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FACTORS ASSOCIATED WITH THE HONEY BEE COLONY AND THE FORAGING BEE THAT CONTRIBUTE TO THE SPREAD OF BLUEBERRY LEAF MOTTLE VIRUS TO HIGHBUSH BLUEBERRIES

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by

Walter Lee Pett

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ABSTRACT

FACTORS ASSOCIATED WITH THE HONEY BEE COLONY AND THE FORAGING BEE THAT CONTRIBUTE TO THE SPREAD OF BLUEBERRY LEAF MOTTLE VIRUS TO HIGHBUSH BLUEBERRIES

By

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Factors associated with honey bees that facilitate the spread of pollen-borne blueberry leaf mottle nepovirus (BBLMV) to highbush blueberries were examined. These factors included the longevity of infectious BBLMV in the honey bee colony and in-hive virus transfer within and between colonies. Blueberry leaf mottle virus remained infectious for at least 10 days within colonies of the honey bee, which is the primary pollinator. Flowering blueberry plants were caged for various periods with colonies of honey bees derived from a blueberry farm where a high percentage of bushes were infected by BBLMV. Twelve of 84 plants later tested enzyme-linked immunosorbent assay (ELISA)-positive for BBLMV. Counts of pollen grains washed from "house bees" (bees that have never left the colony) resulted in a average of 5,149 pollen grains per bee, indicating that in-hive pollen-virus transfer occurs and can be a source of spread of virus -contaminated pollen within a colony. Evidence of pollen transfer between colonies and to a lesser extent between apiaries was also documented by observing the drifting

(wandering far away from the bee's hive and/or visiting other hives) behavior of honey bees. Two 6-colony apiaries were situated at similar blueberry plantings. Three colonies at each apiary were fitted with pigment dispensers which marked the bees' thorax with colored pigment (unique for each colony) as they entered or left the colony. The dispensers proved to be 99.7% effective in marking bees. Counts at non-marking colony entrances were made by recording the number and color of foragers. The percentage of foragers originating from different colonies within an apiary ranged from 32 to 63%. Bees originating from colonies located 600 m away comprised 1.3 to 4.5 % of the foraging force of the unmarked colonies. This drifting phenomenon could contribute to the maximum spread of BBLMV by bee-to-bee contact and multiple colony visits.

To my sons Will and Liam.
I love you.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Michigan is a major producer of highbush blueberries (*Vaccinium corymbosum* L) in the US. with over 7,300 hectares in blueberry production. Michigan blueberry growers face similar problems with weeds, insects, and plant pathogens as other fruit and vegetable producers do. However, with a perenial crop such as blueberries, where peak production is not reached for seven to twelve years after plant establishment, certain plant pathogens can have a devastating effect compared to weed or insect damage. Growers can obtain some control against weeds, insects and certain plant pathogens with the use of a varity of pesticides. However, for several plant pathogens there are no chemical means of control. In 1979, Ramsdell and Stace-Smith described the physical and chemical properties of one such plant pathogen, a virus, that was detected in a Michigan blueberry field and named the virus blueberry leaf mottle virus (BBLMV). BBLMV causes a devastating disease to at least six cultivars of highbush blueberry ('Jersey', 'Rubel', 'Bluecrop', 'Elliot', 'Spartan', and 'Blueray') (Sandoval Briones 1992). The symptoms are most severe in 'Rubel'. Stems are killed to the ground. Sparse regrowth from the crown exhibits leaf distortion and mottling. Yield is nil. In 'Jersey', stem-kill occurs, but leaf symptoms are very mild. The regrowth from the crown exhibits shortened internodes and rosetting of leaves which are small and yellow-

green instead of deep green. The symptoms in all other known susceptible cultivars are similar to 'Jersey'. 'Jersey' is the most important cultivar in Michigan because it makes up 45% of the total acreage. The disease causes extreme reduction of the bearing surface of bushes, yield is greatly reduced with up to a 75-100% crop loss, and eventual death of the bush. The causal virus has been detected and serologically diagnosed in over 27 fields in the state (D. C. Ramsdell, unpublished data).

Blueberry leaf mottle virus is classified as a nepovirus (Ramsdell and Stace-Smith 1981 and 1983). Infective virus was associated with pollen collected from diseased blueberry, and BBLMV antigen was detected in triturated pollen (Childress and Ramsdell 1986). It has been shown that the honey bee (*Apis mellifera* L.) can transmit BBLMV from blueberry to blueberry during pollination (Childress and Ramsdell 1987).

The importance of the honey bee as a pollinator for highbush blueberries has been well documented (Dorr and Martin 1966). Michigan blueberry growers use 2.5 to 10 colonies per hectare to ensure adequate pollination (McGregor 1976). Due to the necessity of the honey bee for pollination and proper fruit development, it is impossible to remove the virus vector from the blueberry system. Therefore, we must understand the factors that promote the spread of BBLMV, so possible means of decreasing the spread of the virus can be devised.

There are several factors associated with the honey bee colony and the foraging bee that may contribute to the spread of BBLMV these include, but are not limited to: Longevity of infectious BBLMV within a colony; the in-hive transfer of virus and/or pollen from bee to bee; and the transfer of virus between colonies via honey bee drifting (movement of

foragers from their own colony to adjacent colonies). This study was initiated to examine the role of honey bee colonies and foraging bees in the spread of BBLMV to highbush blueberries under field conditions and to develop strategies to minimize the spread of virus in spite of the necessary widespread use of honey bees in commercial blueberry culture.

This dissertation is a compilation of three published papers. The papers describe results of experiments that were designed to investigate the role of honey bees and the behavior of honey bee colonies with respect to pollination and the movement of BBLMV.

The first paper is titled "A self marking system to determine foraging populations of honey bees (*Apis mellifera* L) (Hymenoptera: Apidae)" and was published in BeeScience (Boylan-Pett *et al.* 1991). This paper describes the fluorescent pigment dispenser used to mark foragers so that they could be distinguished from non-foraging bees. The method also allows for identifying bees from a particular colony when the colony is fitted with a dispenser containing a pigment that is unique for that colony (six colors were distinguishable in the field).

The second paper is titled "Honeybee foraging behavior, in-hive survival of infectious, pollen-borne blueberry leaf mottle virus and transmission of the virus in highbush blueberry" and was published in Phytopathology (Boylan-Pett *et al.* 1991a). This paper reports on the longevity of infectious virus in the honey bee colony, the behavior of the foraging honey bee, and the behavior of the colony itself, as they relate to the spread of the virus. It is shown that these factors all play a critical role in the short range spread (within the foraging range of the colony) of the virus. It is also shown how these factors can contribute to the long range (well beyond the foraging range of the colony) spread of the virus.

The third paper was published in Acta Horticulturae from the proceedings of the 6th Pollination Symposium (Boylan-Pett and Hoopingarner 1991). This paper was the first to report on the long range drifting of honey bees from one apiary to another located 600 m apart. This drifting behavior, together with the in-hive transfer of pollen/virus, provides us with information that helps us explain the movement of the virus from field to field.

The three papers noted above provide information on methods that examine the BBLMV/honey bee interaction, the interaction itself, and discusses methods that can help minimize the spread of the virus.

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LITERATURE REVIEW

Associations between plant viruses and pollen have been known for over fifty years (cited in; Cooper *et al.* 1988). Since that time, the virus-pollen interaction has been established for at least 46 different viruses (Cooper *et al.* 1988). The two virus groups most often utilizing this biological means of transmission are those belonging to the ilarvirus (Fulton 1983) and nