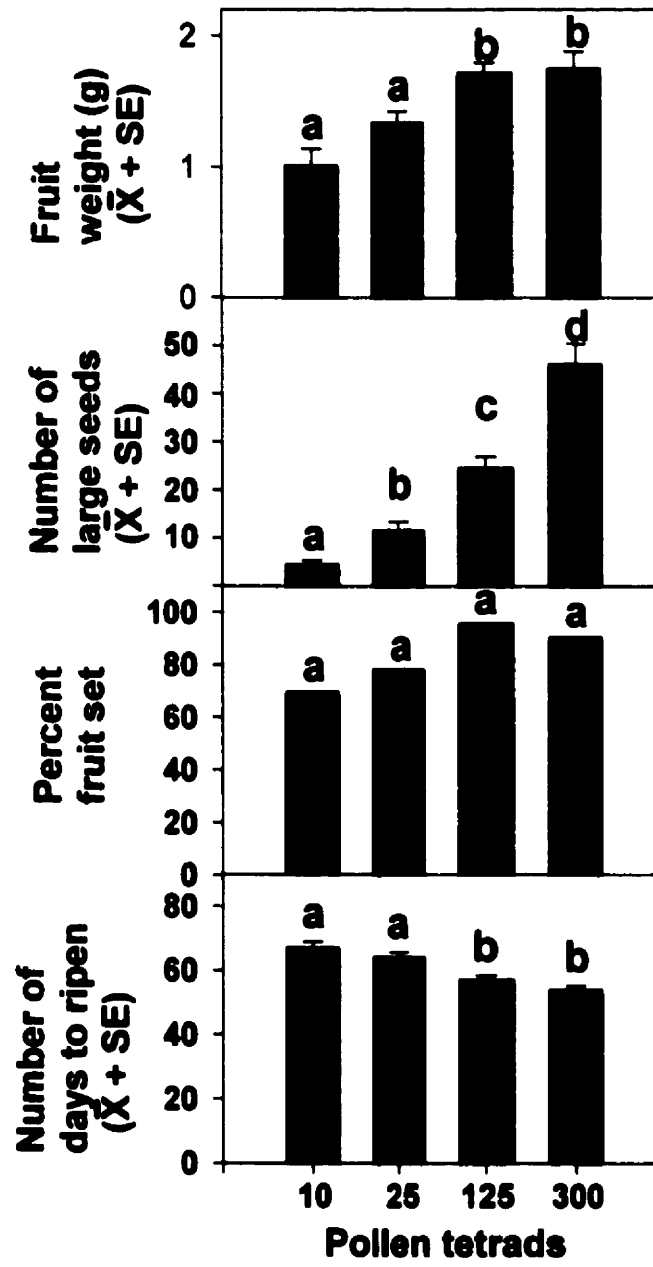

Figure 8. Fruit weight, number of large seeds, percent fruit-set and number of days to ripen for highbush blueberry variety 'Bluecrop' after loading of 10, 25, 125 or 300 'Patriot' variety pollen tetrads onto flower stigmas. Bars with same letter did not differ significantly ($P > 0.05$) by GLM procedures with Ryan's Q test for differences between means and Chi-square Fisher's Exact test for differences between percent fruit set.

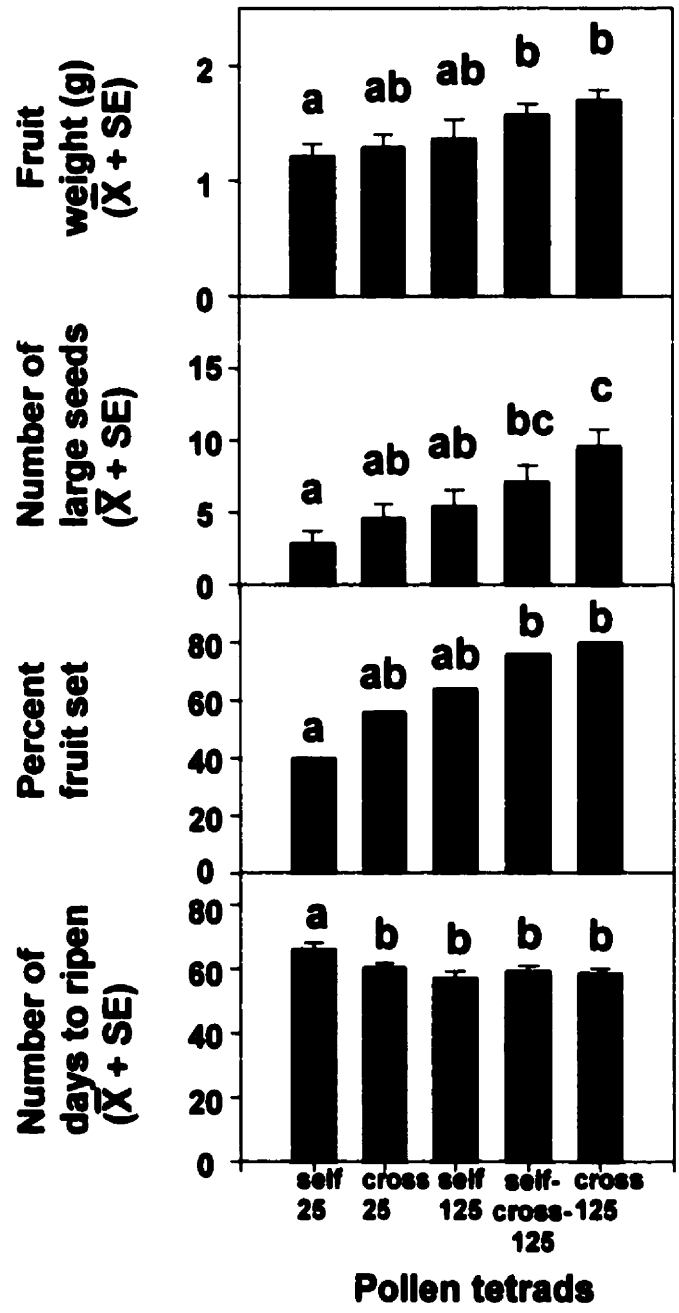

Figure 9. Fruit weight, number of large seeds, percent fruit-set and number of days to ripen for 'Bluecrop' highbush blueberries after loading 25 'Bluecrop' pollen tetrads (SELF-25); 25 'Patriot' pollen tetrads (CROSS-25); 125 'Bluecrop' pollen tetrads (SELF-125); 63 tetrads of 'Bluecrop' plus 63 'Patriot' pollen tetrads (SELF-CROSS-125); and 125 'Patriot' pollen tetrads (CROSS-125); on 'Bluecrop' variety stigmas. Bars with same letter do not differ significantly ($P > 0.05$) by GLM procedures with Ryan's Q test for differences between means and Chi-Square Fisher's Exact test for differences between percent fruit set.

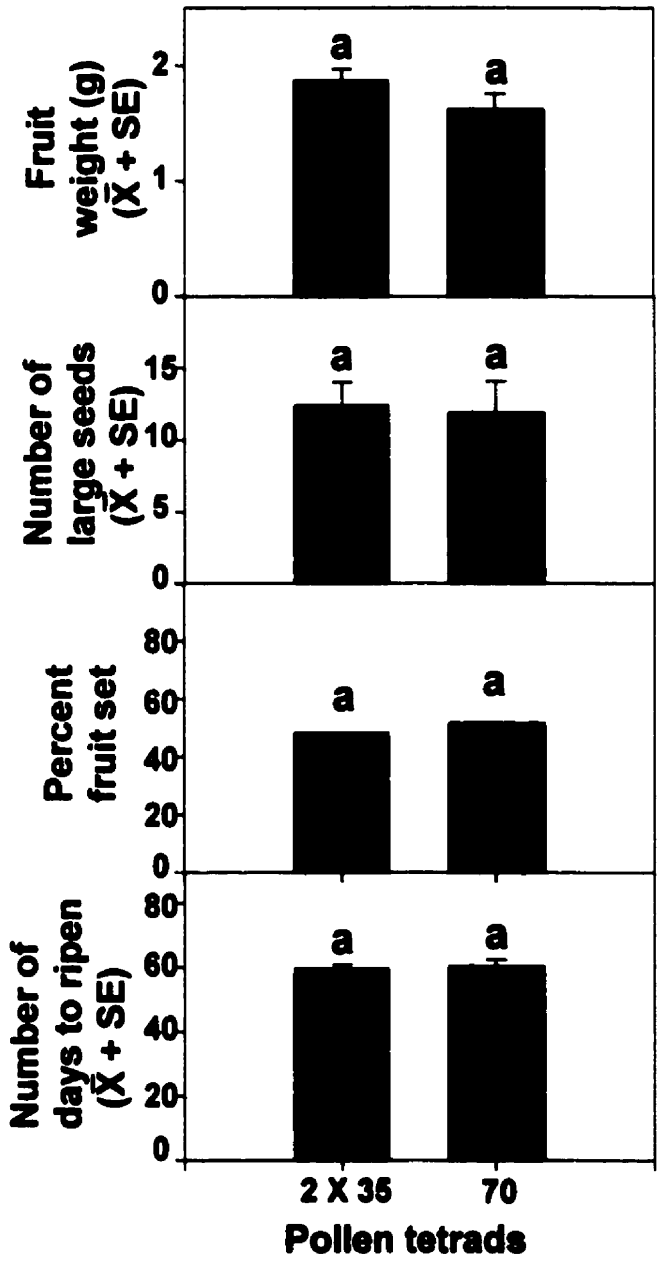

Figure 10. Fruit weight, number of large seeds, percent fruit-set and number of days to ripen for highbush blueberries after loading 35 tetrads of 'Patriot' pollen twice (2 x 35) with a time interval of 3.5 h or loading 70 tetrads of 'Patriot' pollen (70) on 'Bluecrop' stigmas. Bars with same letter did not differ significantly ($P > 0.05$) by GLM procedures with Ryan's Q test for differences between means, Chi-Square approximation for differences between days to ripen, and Chi-Square Fisher's Exact test for differences between percent germination.

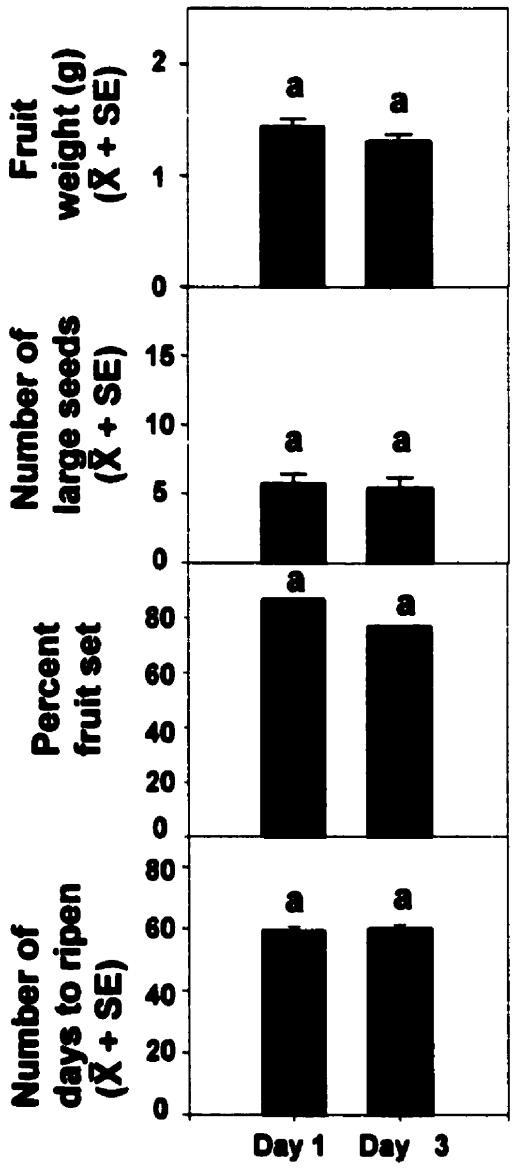

Figure 11. Fruit weight, number of large seeds, percent fruit set and number of days to ripen fruit for 'Bluecrop' highbush blueberries after pollen deposition of 125 'Patriot' tetrads from 1-day and 3-day old 'Patriot' flowers. Bars with same letter did not differ significantly ($P > 0.05$) by GLM procedures with Ryan's Q test for differences between means and Chi-Square Fisher's Exact test for differences between percent fruit set.

(F = 2.187, df = 5,42, P = 0.074, but no plant-treatment interaction was found for any variable.

Characterization of seed types

Three discrete seed types (large, small and flat) were produced by ripe blueberries. These were significantly different between types for length (X^2 = 250.18, df = 2, P = 0.0001) and width (X^2 = 241.11, df = 2, P = 0.0001). 'Large' seeds were plump (length =1.70 $\pm$ 0.02 mm; width = 1.05 $\pm$ 0.01 mm) and dark brown in colour; 'small' seeds were not quite as plump (length = 1.02 $\pm$ 0.01 mm; width = 0.68 $\pm$ 0.01 mm) and were a golden brown in colour; 'flat' seeds were flattened (length = 0.40 $\pm$ 0.01 mm; width = 0.28 $\pm$ 0.01 mm) and pale in colour. Germination occurred in 220 out of 259 large seeds (85%), but none of the 302 small and 123 flat seeds germinated.

Pollen load sizes of 10, 25, 125 and 300 tetrads did not affect mean days to seed germination (P = 0.278; Fig. 12). However, there is weak evidence that there were differences between treatments for percent seed germination (P = 0.077), with treatment differences between 10 and 125 (P = 0.034) and 10 and 300 (P = 0.039) load sizes. There was a treatment response of pollen tetrad number on large seed length (P = 0.04) between treatments 25 and 300 pollen tetrads (P = 0.028), but not for large seed width (P = 0.204).

Pollen viability on agar plates

In-vitro pollen tube germination for 'Bluecrop', as indicated by percent tetrads with one or more pollen tubes, did not change from day 1 (87.8%) to day 2 (93.2%)(Table 1)(P = 0.2352). In contrast, 'Patriot' pollen tube germination increased from 78.9% to 88.8% from day 1 to day 2 (P = 0.0243). There was no difference (1.43 and 1.66; $P > 0.05$) in the number of pollen tubes per tetrad for day 2 between 'Bluecrop' and 'Patriot' tetrads.

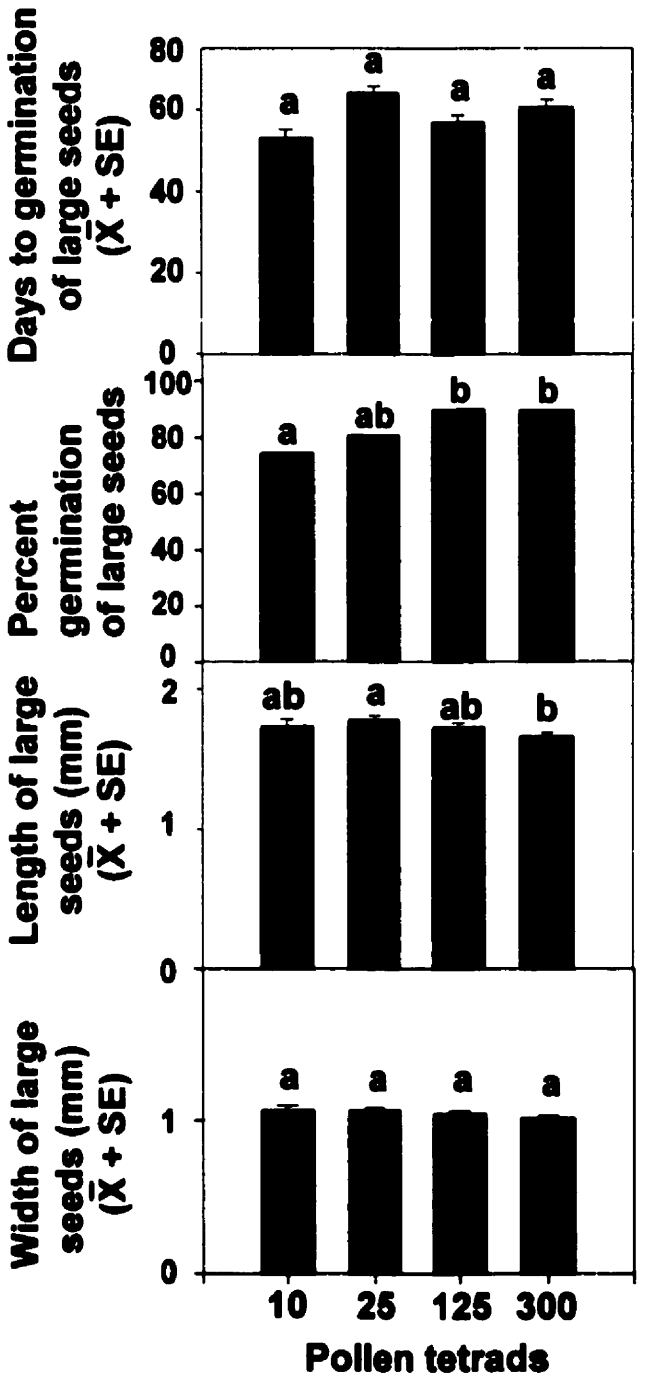

Figure 12. Days to germination, percent germination, length and width of large seeds from 10, 25, 125 and 300 'Patriot' pollen tetrads added to 'Bluecrop' variety flower stigmas. Bars with same letter did not differ significantly ($P > 0.05$) by Chi-Square Fisher's Exact test for percent germination, GLM procedures with Ryan's Q test for differences between mean days to germination, and Kruskal-Wallis Test with PROC NPAR1WAY (Chisquare approximation) procedures for differences between mean width and between mean length.

Table 1. Results of *in-vitro* pollen tube germination test on agar plates for 'Bluecrop' and 'Patriot' pollen tetrads 1 and 2 days after start of incubation.

	Percentage of tetrads with pollen tubes[a]			
	'Bluecrop'		'Patriot'	
Number of pollen tubes per tetrad	Day 1	Day 2	Day 1	Day 2
0	12.2	6.8	21.1	11.2
1	42.6	38.8	43.3	42.9
2	34.9	38.3	26.8	33.5
3	9.4	14.4	7.2	9.9
4	1.0	1.8	1.6	2.4
All tetrads pooled	87.8% a	93.2% a	78.86% a	88.82% b

[a] Four plants and 7 replicates for 'Bluecrop', 3 plants and 19 replicates for 'Patriot'. Percent values within a variety sharing a letter in common are not significantly different. ('Bluecrop', t-test = 1.827, P =0.235; 'Patriot', t-test = 2.427, P = 0.024).

Pollen tube growth in the style from 25 outcross tetrads

A mean of 78.5 $\pm$ 7.25 pollen tubes was found in eight styles that received 25 pollen tetrads. Six out of the eight styles had between 85-92 pollen tubes.

DISCUSSION

My results demonstrate that both pollen source and especially the number of pollen tetrads can be important in improving yield of highbush blueberry fruit. Maximum fruit weight was attained from self, mixed and outcross fertilization with 125 tetrads, with a suggestive trend that selfing of 'Bluecrop' flowers was less productive than outcrossing fertilization. Although both pollen load and source had an effect on fruit production, the effects of pollen load size generally were larger than the effect of pollen donor (Fig. 9). My results indicate that 125 tetrads of 50-100% outcross pollen $\leq$ 3d old are sufficient for optimal fruit quality. Pollinator density would be optimal if blueberry flowers received 125 tetrads per flower during bloom.

The pollen load of 125 'Patriot' tetrads gave the greatest fruit weight and the least days to ripen of highbush blueberry var. 'Bluecrop'. Additional tetrads up to 300 did not increase fruit weight or decrease ripening time, but did result in an increased number of large seeds. Decreased ripening time is an important result for growers, as early fruit is more valuable than late fruit. Loads of 10 or 25 tetrads were not sufficient to optimize fruit characteristics, but I did not determine the precise point between 25-125 tetrads at which fruit quality would reach its maximum level. Conceivably, this threshold could occur anywhere in this range, although the optimal level likely is closer to 125 than 25 because about 1-5 microspores are needed to fertilize each ovule that is present in the ovary (Bertin 1990; Spira et al. 1992).

'Bluecrop' selfing with low and high pollen loads resulted in 40% and 64% fruit set, similar to results in another study (52% and 56% in Knight and Scott 1964). Fruit set improved from 40% for selfing with 25 tetrads to 76% and 80% with outcross tetrads of 125 mixed self/outcross and 125 outcross pollen loads, as in other outcrossing studies (Knight and Scott 1964; Krebs and Hancock 1988). Similarly, fruit weight was increased from 1.05 grams for selfing with 25 tetrads to 1.6 and 1.7 grams for 125 mixed and outcross tetrads. Thus, 25 tetrad selfing sets fewer fruit that weigh less than fruits resulting from 125 self, mixed or outcross loads.

Sequential pollen loading with pollen from the same source did not affect fruit quality in my study, although in *Hibiscus moscheutos* L. the timing of sequential pollen loads determined which pollen grains fertilized the ovules (Spira et al. 1996). Outcross pollen in a mixed load may stimulate the less compatible selfing tetrads (Mulcahy and Mulcahy 1986; Marshall et al. 1996). If outcross pollen is beneficial to fruit production and size, it would be important to have pollinator varieties within average flying distance of bee pollinators during a given foraging trip. In my study, there was no significant difference between fruit quality from self, mixed and outcross loads of 125 tetrads but outcross pollen did produce significantly more fertilized seeds than did self pollen (Fig. 9). Bees enhance fruit production by transferring more pollen, whether self or outcross, thus increasing fruit quality. Knowledge of varietal compatibility is important since blueberry seed set can be reduced when self pollen is present together with outcross pollen (Harrison et al. 1994).

The maximum number of large seeds per 'Bluecrop' fruit was 48 in 1996, which agrees well with a mean seed number for 'Bluecrop' reported in other studies in which excess pollen was used for pollination, 47 in Eaton (1967), 40.5 in MacKenzie (1997), and 46 in Moore et al. (1972), but 26.7 in Krebs and Hancock (1988) and 63 in Darrow (1958). Thus, only about half of the ovules produce seed. Pollen tube attrition, seed abortion, or some other factors may reduce seed production to less than ovule capacity.

The mean number of large seeds produced per fruit from the 125 cross pollen treatment was half the mean in 1997 of a similar treatment in 1996, possibly due to a change in methodology between years. Stigmas left for 24 h after emasculation in 1997, rather than 48 h in 1996, may have been less mature and had insufficient stigma fluid for adequate adsorption of all tetrads (Moore 1964; Young and Sherman 1978). Maternal environmental effects (Stephenson 1981; Roach and Wulff 1987) and perhaps physiological condition of pollen producing plants (Stephenson et al. 1992) also can influence the performance and fate of pollen grains.

Characterization of seed types by length and width is important, as only large seeds are fertile and indicate pollination success. Although large (fertile), medium (not fertile) and collapsed (not fertile) seeds have been described histologically (Bell 1957), seeds are described only qualitatively in most studies (Harrison et al. 1994; MacKenzie 1997). Precise characterization of 'large fertile seeds' with length and/or width measurements should be used to standardize the definition of seed production in blueberry pollination studies, or else seeds should be germinated.

The significant effect of a large pollen load on improved percent germination of large seeds between treatments 10 and 125, and 10 and 300, agrees with the relationship found between increased number of pollen grains deposited onto a stigma and the improved performance of resulting progeny in some plants (Mulcahy and Mulcahy 1987; Palmer and Zimmerman 1994; Johannsson and Stephenson 1997). However, days to germination in my study was not affected by pollen load size. Increased sample size might improve the strength of these relationships, but these data do suggest that pollen transfer is related to at least one fitness characteristic in highbush blueberry, percent germination.

Pollen remains viable until the flower is at least 3 days old, and longer periods (8 days) have been reported (Moore 1964). Prolonged pollen viability would be important if pollinators are scarce, since it would extend the life of the flower and give pollinators more opportunity to pollinate all flowers.

My method of transferring a known number of pollen grains using the black-dot-on-slide technique provided more detailed information on floral pollen requirements for fruit set. Other methods of transferring pollen have been used in pollination studies, but pollen deposition has been characterized only as "low", "high", or "excess". These methods could not determine specific pollen requirements for flowers since the exact number of pollen grains deposited was not known (Bertin 1990; Harrison et al. 1993).

In my *in-vivo* study three to four pollen tubes grew from each tetrad down the style, since the majority of styles produced more than 85 pollen tubes from 25 tetrads. Pollen tube production from tetrads can be less than four (Snow 1986). *In-vitro* pollen tube growth did not confirm *in-vivo* results since only one to two pollen tubes per tetrad were produced in my *in vitro* experiment, in agreement with one study (Brewer and Dobson 1969), but fewer pollen tubes than in other studies (Goldy and Lyrene 1983; Lang and Parrie 1992). Variation in pollen tube production could be due to differences between varieties (Goldy and Lyrene 1983; Lang and Parrie 1992), or subtle chemical nuances of the artificial medium (Mazer 1987) at certain temperatures (Stern and Gazit 1998). *In-vitro* pollen tube production may be used as a rough indicator of pollen viability, although it may be an inaccurate predictor of *in-vivo* pollen tube growth down the style (Mazer 1987).

This is the first study that has used a range of known number of pollen tetrads to examine fruit yield and increase progeny fitness by varying the number of pollen tetrads and pollen source of any cultivated crop, including blueberry. Clearly, the pollen requirement of a cultivated crop is critical in determining how to obtain maximum yield through optimal pollination. Pollen requirements of single flowers can be extrapolated to a plant and then to a field. Together with data on pollinator behavior, such as visitation frequency, pollen load per pollinator visit and diurnal foraging patterns, calculations can be made to quantify plant-pollinator relationships to determine the optimal bee density for each pollinator species.

Two examples from my research demonstrate the impact these types of precise pollen transfer studies can have on pollination-related crop production analyses (Chapter II). First, I determined that considerably fewer than 125 pollen grains were found on stigmas of cultivated highbush blueberries in the field after one pollinator visit, suggesting that more than one pollinator visit is required for maximum levels of fruit set and size and that growers need to provide adequate pollinators to reach this level of stigma loading. Second, I was able to relate single pollen transfer data to bee species, and found that an individual bumblebee is equivalent to four honey bees on a per visit basis. I was then able to extend this analysis to determine the relative economic costs and benefits of renting bumble *vs.* honey bees for blueberry pollination, and found honey bees to be far superior to bumble bees economically, in spite of the better per-bee efficacy of bumble bees because there are so many more honey bees than bumble bees in a colony. These examples indicate the horticultural significance of precise pollen load and variety studies, and suggest that essential knowledge of pollen requirements for crop production can be a powerful tool to enhance fruit and vegetable set, size, time to ripen, and perhaps other economically important crop characteristics.

Chapter IV

Pollen storage and foraging by honey bees *Apis mellifera* L. (Apidae) in highbush blueberries *Vaccinium corymbosum* L. (Ericaceae) 'Bluecrop'

INTRODUCTION

Pollen foraging in the honey bee is closely linked to colony state (Schmid-Hempel et al. 1993). Altering colony state by removing pollen stores (Fewell and Winston 1992) or adding brood (Eckert 1990) increases the demand for pollen, the only protein source for honey bee colonies, and these manipulations result in increased pollen foraging (Free 1967; Barker 1971). Colony size also affects foraging type (Fewell et al. 1991). I examined colony effects on foraging by manipulating pollen levels in small and large honey bee colonies located in a 100 ha field of highbush blueberry var. 'Bluecrop'.

It is generally thought that pollen foragers provide better pollination than nectar foragers in fruit trees (Free 1960) because pollen foragers collect large amounts of pollen and nectar foragers carry incidental amounts of pollen. Thus, flowers have a greater chance of receiving pollen on their stigmas and being pollinated by pollen foragers rather than nectar foragers. However, blueberry pollen and nectar is less directly accessible than on the open flowers typical of tree fruit blossoms. Nectar foraging from the cup-shaped blueberry flower requires that insect visitors move anthers aside in order to reach the nectaries. For efficient harvesting of pollen from the terminal pores of blueberry flowers bumble bees 'buzz' each flower (Cane and Payne 1988). However, honey bees are not known to buzz-pollinate but occasionally have been observed to collect pollen in some plants like cranberry, *V. macrocarpon* Aiton, by tapping the flowers with their forelegs (Cane et al. 1993).

Honey bee colonies are rented for pollinating many crops, including blueberry (McGregor 1976; Free 1993). Apiculturists have long been interested in developing methods of managing colonies to increase the number of pollen foragers on a crop. Genetic selection towards pollen collection is one method of increasing pollen foragers, but this would entail selection programs over a few generations and might decrease honey production (Page and Fondrk 1995). Feeding colonies (Free 1965a; Goodwin and Ten Houten 1991) or spraying crops with sugar syrup (Free 1965b; Goodwin 1997), or spraying crops with synthetic honey bee queen mandibular pheromone (Currie et al. 1992a, 1992b; Winston and Slessor 1992, 1993, 1998) increased the numbers of honey bee foragers on crops, and occasionally pollen foraging (Higo et al. 1992). Artificially altering colony state with increased or decreased amounts of colony stores or contents might be another method of increasing the number of foragers for pollination enhancement.

My objectives were to determine if pollen deprivation in small and large colonies of honey bees placed in a blooming crop of highbush blueberry would a) increase the proportion of pollen foragers, b) increase the proportion of *Vaccinium* pollen carried by foragers, and c) increase the weight of pollen carried per bee. I also investigated whether or not both nectar and pollen foragers carry *Vaccinium* pollen, and in what proportions to other pollen types.

METHODS

During May 1997 in the highbush blueberry growing area of Coquitlam, British Columbia, 32 colonies of honey bees were housed in either one or two supers of standard deep Langstroth equipment and placed adjacent to a field of highbush blueberry, var. 'Bluecrop'. All colony queens were 1-y old and were produced from general stock at Simon Fraser University, Burnaby, B. C. Colony contents (uncapped brood, capped brood,

honey and bees) were measured using a frame-sized, Plexiglas grid consisting of 32 squares, each with an area of 25 cm^2. On 30 April, the contents of 16 small (1-super) and 16 large (2-super) colonies were measured, equalized on 1-2 May for respective areas of uncapped brood (2157 $\pm$ 175 cm^2 and 3417 $\pm$ 175 cm^2), capped brood (2712 $\pm$ 177 cm^2 and 4479 $\pm$ 122 cm^2), honey (3167 $\pm$ 250 cm^2 and 4934 $\pm$ 218 cm^2), and bees (12,100 $\pm$ 729 cm^2 and 29,250 $\pm$ 661 cm^2), respectively. The area of pollen was adjusted to 10 - 20 times greater in the pollen-rich treatments than pollen-poor treatments. For small colonies, the mean area of pollen was adjusted to 245 $\pm$ 48 cm^2 and 1381 $\pm$ 125 cm^2 for the poor and rich pollen treatments, respectively. In large colonies, the mean area of pollen was adjusted to 428 $\pm$ 65 cm^2 and 4031 $\pm$ 124 cm^2 for poor and rich pollen treatments, respectively.

Four colonies of each size and treatment were selected randomly and placed in sequence, in pairs, into each of two "U" formations (4 x 7 m, 2 m apart) to minimize foragers drifting from one colony to another (Jay 1966), with the open part of the U-formation oriented toward the southwest. Additional cues for colony location were provided to foragers by having the entrances of alternate colonies face either towards the center or away from the U formation.

Colonies were placed in the field on 3 May (day 0). Nearby blueberries were between 10% and < 50% bloom. I began observations on day 4 (7 May) since inclement weather prevented flight during the first 3 days after colony manipulation. The relative brood areas, uncapped and capped, honey and adult bees were measured in small and large colonies on 23-24 and 26-27 May, respectively, on days of relatively good weather. Open bloom was 100% at the end of the experiment. Pollen areas were measured on 12, 20 and 23-27 May 1997. All colonies were checked for a healthy laying queen during each assessment.

On the first 5 days of good weather (days 4-8; 7-11 May) and on day 13 (16 May), between 1100 and 1400 h, pollen foragers were counted by one person on one half of the entrance of a colony (marked with tape) for 1 min, and nectar foragers were counted by the

second observer on the other side of the colony entrance. The observers then switched sides and counted for another min. In addition, 10 - 20 foragers per colony were caught at colony entrances after screening entrances for 2 min between 1100 and 1400 h. A sample of incoming foragers (10 -20 bees) was collected by swinging an open Zip lock plastic bag (Dow Brands Canada Inc., Paris, Ontario) in front of each colony through slow-approaching incoming foragers. All captured bees were quick-frozen on dry-ice in the bag used to catch them and kept frozen until later examination. Bees also were kept frozen during sorting and random selection of bees to minimize pollen contamination from one bee to another. If contamination of pollen occurred between bees, *Vaccinium* pollen on nectar foragers would have been diluted by the greater amounts of non-*Vaccinium* pollen present on pollen foragers and thus would not have inflated the number of nectar foragers with *Vaccinium* pollen. Frozen bees were separated under a dissecting microscope into bees without pollen loads (deemed nectar foragers) and bees carrying traces to large amounts of pollen (deemed pollen foragers). All pollen foragers, and then all nectar foragers, were placed onto a clear plastic sheet placed over a template with 20 numbered lines radiating from the center. Five pollen and five putative nectar foragers were selected for recovery of pollen by removing bees located on randomly selected numbered lines. A fresh plastic sheet was placed over the template after every sample of bees.

Individual nectar foragers were placed in a centrifuge tube, covered with 90% ethanol and sonicated ('Tru-Sweep ultrasonic Cleaner', Crest Ultrasonic Corp., Trenton, New Jersey) for 20 min to loosen any adhering pollen. The bee was removed, the remaining alcohol was centrifuged for 20 min, the alcohol was decanted and the pollen pellet placed within the boundaries of a wax pencil circle marked on a glass microscope slide. The slide was warmed on a hot plate until the residual alcohol had evaporated. A small piece of fuchsin-gelatin (about 5 x 5 x 5 mm) was placed over the dried sediment

and a glass coverslip added when the fuchsin-gelatin had partially melted (Kearns and Inouye 1993). Slides were cooled until gelatin set and then stored. Pollen pellets were used for pollen analyses, rather than the whole bee, because preliminary work indicated that pollen on pollen foragers' bodies was minimal, perhaps because of extensive grooming prior to arriving back to the colony. Pollen pellets were removed directly from pollen foragers, weighed, and the pollen was dispersed in a small amount of alcohol. A small drop of pollen - alcohol suspension was dried onto a slide, and fuchsin gelatin added and processed as above. Prepared slides from each bee were examined for the presence of *Vaccinium* pollen tetrads. I did not attempt to distinguish wild *Vaccinium* pollen from highbush blueberry pollen, although it is likely that *Vaccinium* pollen was in fact collected from highbush blueberry because the surrounding area consisted of 245 ha of highbush blueberry and the closest patch of wild *Vaccinium* was located >1.5 km from honey bee colonies. Pollen grains were counted in non-overlapping fields of view on the same slide at 100X magnification, until the whole slide or 300 grains were examined. All pollen grains counted were then classified as *Vaccinium* tetrads (distinctly tetrahedral) or other pollen types. Fifty randomly selected slides from pollen loads on pollen foragers and 50 from putative nectar foragers were examined for the most common pollen types (other than *Vaccinium*), and were identified to family (McAndrews et al. 1973; R. Matthews, Simon Fraser University, B. C., Canada, pers. com.)

Statistics

Data were checked for normality and if necessary were transformed to meet assumptions of parametric statistics. Normally distributed data were analyzed by GLM procedure in SAS software (SAS Institute Inc. 1990) followed by Ryan's Q test for differences between means (Day and Quinn 1989). Differences between treatments

for early and late measurements of colony contents were analyzed by a GLM-MANOVA, because colony contents are not independent of one another. The effects of days were tested with GLM-repeated measures procedure (profile analysis). But, proportion of *Vaccinium* pollen carried by pollen foragers was tested using the non-parametric Kruskal-Wallis Test (SAS Institute 1990) because data could not be normalized. Differences between nectar foragers and pollen foragers with and without *Vaccinium* pollen also were analyzed using Kruskal-Wallis Test (SAS Institute 1990). *P* values were adjusted with Bonferoni corrections where applicable. Unless stated otherwise, $\alpha = 0.05$.

RESULTS

Colony changes in brood, bees and honey

Changes in colony contents from 1-2 May to 23-27 May were significant among treatments for honey and bees, but not for capped ($F_{3,27} = 0.69$, $P = 1.00$) and uncapped brood ($F_{3,27} = 2.26$, $P = 0.519$)(Fig. 13). Honey stores increased more ($F_{3,27} = 6.7$, $P = 0.008$) for large colonies rich in pollen stores, compared to small colonies poor or rich in pollen stores. The change in bee numbers was greater ($F_{3,27} = 14.15$, $P = 0.0005$) for large colonies than small colonies, but it was a population decrease.

Pollen content in colonies

At the start of the experiment, there were differences among treatments ($F_{3,27} = 5.0$, $P = 0.0069$) (Fig. 14); small colonies with poor or rich pollen stores ($F_{1,14} = 4.72$, $P = 0.0475$), and large colonies with poor and rich pollen stores ($F_{1,13} = 149.9$, $P = 0.0004$) were different from each other. Throughout the experiment, pollen stores remained significantly higher for the large-pollen-rich treatment than for the other

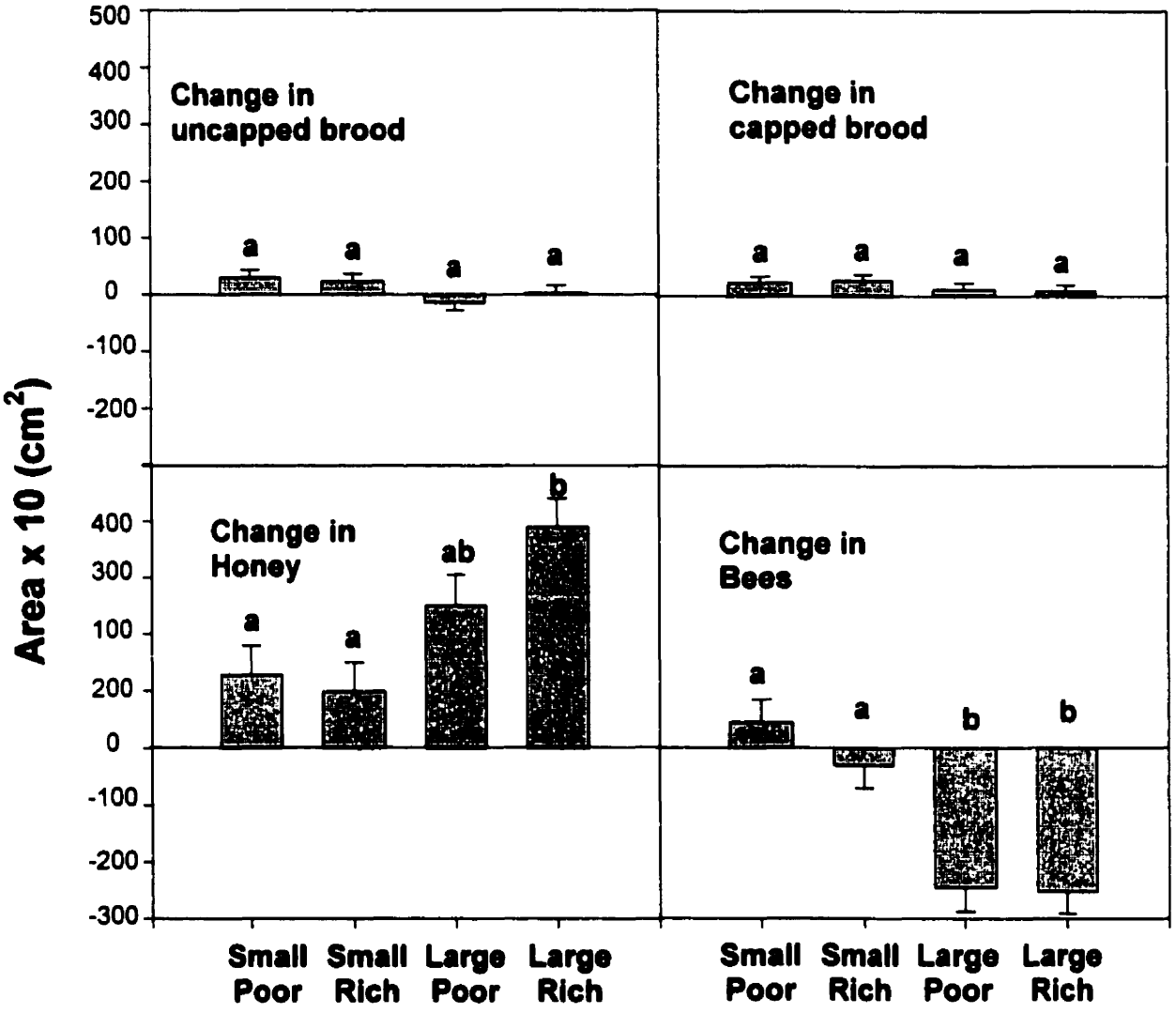

Figure 13. Change in amount of uncapped brood, capped brood, honey and bees over the 22-day study period in small and large colonies of *Apis mellifera* with poor or rich pollen stores. Colony parameters with the same letter did not differ significantly among treatments (GLM -MANOVA procedure with specific comparisons made using the estimate statement, $P < 0.05$).

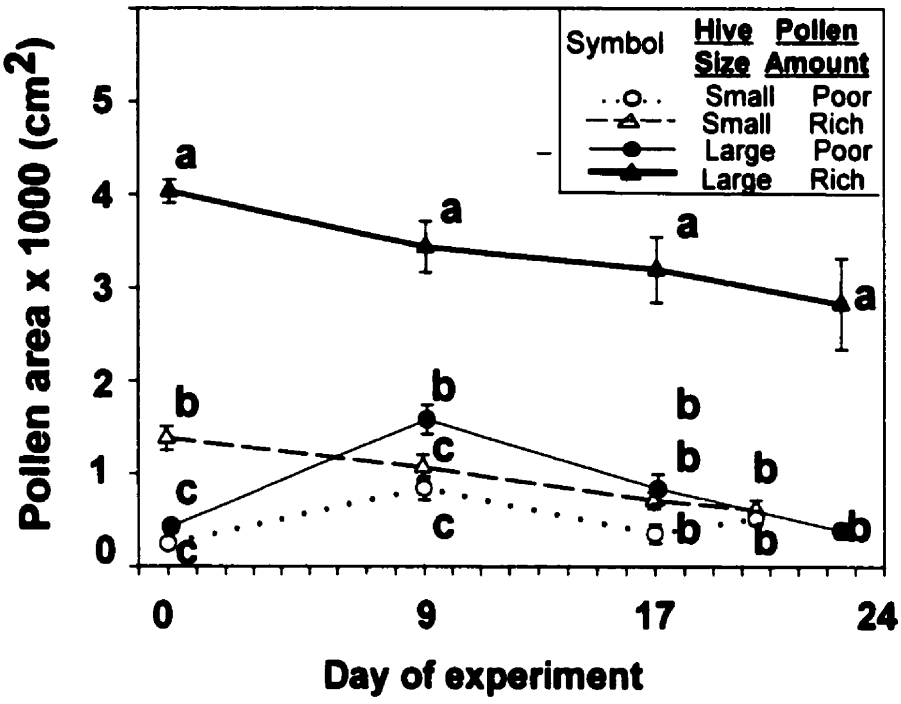

Figure 14. Mean (± SE) area of pollen content in colonies (cm^2) in small and large colonies of *Apis mellifera* with poor or rich pollen stores. Means on the same day with same letter did not differ significantly (Ryan's Q multiple comparison test, $P < 0.05$).

treatments, but after the first measurement date pollen-poor and pollen-rich treatments of small colonies were similar to one another ($F_{1,14} = 1.68$, $P = 1.00$).

Proportion of pollen foragers to nectar foragers at colony entrance

Differences in the foragers were found among treatments only on day 4 ($F_{3,27} = 3.00$, $P = 0.0479$). A significantly larger proportion of mid-day pollen foragers was found in pollen-poor (48.5%) than in pollen-rich treatments (31.5%) of large colonies ($F_{3,28} = 3.13$, $P = 0.0413$)(Fig. 15). In small colonies, there was no difference in percent mid-day pollen foragers between pollen-poor and pollen-rich treatments at any date ($P > 0.05$), although percent pollen foragers was high (46% and 45%). There were no treatment differences for day 5- 13 after colony manipulation. The percentage of mid-day pollen foragers decreased from 31 - 48% to 11 - 21% for all treatments over 10 days, although the statistical evidence for this decrease over time is weak ($F_{3,27} = 2.41$, $P = 0.089$).

***Vaccinium* pollen on pollen foragers**

There was no statistical evidence for treatment differences ($\chi^2 = 2.4875$, $P = 0.4776$, $n = 172$) in percent *Vaccinium* pollen carried by mid-day pollen foragers at any time during the experiment (Fig. 16). However, the proportion of *Vaccinium* pollen carried by pollen foragers on day 4 was large (10 and 29%), diminishing on subsequent days and not exceeding 10% for any day or treatment after day 4. The large range for data on day 4 is in part explained by a small fraction of pollen foragers with a large proportion of *Vaccinium* pollen grains in their load. On day 4, 17% of pollen foragers carried >80% *Vaccinium* load, and this percentage subsequently dropped to 5.3% on day 5 and stabilized on subsequent days to between 1 - 4% of pollen foragers.

The weight of pollen pellets per bee did not differ ($F_{15,19} = 1.378$, $P = 0.249$) between treatments for any day of the experiment. Mean pollen pellet weight per bee ranged from 10 to 18 mg/load.

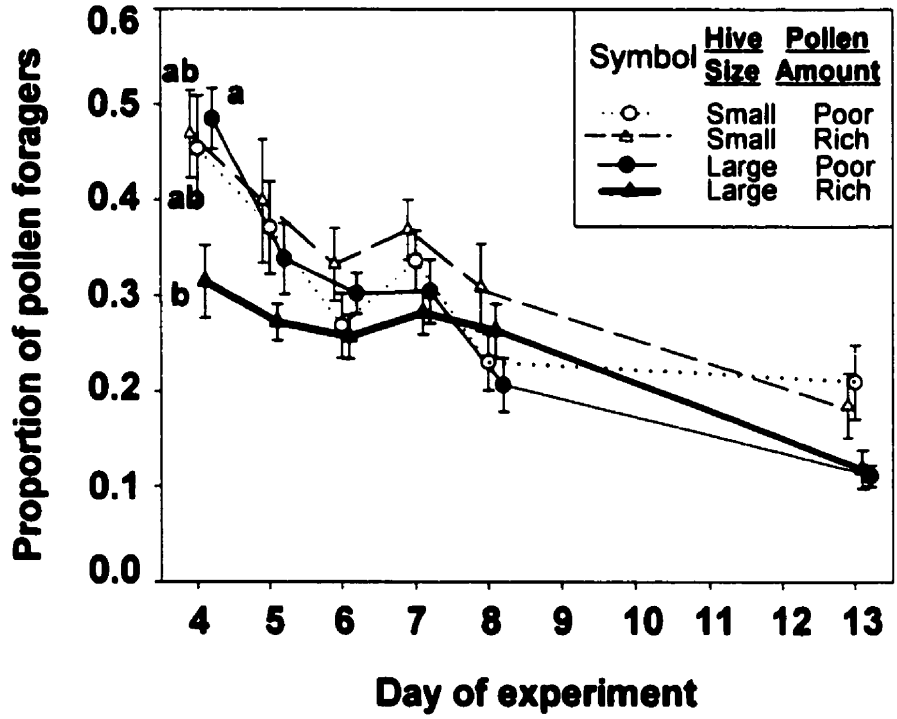

Figure 15. Mean (± SE) proportion of pollen foragers in small and large colonies of *Apis mellifera* with poor or rich pollen stores. Means on the same day with same letter do not differ significantly (Ryan's Q multiple comparison test, $P < 0.05$). Means on same day with no letter assigned to treatments were not statistically different (GLM procedure $P < 0.05$).

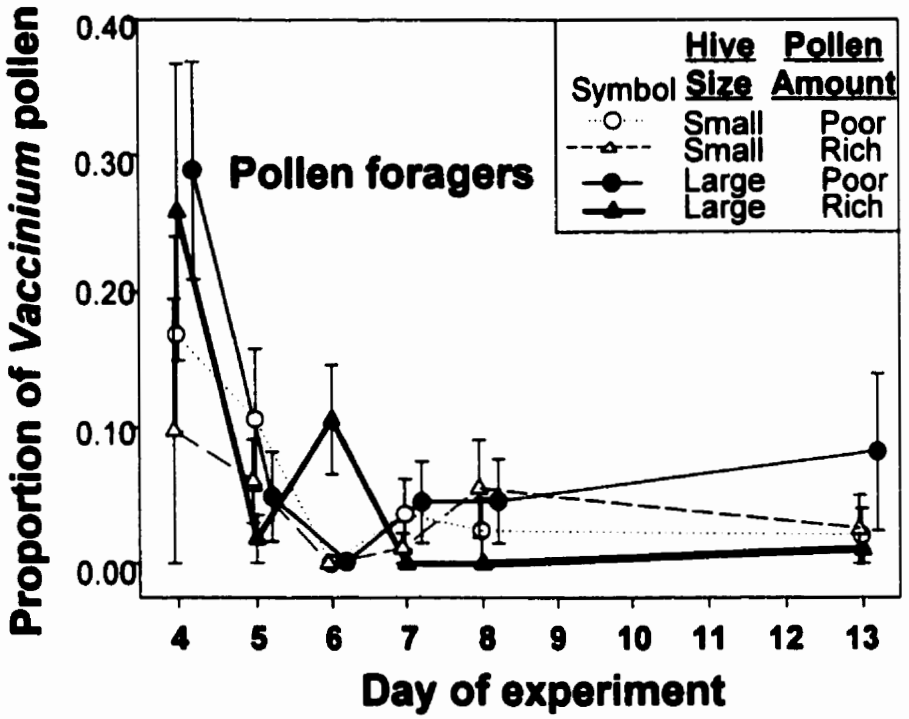

Figure 16. Mean (± SE) proportion of *Vaccinium* pollen loads carried by pollen foragers from small and large colonies of *Apis mellifera* with poor or rich pollen stores. No significant differences were found among treatments (Kruskal-Wallis Test, $P < 0.05$).

Vaccinium pollen on nectar foragers

Pollen storage manipulations did not have an effect ($F_{15,23} = 1.0224$, $P = 0.4689$, $n = 173$) on the percentage of *Vaccinium* pollen found on putative nectar foragers (Fig. 17). The proportion of *Vaccinium* pollen carried by putative nectar foragers did not exceed 13% for any day or treatment.

Percent of bees with *Vaccinium* pollen

Most pollen foragers carried no *Vaccinium* pollen (92.4%) in their loads indicating that only a few of the pollen foragers visited blueberry flowers (Fig. 18). In contrast, about two-thirds of nectar foragers (60.8%) carried some *Vaccinium* pollen on their bodies. Most nectar foragers with *Vaccinium* pollen (97.6%, $n = 502$) carried < 100 pollen tetrads, whereas 59.7% of the 62 pollen foragers with *Vaccinium* pollen carried >100 pollen tetrads. Pollen from the Aceraceae and Roseaceae accounted for 70% of pollen found on pollen and nectar foragers.

DISCUSSION

Bees from large colonies with pollen-poor stores had a significantly higher proportion of pollen foragers 4 days after the experiment began than in pollen-rich colonies, but there was no difference in the proportion of pollen foragers from small colonies with pollen-poor and pollen-rich stores. There were no differences in the weight of pollen pellets or the proportion of *Vaccinium* pollen grains in pollen pellets or on nectar foragers with colony size or pollen storage on all days examined. I found dramatic differences between the small proportion of pollen foragers (7.6%, $n = 819$) and the high proportion of nectar foragers (60.8%, $n = 826$) that carried *Vaccinium* pollen. My results provide evidence that nectar foragers have *Vaccinium* pollen on their bodies and thus may

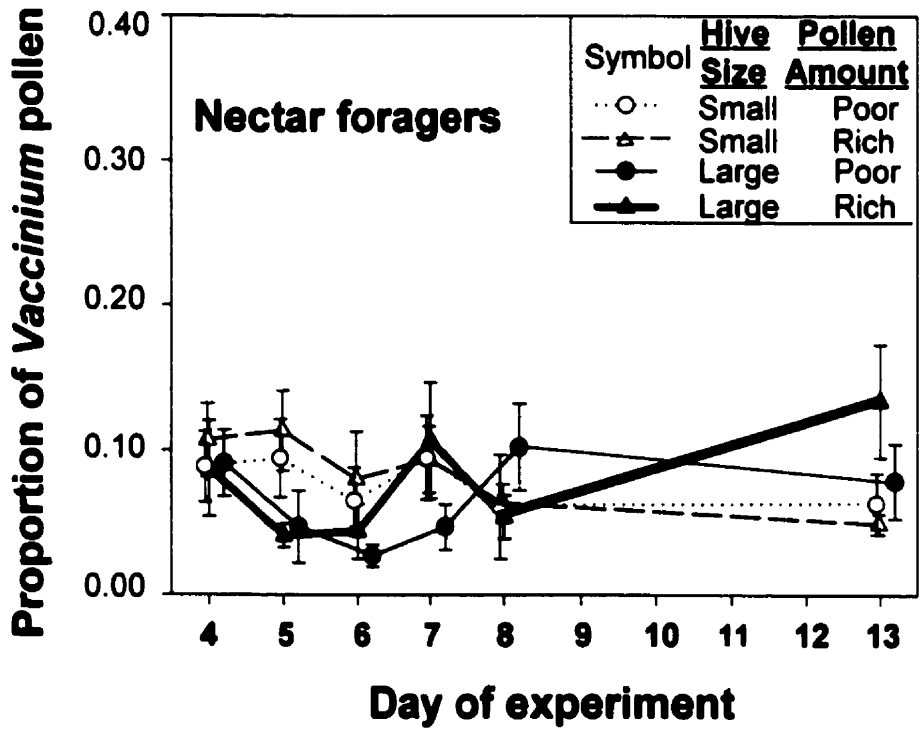

Figure 17. Mean (± SE) proportion of *Vaccinium* pollen on bodies of nectar foragers from small and large colonies of *Apis mellifera* with poor or rich pollen stores. No significant differences were found among treatments (GLM procedure, $P < 0.05$).

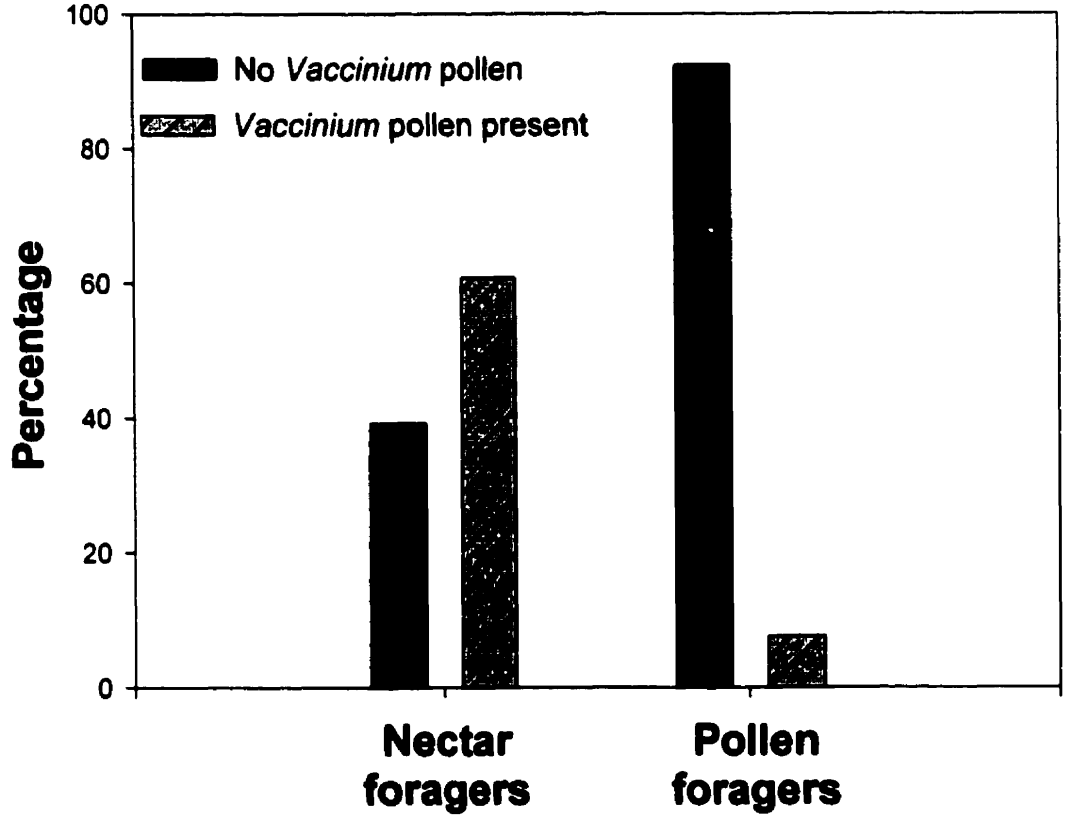

Figure 18. Percent of pollen and nectar foragers with and without *Vaccinium* pollen.

be important blueberry pollinators since returning pollen foragers rarely carried pollen from blueberry, and so their significance as pollinators of blueberry is questionable.

Pollen foraging in relation to pollen stores

The initial proportion of foragers returning with pollen loads was significantly higher for large colonies with pollen-poor stores (48.5%) than with pollen-rich stores (31.5%), but this difference diminished to non-significance after 5 days. A similar difference in the proportion of pollen foragers also was found in another study with the same large size colonies (Fewell and Winston 1992).

Honey bees show a high degree of resilience in responding to changes in parameters such as colony population size (Winston et al. 1985), with workers foraging at younger ages when populations are suddenly reduced (Winston and Ferguson 1985). In addition, the presence of reserve bees is thought to provide colonies with the task force needed to combat unexpected external disturbances (Schmid-Hempel et al. 1993; Seeley 1995; Kuhnholz and Seeley 1997). In my study, the increase in proportion of pollen foragers in large colonies is likely a colony level response to an altered level of colony state, in this case pollen stores.

The smaller colonies in my study showed no difference in the proportion of pollen foragers in response to pollen-rich (46% pollen foragers) and pollen-poor (45% pollen foragers) stores, although these proportions were similar to those in the large colony with poor pollen stores. The response resiliency of large colonies, with abundant reserve bees (Camazine 1993) does not appear to be an option for small colonies, presumably because small colonies do not have sufficient reserve bees to respond to colony perturbations. Small colonies respond to stress with larger nectar loads carried by individual foragers (Fewell et al. 1991), but do not increase the total number of foragers. In general, small colonies strive to increase their populations by brood rearing (Free and Racey 1968, Harbo 1986) in order to collect sufficient stores for successful over-wintering. The colony

responds to increased brood rearing by placing a high value on pollen resources, and increasing the number of pollen foragers (Eckert 1990). Thus, small colonies forage for pollen at maximum levels to increase their brood rearing and population growth, and my results indicate that these colonies appear to be unable to further increase their number of pollen foragers after removal of pollen stores.

The proportion of pollen foragers decreased to < 38% in 7 days after colony manipulations and further decreased to 10-25% in 14 days. This trend was similar for both large and small colonies, although more dramatic for large colonies with poor pollen stores and small colonies with poor and rich pollen stores. However, large colonies had 35% more pollen foragers than in small colonies. After a disturbance or change in colony parameters, workers of a colony adjust their behavior so that the colony returns to the preferred colony state (Schmid-Hempel et al. 1993). The return to this state was reached in 7 days in this study and 16 days in Fewell and Winston (1992). It was surprising that large colonies with high pollen stores also responded to manipulations with more pollen foragers early in the experiment. Perhaps the 4 days of inclement weather at the start of the experiment placed colony state into an apparent low pollen level, and colonies responded with an increased proportion of pollen foragers.

Proportion of *Vaccinium* pollen collected in relation to colony need.

My results on the proportion of *Vaccinium* pollen carried by individual foragers are important because they indicate that this parameter for both pollen and nectar foragers is not affected by colony size or levels of colony pollen stores. These results are similar to a study in which 10% and 9% of pollen pellets were identified as those of blueberry (Eaton and Stewart 1969; Olsen et al. 1979). In my study, pollen other than from the target crop was mostly tricolporate predominantly from Rosaceae and Aceraceae. These open flower types would be preferred by pollen foragers in particular since they provide bees with easy access to pollen, in comparison to blueberry flowers with their hidden nectaries and anthers

that are less accessible to honey bees. Non-crop pollen may also be obtained from within hive transfer (DeGrandi-Hoffman et al. 1986) and from non-target crop plants, especially if non-target plants are more attractive or if colonies are not placed adjacent to the target crop (Free and Williams 1972).

***Vaccinium* pollen carried *versus* forager type.**

Honey bee pollen foragers are assumed to be better pollinators than nectar foragers, and in general more pollen foragers is considered to be an indicator of improved pollination for many crops (Free 1993), although there are no clear data that support this assumption. My results indicate that pollen analyses are needed to examine whether this is true for all crops. Although my results do not indicate whether or not pollen foragers are better pollinators than nectar foragers on highbush blueberry, my study does indicate that nectar foragers are the major visitor of blueberry, since few honey bees foraged for blueberry pollen.

The large variation on day 4 resulted in part from the few pollen foragers with nearly pure *Vaccinium* pollen loads. Bees with these loads (2-3% of pollen foragers) were consistent throughout the study, indicating that there is a small proportion of specialist bees that forage for blueberry pollen.

Because the value of a pollinator depends on the deposition as well as the collection of pollen grains, the true value of a pollen *versus* nectar forager in blueberry is not known. If the total amount of pollen a forager carries is indicative of her potential pollination capabilities, pollen foragers would be the better pollinators. The sheer number of nectar foragers with some blueberry pollen would likely outweigh the few pollen foragers with large loads, and indicate that nectar foragers could be far more valuable for blueberry pollination than pollen foragers.

Nectar *versus* pollen foraging.

My data suggest that it would be advantageous to improve nectar foraging rather than general pollen foraging when honey bees are placed into blueberries, because 61% of nectar foragers carried blueberry pollen on their bodies and therefore should be able to pollinate blueberry. Nectar foragers visit 'Bluecrop' highbush blueberry and deposit *Vaccinium* tetrads, and thus are pollinators of the crop (Chapter IV). However, these results may not be applicable to varieties with long corollas and/or with narrow entrances that restrict honey bee foraging. Methods of increasing foragers such as feeding or spraying sugar syrup to colonies or spraying queen mandibular pheromones on blooming crops ('Fruit Boost') might be beneficial to blueberry pollination if nectar foraging were increased. Depending on the crop and forager behaviour, either nectar and or pollen foragers may be the most efficient pollinator(s) of a crop (Free and Williams 1974). Thus, it is of interest to determine which forager type is stimulated to forage and whether or not methods of increasing foragers are appropriate for the target crop. For example, syrup feeding increases pollen collection in kiwi fruit (Goodwin and Ten Houten 1991), and perhaps bees respond similarly in other crops. However, if syrup were fed to colonies placed into blueberry fields, I predict a *decrease* in blueberry pollination because blueberry nectar foraging would decrease and pollen foraging would increase, but not on blueberry. Thus, feeding syrup to colonies could be counter-productive to blueberry pollination. Nectar foraging could be promoted by having large colonies (Harbo 1986) with empty comb for storing nectar (Rinderer et al. 1979). Also, attractants such as "Fruit Boost" sprayed on blooming highbush blueberry increases bee counts and yields (unpublished, J. Pettis, USDA, Beltsvile, Maryland), suggesting that nectar foraging may be increased by this spray.

Although decreasing pollen stores and increasing brood area prior to placement into fields of blooming blueberry will stimulate pollen foraging, these manipulations do not

benefit blueberry pollination because pollen foragers rarely forage on blueberry. Growers might benefit from renting large colonies with excess pollen stores and empty comb, to enhance pollination by maximizing the number of nectar foragers on the crop.

Chapter V

Population biology and potential for managed pollination of the solitary mason bee *Osmia lignaria propinqua* Cresson

INTRODUCTION

The decreased availability of honey bees due to infestations by two parasitic mites and the impending threat of the Africanized "killer" bees in North America (Torchio 1990a; Winston 1992) has prompted an increased interest by growers in alternative pollinators. My study of the solitary mason bee, *O. l. propinqua*, in southern British Columbia was initiated because of a desire by fruit and berry growers to obtain an alternative or supplementary pollinator to honey bees (Levin 1957; Linsley 1958; Bohart 1972; Torchio 1990a). In Canada and western North America, the solitary alfalfa leafcutter bee, *M. rotundata*., is the major commercial alternative to honey bees as a pollinator of alfalfa (Hobbs 1967; Richards 1984; Peterson et al. 1992), but requires warm temperatures to fly and is unsuitable for early spring pollination in crops such as almonds, apples and blueberries. *Osmia* spp. fly at cool temperatures and have been recommended in North America as commercial pollinators of tree fruits (Phillips and Klostermeyer 1978; Kuhn and Ambrose 1984; Torchio 1987). They are not yet commercially available in large numbers.

The Japanese *O. cornifrons* Radoszkowski is used for pollinating fruit crops in Japan (Maeta and Kitamura 1964, 1965, 1974, 1981; Yamada et al. 1971; Sekita and Yamada 1993), and the European *O. cornuta* Latreille is under investigation for orchard pollination in Europe (Krunic et al. 1991; Bosch 1994a). *Osmia lignaria* in North America can be a successful pollinator of tree fruits (Kuhn and Ambrose 1984, Torchio 1982b, 1991b) and *O. ribifloris biedermannii* Michener, also has been suggested as a good candidate species for highbush blueberry pollination (Torchio 1990b).

Commercial pollination by any bee species depends on the availability of bees in large numbers at times of bloom, because flowering time can be brief (1-2 days) and crop areas large. Apple pollination, for example, requires 620 *O. l. propinqua* females per ha of apple trees (Torchio 1991a); 4.5 million would be required to pollinate British Columbia's 7,285 ha of apple orchards (Statistics Canada 1997). If large numbers can not be collected in the wild or increased while pollinating the crop, these bees would require artificial rearing under controlled conditions.

Osmia spp. nest in natural sites such as insect holes in wood and or cavities in rocks (Rust 1974), and can be collected in artificial nests (Torchio 1982a; Torchio 1982b). *Osmia* spp. occur in the Okanagan (Scott 1986) and coastal regions of British Columbia (MacKenzie and Winston 1984), but their density, distribution and nesting habits have not been studied. The most common of these species, *O. l. propinqua,* is the most convenient for commercial pollination because of its abundance (Rust 1974; Torchio 1987).

This research was part of a long-term study to test the suitability of *Osmia* spp. as a supplementary and/or alternative pollinator of commercial crops in British Columbia, and to determine whether numbers can be increased to sufficient levels for commercial pollination. My specific objectives were to determine: 1) locations in southern B. C. where native populations are concentrated; 2) nest type and height preferences; 3) period of *Osmia* nest building activity; 4) rate of emergence at different temperatures; and 5) the sex ratio of wild *Osmia* spp.

METHODS

Occupancy of nest types at different locations

Experiments were done at 14 locations (80 sites) in five regions within five biogeoclimatic zones of southwestern British Columbia (Table 2). At each site I

Table 2. Occurrence and numbers of *Osmia* spp. cocoons recovered from five geographic regions and five biogeoclimatic zones in southwestern B. C.

Region and location (biogeoclimatic zones)[a]	Total sites	% of sites with *Osmia* spp.	Mean cocoons per occupied site[b]
Southern Vancouver Island[c] (six locations combined) (CDF, CWH)	6	100.0	339.8 a
Lower Mainland			
Maple Ridge (CWH)	5	40.0	54.5 b
Southern Interior			
Pemberton (CWH)	6	66.6	15.0 bc
Shushwap-North Okanagan			
Armstrong & Coldstream (IDF)	6	50.0	5.0 bc
Chase (IDF)	5	60.0	9.3 bc
Enderby (IDF)	6	83.3	154.2 ab
Vernon (IDF)	6	33.3	10.5 bc
Winfield & Oyama (IDF)	6	16.7	12.0 c
COMBINED FOR REGION	**29**	**48.3**	**60.5**
Similkameen-South Okanagan			
Cawston (BG)	6	66.6	11.0 bc
Keremeos (BG)	5	40.0	49.5 b
Naramata (PP)	6	33.3	2.5 c
Oliver (BG)	5	20.0	2.0 c
Summerland (IDF, PP)	6	66.6	18.8 b
Okanagan Falls (BG)	6	83.3	21.6 b
COMBINED FOR REGION	**34**	**52.9**	**18.5**
TOTAL	80	55%	77

[a] Symbols for biogeoclimatic zones (Meidinger and Pojar 1991) as follows: Coastal Western Hemlock CWH; Bunchgrass BG; Ponderosa Pine PP; Interior Douglas Fir IDF; Coastal Douglas Fir CDF.

[b] Means followed by the same letter are not significantly different, Kruskal - Wallis Test $P < 0.05$.

[c] Sites for southern Vancouver Island are Mill Bay, Shawnigan Lake, Sooke and three sites in Victoria.

compared three types of wooden block nests with three nest types that consisted of bundles of straws held in a pipe (details in Table 3). One of each type of artificial nests were placed at each of five to six sites in every location by February 1994 in southern Vancouver Island and the Lower Mainland, and by late March 1994 in the four inland regions. Nests were attached to fence posts or buildings in sunny locations, and to trees if no other structures were available, at 50-200 cm above ground and generally facing south. Nests were removed from all locations at the end of August, stored under cover at ambient temperatures in Burnaby, B. C. until early October 1994, and then stored at 3- 4°C until occupancy was determined by the presence of cocoons in February 1995.

Period of nest building activity

WOOD-CARDBOARD nests (7 at each site) were set out in February 1995 at four sites on southern Vancouver Island and four sites in Enderby to monitor the period of nest building by *O. l. propinqua*. Weekly observations were made on the number of filled straws until no further straws were filled. A nest was deemed filled when mud plugs were visible at the exposed end of each straw.

Nest height preference

WOOD-CARDBOARD nests were placed on a post in 1995 at 0, 30, 60, 90, 120, 150 and 180 cm above ground, in sunny locations facing south at three sites each in Enderby and southern Vancouver Island (at Mill Bay and two sites in Victoria) where bees had been abundant in 1994. Both wild and released *O. l. propinqua* were present as adults in 1995. Released bees were obtained from cocoons collected from the same locations in 1994. Prior to placing cocoons in a cardboard box, adjacent to nests, cocoons were rinsed in 0.05% NaOCl (bleach), followed by two rinses of tap water to remove pollen eating mites (*Chaetodactylus* spp.) that can kill *Osmia* eggs (Sekita and Yamada 1993). Rinsed

Table 3. Description of nest types tested for preference by *Osmia lignaria propinqua* in southwestern B. C.

Name	Description	Source
WOOD-PLASTIC	Laminated wood block (13 x15 x 15 cm) with 42 plastic drinking straws [14 cm long 7.5 mm internal diameter (ID)] inserted into smoothly drilled holes (14 cm deep)	Constructed by the authors
WOOD-CARDBOARD	Laminated wood blocks as above with 42 cardboard straws (14 cm long, 7.5 mm ID, walls 0.5 mm thick).	Constructed by the authors. Cardboard straws from Custom Paper Tubes Inc. 15900 Industrial Parkway Cleveland, Ohio 44135 USA
PAWOOD-PLASTIC	Commercially-made pressed block manufactured from wood-paper mixture with 36 plastic straws (7.5 mm ID) inserted into holes (13.5 cm deep).	F. Pederson, PO Box 415, Ambrose, ND 58833 USA
PIPE-PLASTIC	Bundle of 42 plastic drinking straws[a] (14 cm long, 7.5 mm ID) inserted into black irrigation pipe (10 cm ID) with a cap at one end.	Constructed by authors.
PIPE-CARDBOARD	Pipe as above with a bundle of 42 cardboard straws[a] each wrapped in plastic (14 cm long, 7.5 mm ID, walls 0.25 mm thick).	D. Mayer, Washington State University., 24106 N. Bunn Rd., Prosser WA 99350 USA
PIPE-CARDBOARD-P	Pipe as above with a bundle of 42 cardboard straws[a] (14 cm long, walls 1 mm thick, 7.5 mm ID)	Constructed by authors.

a A bundle of straws was held together with an elastic band and was wrapped in a 2.5 cm thick piece of upholsterer's cotton to insulate nesting bees from extreme temperatures.

cocoons were air dried for 2-3 h, and stored at 3- 4°C until placed into the field adjacent to artificial nests. Nests were examined weekly for occupancy, between mid-March and late June. Nests were considered complete when a mud plug was visible at the exposed end of each straw.

Emergence at different temperatures

Cocoons were removed from WOOD-CARDBOARD nests placed in Enderby and southern Vancouver Island in early 1994. Nests with cocoons were stored as above. In late January 1995 cocoons were removed from nests, separated by sex and location, and placed in petri-dishes (10 cm diam.). These were incubated until adult emergence or termination of the experiment after 12 days at three temperatures and light treatments: 1) 22°C with 60% RH and 16:8 L:D photo regime; 2) 22-25°C, 70-75% RH and 16:8 L:D photo regime; and 3) 26-30°C, 90% RH and 12:12 L:D photo regime. A fourth treatment exposed cocoons to natural light conditions (12:12 L:D) in a greenhouse. There were 16 to 26 cocoons of each sex per location per treatment.

Sex ratio

Cocoons were removed in February 1996 from WOOD-CARDBOARD nests from Enderby and Southern Vancouver Island and their sex estimated by size and position in cardboard straws (bees from 1995 nest height preference). Females are located in large inner cells and fit tightly into the 7.5 mm diam. cardboard straws, whereas males are located in small outer cells and fit loosely in the straw. The sexual identity was confirmed by opening a few cocoons and identifying male bees by their abundant, white facial pilosity, and females by their scant, black pilosity (Bosch 1994b).

Statistics

Proportional data for nest type preference were analyzed by multiple-comparison test for proportions and the Bonferroni inequality procedure (Jones 1984). Thirty-two sites with all six nest types and the presence of *Osmia* spp. were used for analyses of nest type preference. Cocoon densities in plastic and cardboard straws in completely filled WOOD-PLASTIC and WOOD-CARDBOARD nests (from Southern Vancouver Island and Enderby), were transformed by ln x and were analyzed using the GLM (SAS Institute 1990) procedure followed by Ryan's Q test for differences between means (Day and Quinn 1989). Differences between mean number of cocoons produced in 14 locations and differences between sex ratio at two locations were analyzed using a Kruskal-Wallis Test with PROC NPAR1WAY (Chi-square approximation)(SAS institute 1990, Schlotzhauer and Littell 1987). The LIFETEST analysis (SAS Institute 1990) was used to determine if there were differences in the rate of nest occupancy (number of straws filled per week) at different heights between southern Vancouver Island and Enderby, and between sites within locations. LIFETEST was also used to determine the rates of bee emergence at different temperature and light treatments. The Wilcoxon and the log rank tests were used in this procedure, with the Wilcoxon indicating early differences and the log rank test emphasizing differences later in nest occupancy and bee emergence respectively. In all cases, $\alpha = 0.05$.

RESULTS

Occupancy of WOOD-PLASTIC and WOOD-CARDBOARD nest types was higher than in all other nest types (Fig. 19). The number of cocoons in occupied straws was higher in WOOD-CARDBOARD nest blocks with cardboard straws (5.67 cocoons /straw) than WOOD-PLASTIC nest blocks with plastic straws (2.33 cocoons/straw).

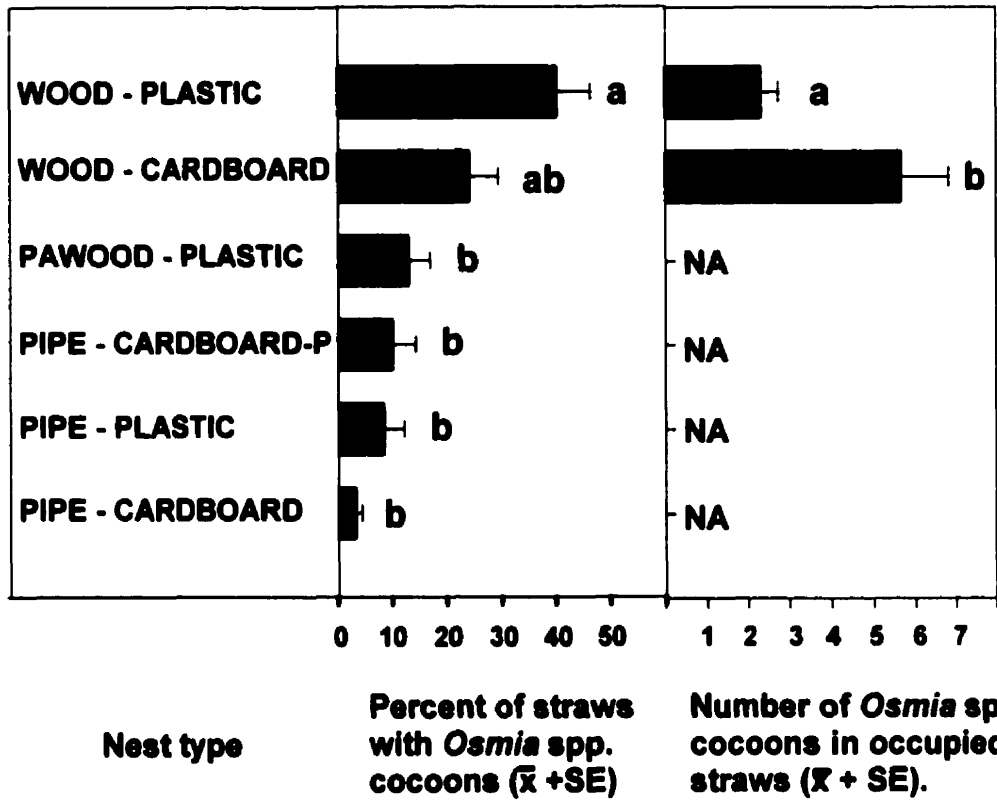

Figure 19. Percent of straws with *Osmia* spp. cocoons in six nest types, and the number of *Osmia* spp. cocoons in occupied plastic and cardboard straws. NA, not assessed. Bars with same letter did not differ significantly by multiple-comparison test for proportions and the Bonferroni inequality procedure, $P < 0.05$, for percent straws with cocoons in nest types n = 32; and by GLM procedure with Ryan's Q test for differences between means of number of cocoons per straw type, n = 164 and 82 for WOOD-PLASTIC and WOOD-CARDBOARD nests, respectively.

Osmia l. propinqua were found in each of the five biogeoclimatic zones, in all 14 locations, and at 55% of the 80 sites (Table 2). All six sites of the southern Vancouver Island region were occupied by *O. l. propinqua* whereas all other regions had at least one site with no *O. l. propinqua*. The number of cocoons ranged from 2 - 712 in occupied sites. More cocoons were collected from sites in Southern Vancouver Island (2039) and Enderby (771), than from any other location ($X^2 = 28.61$, df = 13, $P = 0.0074$), especially from three sites on southern Vancouver Island (478, 572 and 712 cocoons); and two sites in Enderby (345 and 409 cocoons). The remaining locations had a mean of 17.5 cocoons per occupied site.

Nest building began in late March to early April on coastal southern Vancouver Island and in late April to early May at Enderby in the Shuswap-North Okanagan region (Fig. 20). Nesting continued for 4-8 weeks depending on the site.

Rates of occupancy between Enderby and Victoria were significantly different (LIFETEST, $P < 0.05$) at 0, 30, 120 and 150 cm above ground. On Southern Vancouver Island, nests generally were occupied earlier at heights < 90 cm and were occupied later at heights of 120 cm above ground (Fig. 21). Peak establishment of residency occurred between weeks 2 and 6 at all heights. In Enderby, occupancy was progressively earlier with height except at 180 cm. Peak establishment of residency was progressively earlier with height, from week 5 at 0 cm to week 2 at 90 cm, but switched to week 4 at 180 cm, similar to the residency establishment curves at 30 and 60 cm. Ultimately, there was no difference in percent occupancy with height on southern Vancouver Island (> 78%), but in Enderby, nests at 120 cm above ground were more heavily occupied (87%) than those at 0 cm (56%).

Under a common incubation regime, male and female bees emerged from cocoons originating at Enderby faster than from cocoons originating from southern Vancouver Island (Fig. 22). For female cocoons from Southern Vancouver Island, bees emerged significantly faster in warmer than cooler temperatures. Under greenhouse conditions at

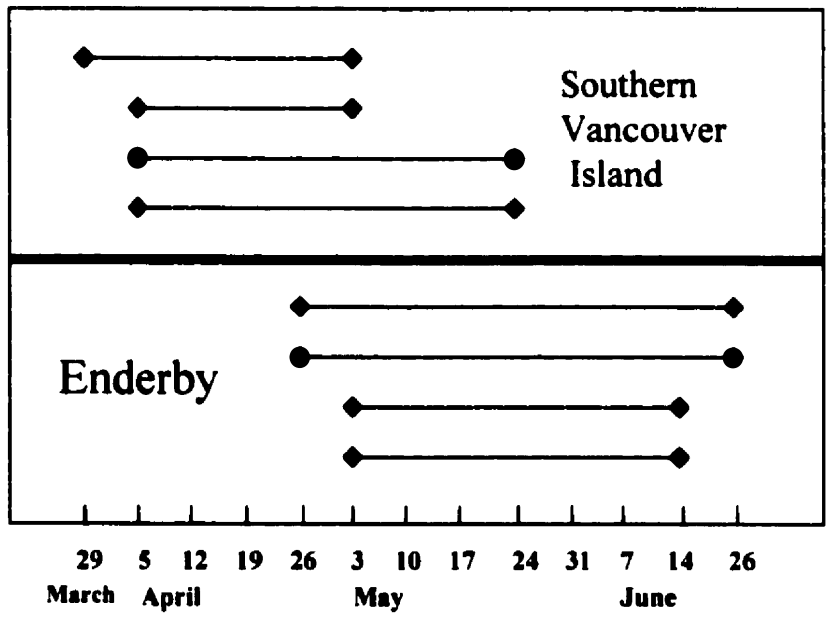

Figure 20. Periods of nesting activity by *Osmia lignaria propinqua* at four southern Vancouver Island sites and at four Enderby sites in 1995.

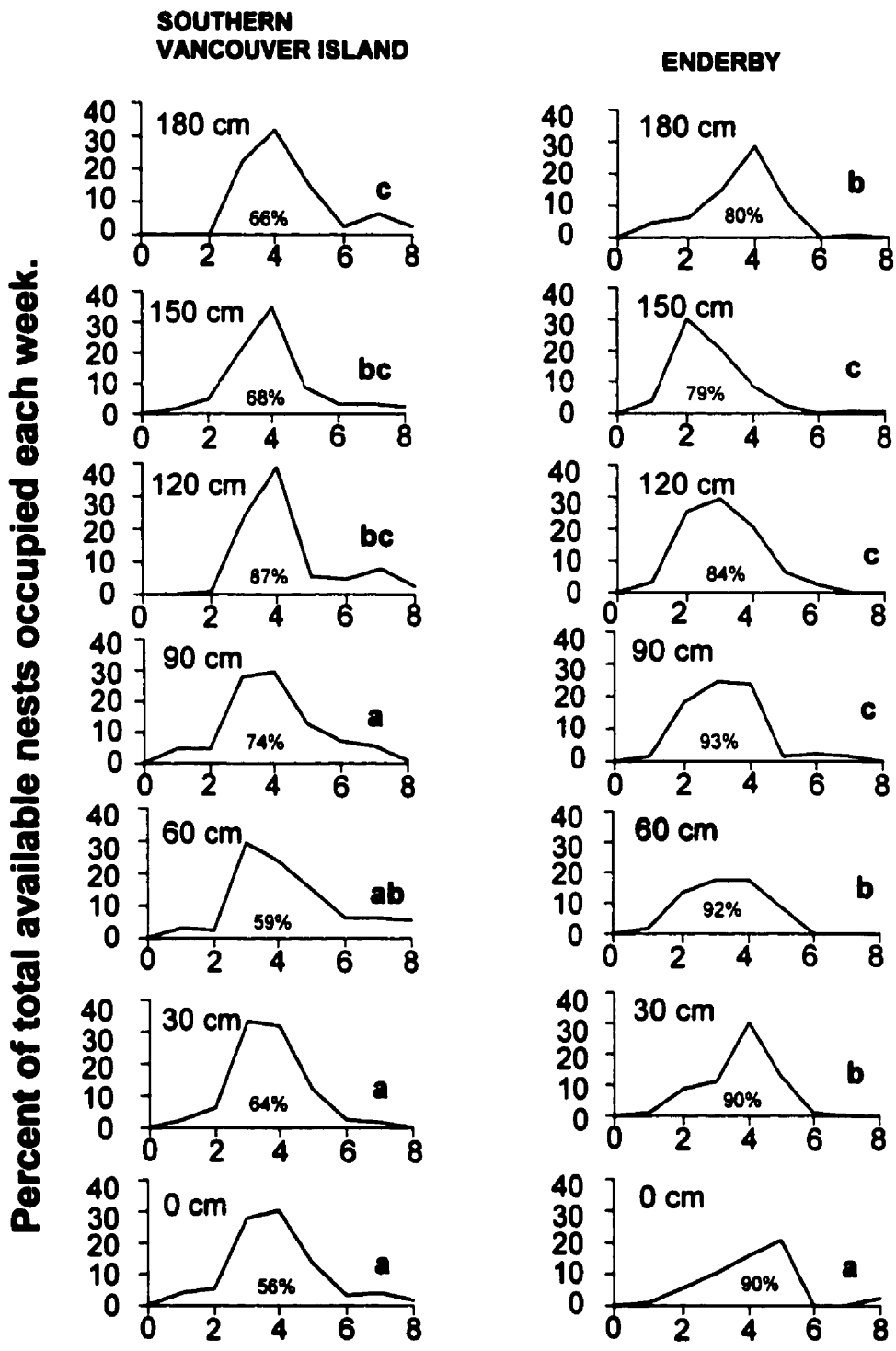

Figure 21. Percent of total available nests occupied each week for nests at ground level and six above-ground heights for populations of *Osmia lignaria propinqua* at Enderby and southern Vancouver Island. Curves within a column with the same letter were not significantly different, LIFETEST, $P < 0.05$. Percents under curves show final proportion of available nests occupied after 8 weeks

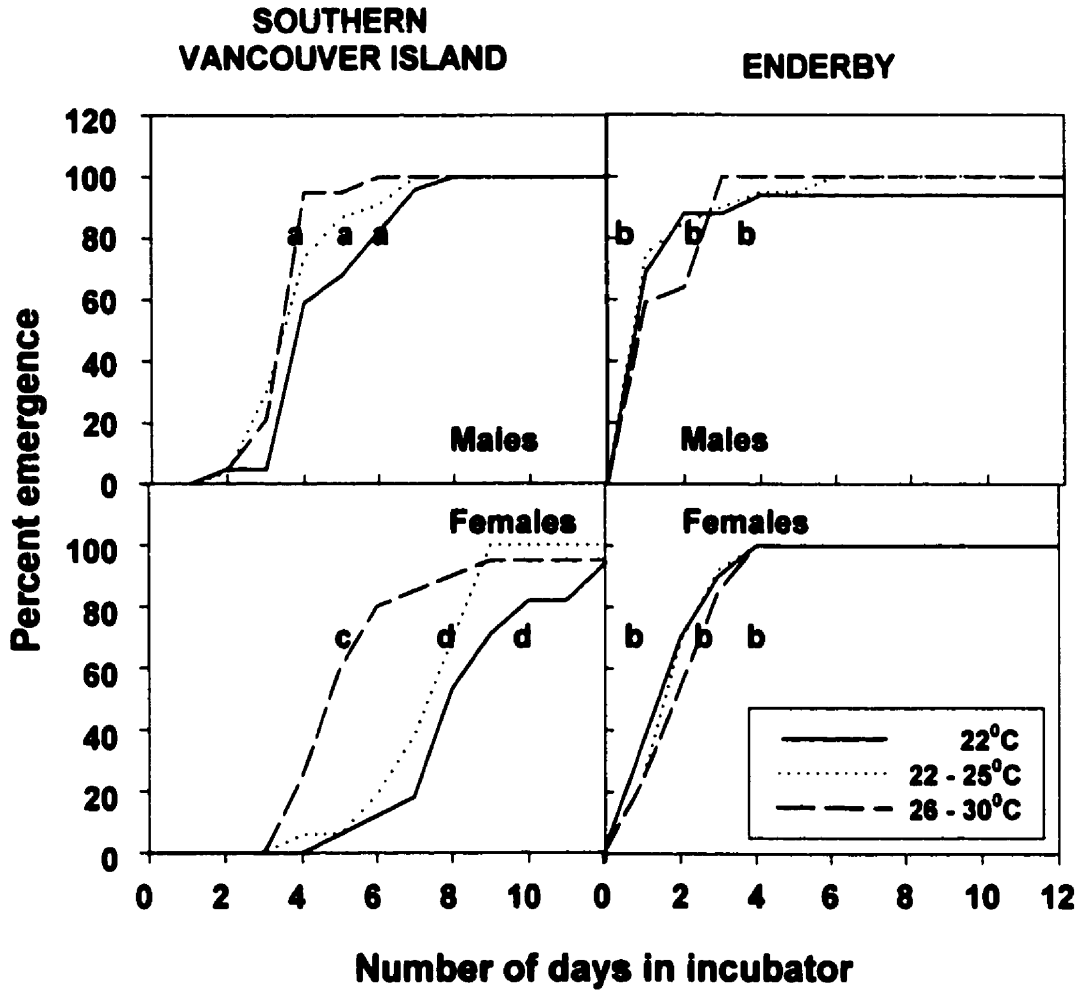

Figure 22. Cumulative percent emergence of *Osmia lignaria propinqua* from populations collected at southern Vancouver Island and Enderby at incubator temperatures and light regimes: 22°C with 60% RH and 16:8 L:D photo regime; 22-25°C, 70-75% RH and 16:8 L:D photo regime; and 26-30°C, 90% RH and 12:12 L:D photo regime. For entire figure, lines with the same letter were not different, LIFETEST, $P < 0.05$.

20°C (Fig. 23), southern Vancouver Island male and female emergence was incomplete (77% and 44%, respectively), whereas 94% of Enderby male and females emerged within 12 days. The sex ratio was not significantly different ($X^2 = 10.193$; df = 5; $P > 0.070$) between Enderby (1.66 males : 1 female, n = 1951) and Southern Vancouver Island (1.70 males : 1 female, n = 3518) populations.

DISCUSSION

The commercial use of *Osmia* spp. for pollination requires that sufficient numbers of females (approx. 620 / ha for orchard crops; Torchio 1991a) be available for pollinating large acreages. In 1994, the 3388 *O. l. propinqua* collected from nests at 80 sites in 14 locations would have been sufficient to pollinate only 5.5 ha of orchard. Thus, it would be unwise to initially rely on wild-trapped *O. l. propinqua* for commercial pollination until adequate numbers are available. However, use of *Osmia* spp. could provide a supplement to other pollinators. The largest number of cocoons was collected from southern Vancouver Island, where nest blocks had been provided in the previous 3 years, suggesting that populations can be increased by providing nesting sites. Similarly, a permanent water source, available mud and an abundance of forage probably enhanced the population levels in Enderby. The low occupancy of nests in most locations could be due to absence of bees, lack of mud and water, insufficient forage, inclement weather conditions and adverse local temperatures (Torchio 1981).

Osmia produced cocoons in all nest types tested but cardboard straws, whether inserted into wooden blocks or bundled together, produced more than double the number of cocoons than plastic straws in wooden blocks. Low cocoon densities in plastic straws

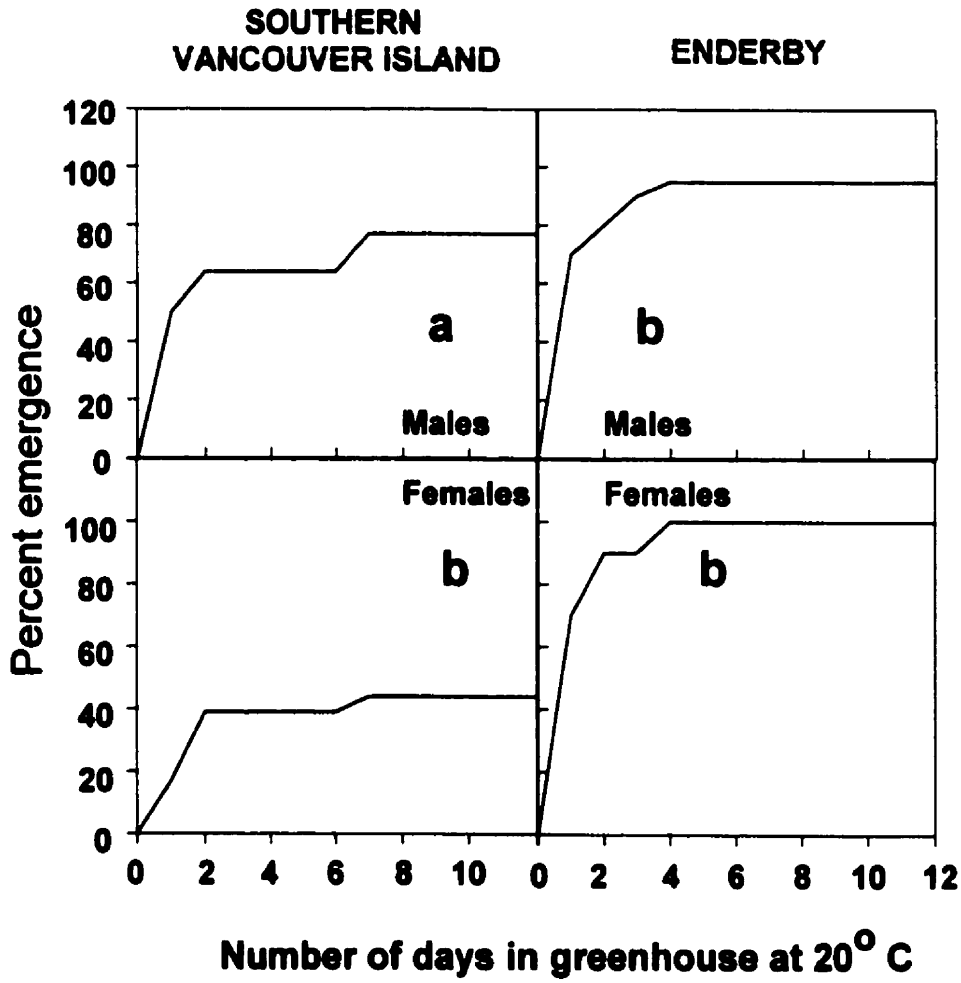

Figure 23. Cumulative percent emergence of *Osmia lignaria propinqua* from populations collected at southern Vancouver Island and Enderby under natural light conditions (12:12 L:D) inside a greenhouse with day time temperatures set at 20°C. For entire figure, lines with the same letter were not different, LIFETEST, $P < 0.05$.

could have been caused by lack of adequate footing on the slippery straw surface. Mud plug construction also may be more difficult on a slippery surface. Low occupancy of straws in irrigation pipe containers could have been due to the cap at the end of the container not being light proof, even though it had been painted black (W.Shuge, Shijiazhuang Fruit Trees Research Institute, Shijiazhuang city, Hebei Province P. R. China, pers. comm.). In 1995, occupancy of irrigation pipes was improved by bundling cardboard straws with glued newspaper and gluing a piece of cardboard onto the end, making the nest light-proof.

Sex ratio may have a significant impact on future population size. The 1.7 male :1 female sex ratio in this study favored females more than sex ratios typically reported for *O. l. lignaria* Say (2.14 male : 1 female)(Torchio 1976) and *O. l. propinqua* (2.1:1; 2.45:1; 3.39:1)(Torchio 1982b, 1984, 1985, respectively). Variation in sex ratio could be due to different nest types (Torchio 1976, 1982a, 1984, 1990b; Torchio and Asensio 1985; Bosch 1994a), intertunnel distance (Tepedino et al. 1994), or population size (Grechanyi and Pogodaeva 1996). In my study, the percent female cocoons from Enderby increased as the population (indicated by the total number of cocoons produced at a particular site) decreased; percent females increased from 34.6% to 38.4% and 44.3% in populations of 980, 777, and 194 cocoons, respectively. The remaining sites on southern Vancouver Island could not be estimated in the same way since non-research nests were not examined for cocoon number. Nonetheless, sites on southern Vancouver Island consisted of 38.8% females with populations well over several thousand *Osmia*. Further research is needed to determine optimum population size, if any, that will maximize female production.

The different rates of emergence at the same temperatures and the differences in nest height preferences suggest that the geographically isolated populations of *O. l. propinqua* in Enderby and southern Vancouver Island consist of two different populations, and could require population-specific management regimes. It also therefore suggests that diapause and incubation requirements might be tailored through selection for local needs.

Other studies have found differences between populations for emergence time for *O. cornuta* (Bosch and Blas 1994; Tasei 1973), *M. rotundata* (Rank and Rank 1989; Johansen and Eves 1973; Tasei and Masure 1978), for overwintering mortality of *O. lignaria* (Rust 1995), and for temperature response in other insects (Boivin et al. 1986).

Bees that emerge synchronously, dependably and successfully are necessary if *Osmia* spp. are to be used commercially. However, it would be unwise to use Enderby *Osmia* in cool, wet regimes like southern Vancouver Island, where premature emergence during an early spring warm period might trap the bees in prolonged unfavorable weather. If the southern Vancouver Island population is used, greenhouse emergence should be avoided; incubation temperatures of 26°C would maximize emergence. The 20°C temperature maintained in the greenhouse may be near the threshold for emergence for the southern Vancouver Island population. In addition, greenhouse temperatures may have cooled at night, and prolonged emergence time. Bees may have emerged within an additional number of days under warmer incubator conditions (Stephen and Osgood 1965).

Although Enderby males emerged 28% faster than females on day 1 (Figure 22), there was no statistical evidence of protandry, the emergence of males in advance of the females, presumably an adaptation to assure immediate fertilization (Linsley 1958). In contrast, the Vancouver Island population was distinctly protandrous.

My findings of differences in nest height preferences for southern Vancouver Island and Enderby populations again indicate another contrast between these populations. Preferred nest height was 60 cm for *O. cornifrons* in Japan (Maeta and Kitamura 1974). Although there are no data on nest height preferences for *O. lignaria* in N. America nor *O. cornuta* in Europe, specific heights were used in studies of nest type comparisons: 1 m (Bosch 1994a), 1.3 m (Torchio 1976; Vicens et al. 1994), and 1.83 m (Torchio 1990b). Any of these heights would probably be acceptable in Enderby, but a height > 1 m might be sub-optimal in Vancouver Island and 1.83 sub-optimal for Enderby. Proper nest height may be critical, because it would increase the number of bees produced and decrease

dispersal of nesting females, a major problem for successful commercial use of *Osmia* spp. (Bosch 1992; Torchio and Asensio 1985).

If *O. l. propinqua* were available in large numbers, these bees would be a valuable and marketable addition to available commercial pollinators. I found that *Osmia* are present at most sites but they are not usually abundant, and a concerted effort was required to collect the initial population. Further, there are two distinct populations in southern British Columbia and these have different nesting and emergence requirements. These differences increased the probability of collecting *Osmia* spp. under favorable environmental conditions such as abundant forage and mud, together with the best nest type. Thus, the use of *Osmia* spp. as a commercially managed pollinator would be enhanced by collections at favorable sites using appropriate nest type and height. The success of these *Osmia* spp. would depend on a good match between population characteristics and local site conditions.

Chapter VI

Conclusions and recommendations for highbush blueberry growers

REVIEW OF RESULTS

Comparison of bee species

Overall, bumblebees are the most effective pollinator of blueberry. Bumble bees deposited twice the amount of blueberry pollen grains and handled flowers 50 percent faster on flowers than honey bees. Mason bees deposited variable amounts, generally the same as honey bees but less than bumble bees, and had variable floral visitation rates. Bumble bee activity was not deterred by cool weather during bloom, whereas honey bees, mason bees, and alfalfa leaf cutter bees require warmer temperatures before foraging begins. Individual foraging distances also differed between species. The median distances flown from nest sites were different between honey bees (130 m) and bumble bees (69 m). Alfalfa leaf cutter bees were not observed in the field. Mason bees foraged at the shortest distance (58 m). Blueberry visitation as estimated by the proportion of bees at nest entrances with blueberry pollen, was greatest for bumble bees and mason bees (62 and 65%) and less for alfalfa leaf cutter bees (36%) and honey bees (7%).

Pollen requirements of highbush blueberry fruiting

I determined that maximum fruit weight can be attained from self, mixed and outcross fertilization with 125 pollen grains, with a trend that selfing of 'Bluecrop' flowers was less productive than outcrossing fertilization with variety 'Patriot'. The effect of pollen load size generally was larger than the effect of pollen donor; 125 pollen grains of 50-100% outcross pollen < 3 days old are sufficient for optimal fruit quality.

Pollen storage and foraging by honey bees.

I examined whether blueberry pollination could be improved by increasing the number of pollen foragers in large and small honey bee colonies by depriving colonies of pollen. The proportion of pollen foragers increased in large colonies with small pollen stores, but not in small colonies with either small or large pollen stores. Overall, there were no differences between small and large pollen stores in small and large colonies for pollen pellet weight, proportion of blueberry pollen grains in pollen pellets or on nectar foragers. However, I found dramatic differences between the small proportion of pollen foragers (8%) and the high proportion of nectar foragers (61%) that carried some bodily blueberry pollen. My results provide evidence that honey bee nectar foragers are the major pollinators of blueberry and pollen foragers go elsewhere for their pollen.

Collection and use of the native solitary mason bees.

I found mason bees at most locations, but they were not usually abundant, and a concerted effort was required to collect the initial population. The largest number of cocoons was collected from southern Vancouver Island where nest blocks had been provided three in previous years. Populations of this bee may be increased if released in suitable environments with abundant forage, a permanent water source and an available mud source. More research is needed to develop efficient methods for increasing populations suitable for commercial pollination. If they multiply on the crop, once they are introduced, they could be self-sustaining or perhaps marketable. However these bees would have to be managed since parasites decrease populations over several generations.

Economic analyses between honey bees, bumble bees and mason bees.

Since alfalfa leaf cutter bees are not suitable for blueberries because cool weather conditions limit their foraging, only bumble bees, honey bees and mason bees were evaluated for economic suitability for blueberry pollination.

Bumble bees deposit twice the number of pollen grains on individual blueberry flowers than honey bees, and work flowers 50% faster than honey bees. Thus, one bumble bee is equivalent to 4 honey bee foragers per unit foraging time. There are approximately 8,000 honey bee foragers in a honey bee colony, so 2,000 bumble bee foragers are required to equal one honey bee colony, but there are approximately 100-200 foragers in a bumble bee colony. The current cost of a bumble bee colony is about Can. $250 (1998; B. Macadam, Westgro Sales, Ltd. Delta, B.C., Canada). Therefore the cost of 2,000 bumble bees is about $2,000 - 5,000 depending on the number of foragers in a colony. In comparison, one honey bee colony with about 8,000 foragers costs $50 to rent, and 5 honey bee colonies per ha are recommended for blueberry, costing $250. The equivalent bumble bee colonies would cost Can.$12,500 - 25,000 or a mean cost of Can.$18,750/ha. Mason bees would cost Can.$414 /ha (based on Japanese costs of Can. $0.14/bee; 740 nesting bees per ha (Torchio 1990b) 2,960 bees, 50:50 male-female ratio, with 50% mortality of female bees) slightly higher than the cost of honey bees per ha ($250 Can.). However, the cost of mason bees in North America is $0.75 - $1.50 Can./bee since these are produced in small numbers and sold only to home gardeners. Using the higher North American costs of bees, pollinating 1 ha in North America would cost $2,220 Can. (2,960 bees x $0.75) to $4,440 Can. (2,960 bees x $1.50), or a mean of $3,330 Can.

Bumble bees would be more competitive if colony costs could be divided over 2 or 3 crops. Since the life of a bumble bee colony is no more than 1-3 months, short blooming periods for individual crops would be essential. Similarly, cost of mason bees could be reduced if nest cells are recovered and then could be rented out to other growers. Unless bumble bee colonies and mason bees are reduced in cost, these species will remain uneconomical. Biologically an individual bumble bee is superior to a honey bee or mason bee for blueberry pollination.

RECOMMENDATIONS

Choice of bee and management.

Honey bees, although not the most effective biologically, are economically the best highbush blueberry pollination in today's market because of their availability and ease of placement into the field wherever bees are required. Since nectar foragers are the major honey bee visitors at blueberry, large colonies would provide a larger work force of nectar foragers. Nectar collection could be promoted by having adequate pollen stores and excess room for nectar storage. Small colonies would be less suitable for blueberry pollen foraging since the focus of small colonies is pollen collection and the production of bees rather than nectar collection and honey storage (Free and Racey 1968; Harbo 1986). The demand for pollen in small or pollen-deprived colonies would drive pollen foragers from the crop to competing bloom.

Traditional methods of increasing foragers such as feeding or spraying sugar syrup into colonies would be beneficial to blueberry pollination if nectar foraging were increased. However, when colonies are fed sugar, the opposite may occur, and pollen foraging may be stimulated. Nectar fed to colonies decreases their immediate demand for nectar, decreasing nectar foraging and as a result increasing the proportion of pollen foragers. Since pollen foragers are not the major blueberry visitors, I would predict a decrease in blueberry pollination if syrup were fed to colonies placed into blueberry fields. Thus, feeding syrup to colonies could be counter-productive to blueberry pollination. Nectar foraging could be promoted by having large colonies with empty comb for storing nectar or spraying Fruit Boost on blooming highbush blueberry to increase bee numbers and yields, perhaps because nectar foraging may be increased by this attractant spray.

Assessment of bee density.

A pollen count on floral stigmas can be used to measure bee effectiveness in a field of blueberries. Since 125 pollen grains are optimum for fruit weight and fruit ripening time whereas 25 pollen grains were insufficient, lower counts indicate inadequate bee density in the field. More colonies could be rented and moved into the field to boost bee numbers where stigmatic pollen loads are found to be inadequate.

Overall, pollination effectiveness differed between bumble bees, honey bees, mason bees and alfalfa leafcutter bees because these bees differ in their behaviour at the blueberry flower. The high proportion of returning bumble bee and mason bee foragers at the nest that carry blueberry pollen indicates that individuals of these species are faithful blueberry pollinators. However, if inclement weather conditions prevail, bumble bees are the preferred pollinators since cool temperature did not deter their foraging activity. An additional advantage of bumble bees and honey bees over mason bees is that flight distance was further, eliminating the need for multiple nest locations which increases management costs.

Few honey bees at colony entrances carried blueberry pollen. However, most honey bee foragers on blueberry flowers did carry blueberry pollen, indicating that foragers in the crop are foraging on blueberry. Overall, individual honey bees were not as effective pollinators as individual bumble bees and mason bees, but of honey bees are more readily provided in greater numbers-due to their large colony population.

If mason bees were available in large numbers, these bees would be a valuable addition to commercial pollination of blueberry, but the difficulty of collecting and maintaining managed populations suggests they would not be a good target species for commercial blueberry pollination management unless populations could consistently be increased in areas of preferred forage plants or reared in commercial production facilities.

Although I did find blueberry pollen on alfalfa leafcutter bees, indicating that they do visit blueberry flowers, their high temperature threshold of 18°C makes them unsuitable

for blueberry pollination, since cooler ambient air temperatures are frequent when highbush blueberry blooms.

Honey bees remain the best pollinator for blueberry because they cost less than other managed bee species, even though biologically, honey bees rank second with mason bees to bumble bees. Even though individual honey bees are a relatively poor pollinator of blueberry, they remain the best economically, since the overriding factor that determined its superiority was the cost of bringing bees into the crop.

CONCLUSIONS

The major accomplishments of this study have been to elucidate the minimal number of pollen grains required by blueberry flowers for maximal fruit production, and determine how well each bee species performs as a pollinator. These research results will allow stigmatic pollen loading to be assessed and determine if pollinator density is adequate and more importantly determine if pollination or other factors are the cause of poor fruit set or low yields. More specifically, pollen counts of flowers can be used to determine optimal colony placement, number of colonies at each location and overall colony density.

This study indicates that bumble bees and blueberry flowers have the most closely linked relationship, which has likely evolved from the development of a mutualistic relationship between plant and its pollinator. The buzz behaviour of bumble bees allows them to forage for both pollen and nectar, and deposit larger amounts of pollen onto blueberry flower stigmas than honey bees, mason bees and alfalfa leaf cutter bees. The major type of foraging activity by honey bees is for nectar, and this does not lend itself to removing pollen from the anthers of blueberry flowers. However, blueberry flowers can still be adequately pollinated by an increased number of bee visits to flowers. More bee visits to a flower would make-up for the lower number of pollen grains deposited.

Not only is the pollinating behaviour of the bee important while she is visiting a flower, but other parameters also are critical in whether a bee species would be a good pollinator of a crop. The localized foraging of mason bees would be an asset to farmers since it is less likely that these bees would stray out of the crop. Conversely, honey bees communicate alternative forage to other bees in their colony and thus are more likely to stray away from the crop. Nevertheless, this could be countered by placing more honey bee colonies into the crop.

There are important implications in knowing the pollen loading requirements of individual blueberry flowers and the effect of selfing and crossing between blueberry varieties. Knowing that 125 blueberry tetrads is sufficient for optimal fruit quality growers could increase bee density until this level was reached in the crop. In addition, the usual practice of planting highbush blueberry in solid blocks of a single vegetatively-propagated variety, would decrease the chance of bees outcrossing with other blueberry varieties. Thus my work, indicates the importance of having a different variety of blueberry interspersed within a block of a blueberry plants.

Since honey bees remain the best commercial pollinator of blueberry, because these bees are the most economical, more research is needed to determine how honey bee pollination can be improved. Colony manipulations such as pollen removal (this study), pollen addition, and honey removal need to be examined in blueberry and other crops to determine the best management strategy for optimal pollination using honey bee colonies.

It is surprising that no other study could be found that simultaneously determined a plant's needs for pollen and determined a bees' capabilities to fulfill these needs in a crop plant (literature search from 1980, and references therein). Generally, pollen requirements of a flower does not simply equal ovule number; pollen requirement is usually more than ovule number. The continued demand for improved yields will no doubt drive research into determining pollen requirements for crops such as raspberry and greenhouse tomato. Pollen requirements for raspberry and tomato are not known, nor have individual pollinators been examined to determine their pollen loading capabilities. Pollen requirements for cranberry flowers is known and is soon to be published (D. Schiffhauer, Ocean Spray Cranberries Inc, NJ, pers. comm). Only with these basic data can an assessment be completed that will determine how pollination levels can be optimized for maximum fruit yield.

Enhancing pollination effects to increase yields has been successful by spraying Fruit Boost (honey bee queen mandibular pheromones). Increased recruitment and

increased time spent and flowers visited are two mechanisms identified that improve pollination when Fruit Boost is sprayed on crops (Higo et al. 1995). Perhaps increased bee numbers in the crop are in fact nectar foragers that do the majority of blueberry pollination and may be the reason why Fruit Boost increases yields. The key experiment linking Fruit Boost and increased yields in blueberry would be to determine if the proportion of nectar foragers increase in a colony after spraying with Fruit Boost.

Although mason bees were not economically or biologically the best pollinator, these bees could be a valuable alternative or supplementary pollinator. The availability of mason bees in large numbers will have to be the major focus of future research if this bee is to become a commercial pollinator in North America. In unmanaged populations, numbers drop over a period of 3-4 years because of pollen feeding mites, and populations do not increase without intervention such as mite control. Research is needed on the best crop in which a mason bee population can increase, and mite management. Perhaps mason bee populations could be increased at sites other than in orchards and placed into blooming crops as disposable units. Also, more detailed information is needed on optimal bee numbers for each nest location and density requirements. Since these bees are short distance foragers, they would be excellent pollinators of hybrid crops so that cross pollination does not occur, or smaller orchards where competing bloom is available outside the orchard.

More research on pollination requirements of crop plants and bee capabilities as pollinators of a particular crop is essential to improve crop production of pollinated food crops. These results have answered the major question of blueberry flower requirements, and have brought into focus important research questions that need to be answered for improving crop pollination. Honey bees are the best pollinator of northern highbush blueberries and therefore more research is required into methods of improving their pollination effectiveness on highbush blueberry.

VII APPENDIX

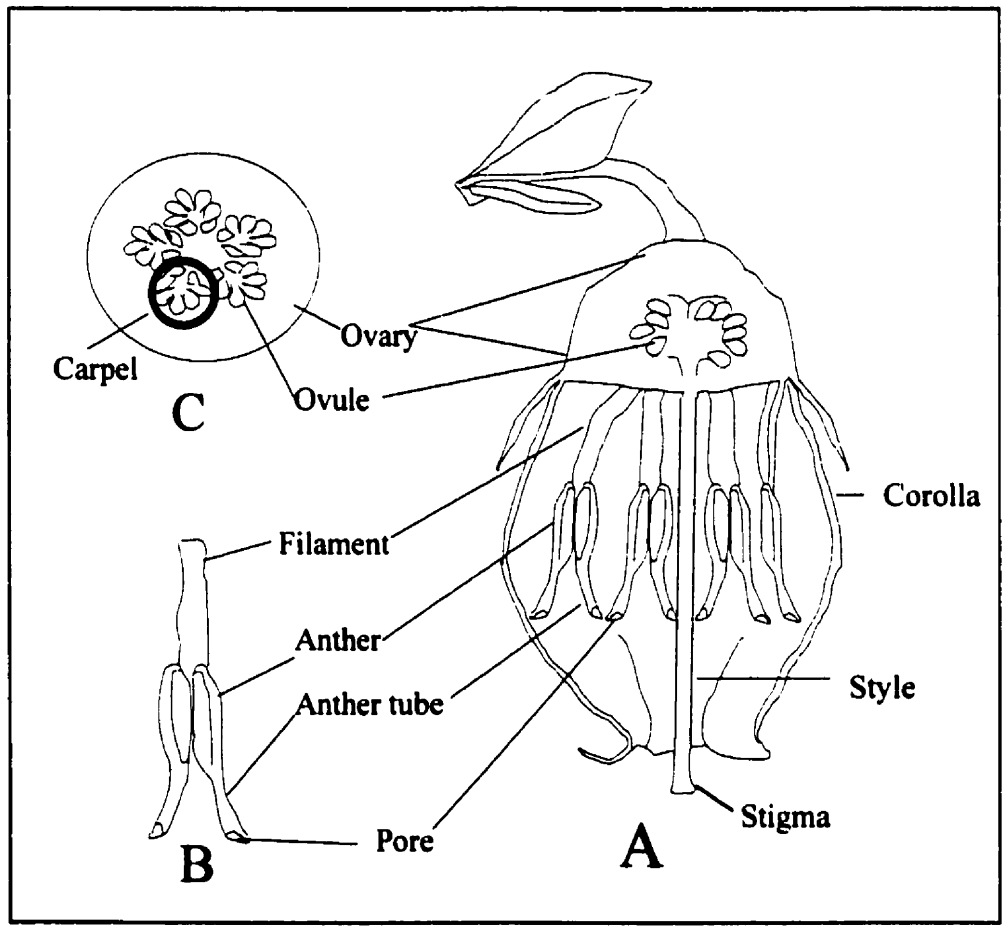

Figure A-1. Longitudinal section of blueberry flower (A), the anther (B) and cross section of ovary (C). (Re-drawn from McGregor 1976).

VIII LITERATURE CITED

Abrol, D. P. and R. P. Kapil. 1994. On homing ability and pollination effectiveness of bees. Mysore Journal of Agricultural Science **28**:249-252.

Aras, P., D. de Oliveira and L. Savoie. 1996. Effect of a honey bee (Hymenoptera; Apidae) gradient on the pollination and yield of lowbush blueberry. Journal of Economic Entomology **89**:1080-1083.

Barker, R. J. 1971. The influence of food inside the hive on pollen collection by a honeybee colony. Journal of Apicultural Research **10**:23-26.

Bell, H. P. 1957. The development of the blueberry seed. Canadian Journal of Botany **35**:139-153.

Bertin, R. I. 1990. Effects of pollination intensity in *Campsis radicans*. American Journal of Botany **77**:178-187.

Bierzychudek, P. 1981. Pollinator limitation of plant reproductive effort. American Naturalist **117**:838-40.

Bohart, G. E. 1972. Management of wild bees for the pollination of crops. Annual Review Entomology **17**:287-312.

Boivin, G., C. Ritchot, P. Martel and D. G. R. McLeod. 1986. Evidence for regional differences in the emergence pattern of the European corn borer (Lepidoptera: Pyralidae) in Quebec. Journal of Economic Entomology **79**:978-980.

Bosch, J. 1992. Parasitism in wild and managed populations of the almond pollinator *Osmia cornuta* Latr. (Hymenoptera: Megachilidae). Journal of Apicultural Research **31**:77-82.

Bosch, J. 1994a. Improvement of field management of *Osmia cornuta* (Latreille) (Hymenoptera, Megachilidae) to pollinate almond. Apidologie **25**:71-83.

Bosch, J. 1994b. The nesting behaviour of the mason bee *Osmia cornuta* (Latr) with special reference to its pollinating potential (Hymenoptera, Megachilidae). Apidologie **25**:84-93.

Bosch, J. and M. Blas. 1994. Effect of over-wintering and incubation temperatures on adult emergence in *Osmia cornuta* Latr (Hymenoptera, Megachilidae). Apidologie **25**:265-277.

Brewer, J. W. and R. C. Dobson. 1969. Pollen analysis of two highbush blueberry varieties *Vaccinium corymbosum*. Journal of American Society for Horticultural Science **94**:251-252.

Brewer, J. W., R. C. Dobson and J. W. Nelson. 1969. Effects of increased pollinator levels on production of the highbush blueberry *Vaccinium corymbosum*. Journal of Economic Entomology **62**:815-818.

Buchmann, S. L. 1983. Buzz pollination in angiosperms. Pages 73-113 in C. E. Jones, Little RD (eds), Handbook of Experimental Pollination Biology. Van Nostrand Reinhold, New York, NY.

Camazine, S. 1993. The regulation of pollen foraging by honey bees: how foragers assess the colony's need for pollen. Behavioural Ecology and Sociobiology **32**:265-272.

Cane, J. H. and J. A. Payne. 1988. Foraging ecology of the bee *Habropoda laboriosa* Hymenoptera: Anthophoridae), an oligolege of blueberries (Ericaceae: Vaccinium) in the southeastern United States. Annals of the Entomological Society of America **89**:419-427.

Cane, J. H., G. C. Eickwort, F. R. Wesley and J. Spielholz. 1985. Pollination ecology of *Vaccinium stamineum* (Ericaceae: Vaccinioideae). American Journal of Botany **72**:135-142.

Cane, J. H., K, Mackenzie and D. Schiffhauer. 1993. Honey bees harvest pollen from the porose anthers of cranberries (*Vaccinium macrocarpon*)(Ericaeae). American Bee Journal **133**:293-295.

Cane, J. H., D. Schiffhauer and L. J. Kervin. 1996. Pollination, foraging , and nesting ecology of the leafcutting bee *Megachile* (*Delomegachile*) *addenda* (Hymenoptera: Megachilidae) on cranberry beds. Annals of the Entomological Society of America **89**:361-367.

Carre, S., I. Badenhausser, J. N. Tasei, J. Le Guen and J. Mesquida. 1994. Pollen deposition by *Bombus terrestris* L, between male-fertile and male-sterile plants in *Vicia faba* L. Apidologie **25**:338-349.

Chaplin, M. F. and J. F. Kennedy. 1994. Carbohydrate analyses. Practical Approach. Oxford University Press, Oxford, UK.

Charest, N. and G. Hergert. 1993. An estimation of the value of pollination by honey bees. Canadian Association of Professional Apiculturists Proceedings.

Conover, W. J. 1980. Practical Nonparametric Statistics. 2nd edition. John Wiley and Sons, New York.

Cripps, C. and R. W. Rust. 1989. Pollen foraging in a community of *Osmia* bees (Hymenoptera:Megachilidae). Environmental Entomology **18**:582-589.

Currie, R. W., M. L. Winston and K. N. Slessor. 1992a. Effect of synthetic queen mandibular pheromone sprays on honey bee (Hymenoptera: Apidae) pollination of berry crops. Journal of Economic Entomology **85**:1300-1306.

Currie, R. W., W. L. Winston, K. N. Slessor and D. F. Mayer. 1992b. Effect of synthetic queen mandibular pheromone sprays on pollination of fruit crops by honey bees (Hymenoptera: Apidae). Journal of Economic Entomology **85**:1293-1299.

Czesnik, E., G. Bounous and D. Gioffre. 1989. A survey of self-incompatibility in highbush blueberry *Vaccinium corymbosum* L. In E. J. Stang [ed.], Proceedings of the Fourth International Symposium on Vaccinium Culture. Acta Horticulturae **241**:56-63.

Dag, A., D. Eisenstein and S. Gazit. 1998. Effect of pollenizer distance and selective fruitlet abscission on outcrossing rate and yield in 'Tommy Atkins" mango. Journal of American Society for Horticultural Science **123**:618-622.

Danka, R. G., G. A. Lang and C. L. Gupton. 1993. Honey bee (Hymenoptera: Apidae) visits and pollen source effects on fruiting of 'Gulfcoast' southern highbush blueberry. Journal of Economic Entomology **86**:131-136.

Darrow, G. M. 1958. Seed number in blueberry fruits. American Society for Horticultural Science **72**:212-215.

Darwin, C. 1876. On the Effects of Cross and Self fertilization in the Vegetable Kingdom. John Murray, London.

Davis, L. E., A. G. Stephenson and J. A. Winsor. 1987. Pollen competition improves performance and reproductive output of the common zucchini squash under field conditions. Journal of American Society for Horticultural Science **112**: 712-716.

Day, R. W. and G. P. Quinn 1989. Comparisons of treatments after an analyses of variance in ecology. Ecological Monographs **59**:433-463.

DeGrandi-Hoffman, G., R. Hoopingarner and K. Klomparens. 1986. Influence of honey bee (Hymenoptera: Apidae) In-hive pollen transfer on cross-pollination and fruit set in apple. Environmental Entomology **15**:723-725.

Dogterom, M. H., J. A. Matteoni and R. C. Plowright. 1998. Pollination of greenhouse tomatoes by the north American *Bombus vosnesenskii*. (Hymenoptera: Apidae). Journal of Economic Entomology **91**:71-75.

Dorr, J. and E. C. Martin. 1966. Pollination studies on the highbush blueberry *Vaccinium corymbosum* L. Michigan Quarterly Bulletin **48**:437-448.

Eaton, G. W. 1967. The relationship between seed number and berry weight in open-pollinated highbush blueberries. HortScience **2**:14-15.

Eaton, G. W. and M. G. Stewart. 1969. Highbush blueberry pollen collection by honey bees. HortScience **4**:95.

Eck, P. 1988. Blueberry Science. Rutgers University Press, New Brunswick and London.

Eck, P. and C. M. Mainland. 1973. Highbush blueberry fruit set in relation to flower morphology. HortScience **6**:494-495.

Eckert, C. D. 1990. The relationship between colony state and individual foraging behaviour in the honey bee, *Apis mellifera* L. M.Sc. Thesis, Simon Fraser University, British Columbia.

El-Agamy, S. Z. A., W. B. Sherman and P. M. Lyrene. 1981. Fruit set and seed number from self-and cross-pollinated highbush (4x) and rabbiteye (6x) blueberries. Journal of American Society for Horticultural Science **106**:443-445.

El-Agamy, S. Z. A., W. B. Sherman and P. M. Lyrene. 1982. Pollen incompatibility in blueberries (*Vaccinium* spp.). Journal of Palynology **18**:103-112.

Faegri K. and L. van der Pijl. 1979. The Principles of Pollination Ecology. William Clowes & Sons Limited, London.

Fewell, J. H. and M. L. Winston. 1992. Colony state and regulation of pollen foraging in the honey bee, *Apis mellifera* L. Behavioral Ecology and Sociobiology **30**:387-393.

Fewell, J.H., R. C. Ydenberg and M. L. Winston. 1991. Individual foraging effort as a function of colony population in the honey bee, *Apis mellifera* L. Animal Behaviour **42**:153-155.

Free, J. B. 1960. The pollination of fruit trees. *Bee World* **41**:141-151, 169-186.

Free, J. B. 1963. The flower constancy of honey bees. Journal of Animal Ecology **32**:119-131.

Free, J. B. 1965a. The behaviour of honey bee foragers when their colonies are fed sugar syrup. Journal of Apicultural Research **4**:61-64.

Free, J. B. 1965b. Attempts to increase pollination by spraying crops with sugar syrup. Journal of Apicultural Research **4**:61-64.

Free, J. B. 1967. Factors determining the collection of pollen by honeybee foragers. Animal Behaviour **15**:134-144.

Free, J. B. 1993. Insect Pollination of Crops. Academic Press, London.

Free, J. B. and P. A. Racey. 1968. The effect of the size of honey bee colonies on food consumption, brood rearing and the longevity of the bees during winter. Entomological Experimentalis et Applicata **11**:241-249.

Free, J. B. and I. H. Williams. 1972. The transport of pollen on the body hairs of honeybees (*Apis mellifera* L.) and bumblebees (*Bombus spp.* L.). Journal of Applied Ecology **9**:609-615.

Free, J. B. and I. H. Williams. 1974. Influence of the location of honeybee colonies on their choice of pollen sources. Journal of Applied Ecology **11**:925-935.

Freitas, B. M. 1997. Number and distribution of cashew (*Anacardium occidentale*) pollen grains on the bodies of its pollinators, *Apis mellifera* and *Centris tarsata*. Journal of Apicultural Research **36**:15-22.

Galletta, G. J. and J. R. Ballington. 1996. Blueberries, cranberries and lingonberries. *In* J. Janick and J. N Moore [eds] Fruit Breeding, volume II: Vine and small fruits crops. John Wiley & Sons, Inc. New York.

Gary, N. E., P. C. Witherell and J. Marston. 1972. Foraging range and distribution of honey bees used for carrot and onion pollination. Environmental Entomology **1**:71-78.

Gary, N. E., P. C. Witherell and J. Marston. 1973. Distribution of foraging bees used to pollinate alfalfa. Environmental Entomology **2**:573-578.

Gary, N. E., P. C. Witherell and J. Marston. 1975. The distribution of foraging honey bees from colonies used for honeydew melon pollination. Environmental Entomology **4**:277-281.

Gary, N. E., P. C. Witherell and J. Marston. 1976. The inter- and intra-orchard distribution of honey bees during almond pollination. Journal of Apicultural Research **15**:43-50.

Goldy, R. G. and D. P. M. Lyrene. 1983. Pollen germination in interspecific *Vaccinium* hybrids. HortScience **18**:54-55.

Goodell, K. and J. D. Thomson. 1997. Comparison of pollen removal and deposition by honey bees and bumblebees visiting apple. *In* K. W. Richards [ed.], Proceedings of the 7th International Symposium on Pollination. Acta Horticulturae **437**:103-107.

Goodwin, R. M. 1997. Feeding sugar syrup to honey bee colonies to improve pollination: a review. Bee World **78**:56-62.

Goodwin, R. M. and A. Ten Houten. 1991. Feeding sugar syrup to honey bee (*Apis mellifera*) colonies to increase kiwi fruit (*Actinidia deliciosa*) pollen collection: effects of frequency, quantity and time of day. Journal of Apicultural Research **30**:41-48.

Grechanyi, G. V. and M. V. Pogodaeva. 1996. Changes in sex ratio in relation to population size in *Drosophila*. Russian Journal of Genetics. **32**:1170-1174. Translated from Genetika **32**:1349-1353.

Gupton, C. L. 1984. Effect of pollen source on fruit characteristics of low-chilling highbush type blueberries. HortScience **19**:531-532.

Gupton, C. L. and J. M. Spiers. 1994. Interspecific and intraspecific pollination effects in rabbiteye and southern highbush blueberry. HortScience **29**:324-326.

Hancock, J. F. and J. H. Siefker. 1982. Levels of inbreeding in highbush blueberry cultivars. HortScience **17**:363-366.

Harbo, J. R. 1986. Effect of population size on brood production, worker survival and honey gain in colonies of honey bees. Journal of Apicultural Research **25**:22-29.

Harrison, R. E., J. J. Luby and P. D. Ascher. 1993. Pollen source affects yield components and reproductive fertility of four half-high blueberry cultivars. Journal of American Society for Horticultural Science **119**:84-89.

Harrison, R. E., J. J. Luby and P. D. Ascher. 1994. Fruit and seed set of half-high blueberry cultivars following repeated pollinations at varying time intervals. Fruit Varieties Journal **48**:176-182.

Hellman, E. W. and J. N. Moore. 1983. Effect of genetic relationship to pollinizer on fruit, seed, and seedling parameters in highbush and rabbiteye blueberries. Journal of American Society for Horticultural Science **108**:401-405.

Heinrich, B. 1979. Bumble bee Economics. Harvard University Press, Cambridge, Mass.

Higo, H. A., S. J. Colley and M. L. Winston. 1992. Effects of honey bee (*Apis mellifera* L.) queen mandibular gland pheromone on foraging and brood rearing. The Canadian Entomologist **124**:409-418.

Higo, H. A., M. L. Winston and K. N. Slessor. 1995. Mechanism by which honey bee (Hymenoptera:Apidae) queen pheromone sprays enhance pollination. Annals of the Entomological Society of America **88**:366-373.

Hood, M. E. and H. D. Shew. 1996. Applications of KOH-aniline blue fluorescence in the study of plant-fungal interactions. Phytopathology **86**:704-707.

Hobbs, G. A. 1967. Domestication of alfalfa leaf-cutter bees. Department of Agriculture Canada Pub. **No.1313**. Ottawa, Ontario.

Jacob-Remacle, A. 1989. Foraging behaviour of honey bees and wild bees in Belgian apple orchards. English summary. Apidologie **20**:271-285.

Jay, S. C. 1966. Drifting of honeybees in commercial apiaries. III. Effect of apiary layout. Journal of Apicultural Research **5**:137-148.

Johansen, C. A. and J. D. Eves. 1973. Effects of chilling, humidity and seasonal conditions on emergence of the alfalfa leafcutting bee. Environmental Entomology **2**:23-26.

Johannsson, M. H. and A. G. Stephenson. 1997. Effects of pollination intensity on the vigor of the sporophytic and gametophytic generation of *Cucurbita texana*. Sexual Plant Reproduction **10**: 236-240.

Johnston, M. O. 1993. Tests of two hypotheses concerning pollen competition in a self-compatible, long-styled species (*Lobelia cardinalis*: Lobeliaceae). American Journal of Botany **80**:1400-1406.

Jones, D. 1984. Use, misuse, and role of multiple comparison procedures in ecological and agricultural entomology. Environmental Entomology **13**:635-649.

Kearns, C. A. and D. W. Inouye. 1993. Techniques for Pollination Biologists. University Press of Colorado, Niwot, Colorado.

Kendall, D. A. and M. E. Solomon. 1972. Quantities of pollen on the bodies of insects visiting apple blossom. Journal of Applied Ecology **9**:609-15.

Knight, R. J. JR. and D. H. Scott. 1964. Effects of temperatures on self- and cross-pollination and fruiting of four highbush blueberry varieties. American Society for Horticultural Science **85**:302-306.

Knuth, P. 1906. Handbook of flower pollination. University of Oxford, London.

Krebs, S. L. and J. F. Hancock. 1988. The consequence of inbreeding on fertility in *Vaccinium corymbosum* L. Journal of American Society for Horticultural Science **113**:914-918.

Krebs, S. L. and J. F. Hancock. 1990. Early-acting inbreeding depression and reproductive success in the highbush blueberry, *Vaccinium corymbosum* L. Theoretical Applied Genetics **79**:825-832.

Krunic, M. D., M. M. Brajkovic and Lj. S. Mihajlovic. 1991. Management and utilization of *Osmia cornuta* Latr. for orchard pollination in Yugoslavia. *In* C. van Heemert and A. de Ruijter [ed.], Sixth International Symposium on Pollination. Acta Horticulturae **288**:190-193.

Kuhn, E. D. and J. T. Ambrose. 1984. Pollination of 'Delicious' apple by megachilid bees of the genus *Osmia* (Hymenoptera:Megachilidae). Journal of the Kansas Entomological Society **57**:169-180.

Kuhnholz, S. and T. D. Seeley. 1997. The control of water collection in honey bee colonies. Behavioural Ecology and Sociobiology **41**:407-422.

Lang, G. A. and R. G. Danka. 1991. Honey-bee-mediated cross- versus self-pollination of 'Sharpblue' blueberry increases fruit size and hastens ripening. Journal of American Society for Horticultural Science **116**:770-773.

Lang, G. A. and E. J. Parrie. 1992. Pollen viability and vigor in hybrid southern highbush blueberries (*Vaccinium corymbosum* L. x spp.). HortScience **27**:425-427.

Levi, A. and L. J. Rowland. 1997. Identifying blueberry cultivars and evaluating their genetic relationships using randomly amplified polymorphic DNA (RAPD) and simple sequence repeat- (SSR-) anchored primers. Journal of American Society for Horticultural Science **122**:74-78.

Levin, D. A. 1990. Sizes of natural microgametophyte populations in pistils of *Phlox drummondii*. American Journal of Botany **77**:356-363.

Levin, M. D. 1957. Artificial nesting burrows for *Osmia lignaria* Say. Journal of Economic Entomology **50**:506-507.

Linsley, E. G. 1958. The ecology of solitary bees. Hilgardia **27**:543-599.

Lyrene, P. M. 1989. Pollen source influences fruiting of 'Sharpblue' blueberry. Journal of American Society for Horticultural Science **114**:995-999.

Lyrene, P. M. 1994. Variation within and among blueberry taxa in flower size and shape. Journal of American Society of Horticultural Science **119**:1039-1042.

Maeta, Y. and T. Kitamura. 1964. Studies on the apple pollination by *Osmia*. Characteristics and underlaying problems in utilizing *Osmia*. Kontyu **33**:17-34.

Maeta,Y. and T. Kitamura 1965. Studies on the apple pollination by *Osmia* I. Ideal and present conditions in utilizing *Osmia* as pollinators of apples in Japan. Tohoku Konchu Kenkyu 1964. **1,2**:45-52.

Maeta, Y. and T. Kitamura 1974. How to manage the Mame-kobachi, *Osmia cornifrons* Radoszkowski for pollination of the fruit and crops. Asuku, Industrial Co. Ltd., Nagano-Shi, Japan. 16 pp. Translation by Saad Publications Karachi, Pakistan.

Maeta, Y. and T. Kitamura. 1981. Pollination efficiency by *O. cornifrons* Radoszkowski in relation to required number of nesting bees for economic fruit production. Honey Bee Science **2**:65-72.

MacKenzie, K. E. 1997. Pollination requirements of three highbush blueberry (*Vaccinium corymbosum* L.) cultivars. Journal of American Society for Horticultural Science **122**:891-896.

MacKenzie, K. E. and G. C. Eickwort. 1996. Diversity and abundance of bees (Hymenoptera: Apidea) foraging on highbush blueberry (*Vaccinium corymbosum* L.) in Central New York. Journal of the Kansas Entomological Society **69**:185-194.

MacKenzie, K. E. and M. L. Winston. 1984. Diversity and abundance of native pollinators on berry crops and natural vegetation in the Lower Fraser Valley, British Columbia. The Canadian Entomologist **116**:965-974.

Marshall, D. L. 1991. Nonrandom mating in wild radish: variation in pollen donor success and effects of multiple paternity among one- to six-donor pollinations. American Journal of Botany **78**:1404-1418.

Marshall, D. L., M. W. Folsom, C. Hatfield and T. Bennett. 1996. Does interference competition among pollen grains occur in wild radish? Evolution **50**:1842-1848.

Martin, F. W. 1959. Staining and observing pollen tubes in the style by means of fluorescence. Stain Technology **34**:125-128.

Martin, E. C. 1966. Honey bee pollination of highbush blueberry. American Bee Journal **106**:366-367.

Mayer, D. F. and J. D. Lunden. 1997. A comparison of commercially managed bumblebees and honey bee (Hymenoptera: Apidae) for pollination of pears. *In* K.

W. Richards [ed.], Proceedings of the 7th International Symposium on Pollination. Acta Horticulturae **437**:283-287.

Mazer, S. J. 1987. Parental effects on seed development and seed yields in *Raphanus raphanistrum*: implications for natural and sexual selection. Evolution **41**:355-371.

McGregor, S. E. 1976. Insect pollination of cultivated crop plants. Agriculture handbook **No. 496**. United States Department of Agriculture, Washington, DC.

McAndrews, J. H., A. A. Berti and G. Norris. 1973. Key to the quaternary pollen and spores of the Great Lakes Region. The Royal Ontario Museum, Toronto, Canada.

Meidinger, D. and J. Pojar. (eds.). 1991. Ecosystems of British Columbia. Special Report Series **6**. B. C. Ministry of Forests, Victoria B. C.

Michaelson-Yeates, T. P. T., A. H. Marshall, I. H. Williams, N. L. Carreck and J. R. Simkins. 1997. The use of isoenzyme markers to determine pollen flow and seed paternity mediated by *Apis mellifera* and *Bombus* spp. in *Trifolium repens*, a self-incompatible plant species. Journal of Apicultural Research **36**:57-62.

Michener, C. D. 1974. The Social Behavior of the Bees – A Comparitive Study. Harvard University Press, Cambridge, Mass.

Moffett, J. O. 1999. The small hive beetle. Bee Biz 9:3-4.

Moore, J. N. 1964. Duration of receptivity to pollination of flowers of the highbush blueberry and the cultivated strawberry. American Society for Horticultural Science **85**:295-301.

Moore, J. N. 1993. Blueberry cultivars of North America. HortTechnology **3**:370-374.

Moore, J. N., B. D. Reynolds and G. R. Brown. 1972. Effects of seed number, size, and development on fruit size of cultivated blueberries. HortScience **7**:268:269.

Mulcahy, D. L., P. S. Curtin and A. A Snow 1983. Pollen competition in a natural population. *In* C. E. Jones and R. J. Little [eds.], Handbook of Experimental Pollination Biology, 330 - 337. Van Nostrand Reinhold, N.Y.

Mulcahy, G. B. and D. L. Mulcahy. 1986. Pollen-Pistil Interaction. *In* D.L. Mulcahy, G. B. Mulcahy and E. Ottaviano [eds.], Biotechnology and Ecology of Pollen, 173 - 178. Springer-Verlag, New York, NY.

Mulcahy, D. L. and G. B. Mulcahy. 1987. The effects of pollen competition. American Scientist **75**: 44-50.

Olsen, L. G., R. Hoopingarner and E. C. Martin. 1979. Pollen preferences of honeybees sited on four cultivated crops. Journal of Apicultural Research **18**:196-200.

Page, R. E. and K. M. Fondrk. 1995. The effects of colony-level selection on the social organization of honey bee (*Apis mellifera* L.) colonies: colony-level components of pollen hoarding. Behavioural Ecology and Sociobiology **36**:135-144.

Palmer, T. M. and M. Zimmerman. 1994. Pollen competition and sporophyte fitness in *Brassica campestris*: does intense pollen competition result in individuals with better pollen? Oikos **69**:80-86.

Palser, B. F. 1961. Studies of floral morphology in the *Ericales*. V. organography and vascular anatomy in several United States species of the Vacciniaceae. Botanical Gazette 123:**79**-111.

Parrie, E. J. and G. A. Lang 1992. Self- and cross-pollination affect stigmatic pollen saturation in blueberry. HortScience **27**:1105-1107.

Peterson, S. S., C. R. Baird and R. M. Bitner. 1992. Current status of the alfalfa leafcutting bee, *Megachile rotundata*, as a pollinator of alfalfa seed. Bee Science **2**:135-142.

Phillips, J. K. and E. C. Klostermeyer. 1978. Nesting behaviour of *Osmia lignaria propinqua* Cresson (Hymenoptera: Megachilidae). Journal of the Kansas Entomological Society **51**:91-108.

Rabaey, A. and J. Luby. 1988. Fruit set in half-high blueberry genotypes following self and cross pollination. Fruit Varieties Journal **42**:126-129.

Rank, G. H. and F. P. Rank. 1989. Diapause intensity in a French univoltine and a Saskatchewan commercial strain of *Megachile rotundata* (Fab.). The Canadian Entomologist **121**:141-148.

Richards, K. W. 1984. Alfalfa leafcutter bee management in Western Canada. Agriculture Canada Pub. **No. 1495 / E**. Communications Branch, Agriculture Canada, Ottawa.

Richards, K. W. 1991. Effectiveness of the alfalfa leafcutter bee as a pollinator of legume forage crops. *In* C. van Heemert and A. de Ruijter [ed.], Sixth International Symposium on Pollination. Acta Horticulturae **288**:180-184.

Rinderer, T. E., J. R. Baxter, C. E. Jr. Carter and L. R. Mornhinveg. 1979. Empty comb stimulates honey production. American Bee Journal **119**:40-43.

Roach, D. A. and R. D. Wulff. 1987. Maternal effects in plants. Annual Review of Ecological Systematics **18**:209-235.

Rust, R. W. 1974. The systematics and biology of the genus *Osmia*, Subgenera *Osmia*, *Chalcosmia*, and *Cephalosmia* (Hymenoptera: Megachilidae). The Wasmann Journal of Biology **32**:1-93.

Rust, R. W. 1995. Adult overwinter mortality in *Osmia lignaria propinqua* Cresson (Hymenoptera:Megachilidae). Pan-Pacific Entomologist **71**:121-124.

SAS Institute. 1990. User's Guide: Statistics, version 6.0. SAS Institute, Cary, NC.

Schlotzhauer, S. D. and R. C. Littell. 1987. SAS System for Elementary Statistical Analysis. SAS Institute Inc., Cary, NC.

Schmid-Hempel P., M. L. Winston and R. C. Ydenberg. 1993. Foraging of individual workers in relation to colony state in the social hymenoptera. The Canadian Entomologist **125**:129-160.

Scott, C. D. 1986. Biology and management of wild bee and domesticated honey bee pollinators for tree fruit pollination. Ph.D. Thesis. Simon Fraser University Burnaby, B. C. Canada.

Seeley, T. D. 1995. The Wisdom of the Hive: The Social Physiology of Honey Bees. Harvard University Press, Cambridge, Mass.

Sekita, N. and M. Yamada. 1993. Use of *Osmia cornifrons* for pollination of apples in Aomori Prefecture, Japan. Japan Agricultural Research Quarterly **26**:264-270.

Smith-Huerta, N. L. 1997. Pollen tube attrition in *Clarkia tembloriensis* (Onagraceae). International Journal of Plant Science **158**:519-524.

Southwick, E. E. and L Southwick Jr. 1992. Estimating the economic value of honey bees (Hymenoptera:Apidae) as agricultural pollinators in the United States. Journal of Economic Entomology **85**:621-633.

Snow, A. A. 1982. Pollination intensity and potential seed set in *Passiflora vitifolia*. Oecologia **55**:231-237.

Snow, A. A. 1986. Pollination dynamics in *Epilobium canum* (Onagraceae): consequences for gametophytic selection. American Journal of Botany **73**:139-151.

Snow, A. A. 1990. Effects of pollen-load size and number of donors on sporophyte fitness in wild radish (*Raphanus raphanistrum*). The American Naturalist **136**:742-758.

Snow, A. A. and T. P. Spira. 1993. Individual variation in the vigor of self pollen and selfed progeny in *Hibiscus moscheutos* (Malvaceae). American Journal of Botany **80**:160-164.

Spira, T. P., A. A. Snow and M. N. Puterbaugh. 1996. The timing and effectiveness of sequential pollinations in *Hibuscus moscheutos*. Oecologia **105**:230-235.

Spira, T. P., A. A. Snow, D. F. Whigham and J. Leak. 1992. Flower visitation, pollen deposition and pollen-tube competition in *Hibiscus moscheutos* (Malvaceae). American Journal of Botany **79**:428-433.

Statistics Canada. 1997. Fruit and vegetable production. **No. 22-003SXPB**. Ottawa, Canada. **66**:17.

Stephen, W. P. and C. E. Osgood. 1965. The induction of emergence in the leafcutter bee *Megachile rotundata*, an important pollinator of alfalfa. Journal of Economic Entomology **58**:284-286.

Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annual Review of Ecological Systematics **12**:253-79.

Stephenson, A. G., T-C. Lau, M. Quesada and J. A. Winsor. 1992. Factors that affect pollen performance. *In* R. Wyatt [ed.], Ecology and Evolution of Plant Reproduction, 119-136. Chapman & Hall, New York, NY.

Stephenson, A. G., J. A. Winsor and L. E. Davis. 1986. Effects of pollen load size on fruit maturation and sporophyte quality in zucchini. *In* D. L. Mulcahy, G. B. Mulcahy, and E. Ottaviano [eds.], Biotechnology and Ecology of Pollen, 429 - 434. Springer-Verlag, New York, NY.

Stern, R. A. and S. Gazit. 1998. Pollen viability in Lychee. Journal of American Society for Horticultural Science **123**:41-46

Stushnoff, C. and A. J. Feliciano. 1968. A simple technique for observing mitotic division of the generative nucleus in pollen tubes of *Vaccinium* spp. HortScience **3**:174.

Stushnoff, C. and B. F. Palser. 1969. Embryology of five Vaccinium taxa including dipoid, tetraploid and hexaploid species or cultivars. Phytomorphology **19**:312-331.

Tasei, J. N. 1973. Observations sur le developpement d'*Osmia cornuta* Latr. et *Osmia rufa* L. (Hymenoptera: Megachilidae). Apidologie **4**:295-315.

Tasei, J. N. and M. M. Masure. 1978. Sur quelques facteurs influencant le developpement de *Megachile Pacifica* Panz (Hymenoptera, Megachilidae). Apidologie **9**:273-290.

Tepedino, V. J., D. R. Frohlich and C. R. Baird. 1994. Effect of intertunnel distance and nest-surface aspect on progeny production rate and sex ratio in the alfalfa leafcutting bee (Hymenoptera:Megachilidae). Journal of Economic Entomology **87**:27-30.

Ter-Avanesian, D. V. 1978. The effect of varying the number of pollen grains used in fertilization. Theoretical and Applied Genetics **52**:77-79.

Thorp, R. W. 1979. Structural, behavioural, and physiological adaptations of bees (Apoidea) for collecting pollen. Annals of the Missouri Botanical Garden **66**:788-811.

Thorp, R. W., D. S. Jr. Horning and L. L. Dunning. 1983. Bumble bees and Cuckoo Bumble Bees of California (Hymenoptera: Apidae). University of California Press, Berkeley, California.

Torchio, P. F. 1976. Use of *Osmia lignaria* Say (Hymenoptera: Apoidea, Megachilidae) as a pollinator in an apple and prune orchard. Journal of the Kansas Entomological Society **49**:475 - 482

Torchio, P. F. 1981. Field experiments with *Osmia lignaria propinqua* Cresson as a pollinator in almond orchards: I, 1975 Studies (Hymenoptera: Megachilidae). Journal of the Kansas Entomological Society **54**:815-823.

Torchio, P. F. 1982a. Field experiments with the pollinator species, *Osmia lignaria propinqua* Cresson, in apple orchards: II, 1976 Studies (Hymenoptera: Megachilidae). Journal of the Kansas Entomological Society **55**:759-778.

Torchio, P. F. 1982b. Field experiments with *Osmia lignaria propinqua* Cresson, as a pollinator in almond orchards: III, 1977 Studies (Hymenoptera: Megachilidae). Journal of the Kansas Entomological Society **55**:101-116.

Torchio, P. F. 1984. Field experiments with the pollinator species, *Osmia lignaria propinqua* Cresson, in apple orchards: III, 1977 studies (Hymenoptera: megachilidae). Journal of the Kansas Entomological Society **57**:517-521.

Torchio, P. F. 1985. Field experiments with the pollinator species, *Osmia lignaria propinqua* Cresson, in apple orchards: V (1979-1980), Methods of introducing bees, nesting success, seed counts, fruit yields (Hymenoptera: Megachilidae). Journal of the Kansas Entomological Society **58**:448-464.

Torchio, P. F. 1987. Use of non-honey bee species as pollinators of crops. Proceedings of the Entomological Society of Ontario **118**:111-124.

Torchio, P. F. 1990a. Diversification of pollination strategies for US crops. Environmental Entomology **19**:1650-1656.

Torchio, P. F. 1990b. *Osmia ribifloris*, a native bee species developed as a commercially managed pollinator of highbush blueberry (Hymenoptera: Megachilidae). Journal of the Kansas Entomological Society **63**:427-436.

Torchio, P. F. 1991a. Bees as crop pollinators and the role of solitary species in changing environments. Acta Horticulturae, Sixth International Symposium on Pollination **288**:49-61.

Torchio, P. F. 1991b. Use of *Osmia lignaria propinqua* (Hymenoptera: Megachilidae) as a mobile pollinator of orchard crops. Environmental Entomology **20**:590 -596.

Torchio, P. F. 1994. The present status and future prospects of non-social bee crop pollinators. Bee World **75**:49-53.

Torchio, P. F. and E. Asensio. 1985. The introduction of the European bee, *Osmia cornuta* Latr., into the U.S. as a potential pollinator of orchard crops, and a comparison of its manageability with *Osmia lignaria propinqua* Cresson (Hymenoptera: Megachilidae). J. Kansas Entomological Society **58**:42-52.

Vander Kloet, T. S. P. 1984. Effects of pollen donors on seed production, seed weight, germination and seedling vigor in *Vaccinium corymbosum* L. The American Midland Naturalist **112**:392-396.

Vicens, N., J. Bosch and M. Blas. 1994. Biology and population structure of *Osmia tricornis* Latreille (Hymenoptera: Megachilidae). Journal of Applied Entomology **117**:300-306.

Walsh, N. E. and D. Charlesworth. 1992. Evolutionary interpretations of differences in pollen tube growth rates. Quarterly Review of Biology **67**:19-37.

Waser, N. M., L Chittka, M. V. Price, N. M. Wiliams and J. Ollerton. 1996. Generalizations in pollination systems, and why it matters. Ecology **77**:1043-1060.

Whidden, T. L. 1996. The fidelity of commercially reared colonies of *Bombus impatiens* Cresson (Hymenoptera: Apidae) to lowbush blueberry in southern New Brunswick. The Canadian Entomologist **128**:957-958.

Winston, M. L. 1987. The biology of the honey bee. Harvard University Press, Cambridge, Mass.

Winston, M. L. 1992. Killer bees. The Africanized honey bees in the Americas. Harvard University Press Cambridge Mass.

Winston, M. L. and L. A. Fergusson. 1985. The effect of worker loss on temporal caste structure in colonies of the honeybee (*Apis mellifera* L.). Canadian Journal of Zoology **63**:777-780.

Winston, M. L. and L. H. Graf. 1982. Native bee pollinators of berry crops in the Fraser Valley of British Columbia. Journal of the Entomological Society of British Columbia **79**:14-20.

Winston, M. L., S. R. Mitchell and E. N. Punnett. 1985. Feasibility of package honey bee (Hymenoptera:Apidae) production in southwestern British Columbia, Canada. Journal of Economic Entomology **78**:1037-1041.

Winston, M. L. and K. N. Slessor. 1992. The essence of royalty: honey bee queen pheromone. American Scientist **80**:374-385.

Winston, M. L. and K. N. Slessor. 1993. Applications of queen honey bee mandibular pheromone for beekeeping and crop pollination. Bee World **74**:111-128.

Winston, M. L. and K. N. Slessor. 1998. Honey bee primer pheromones and colony organization: gaps in our knowledge. Apidologie **29**:81-95.

Wood, G. W., D. L. Craig and I. V. Hall. 1967. Highbush blueberry pollination in Nova Scotia. International Society for Horticulturists Symposium **8**:163-168.

Yamada, M., N. Oyama, N. Sekita, S. Shirasaki and C. Tsugawa. 1971. Bulletin of the Aomori Apple Experiment Station **No. 15**. Aomori, Japan.

Young, M. J. and W. B. Sherman. 1978. Duration of pistil receptivity, fruit set, and seed production in rabbiteye and tetraploid blueberries. HortScience **13**: 278-279.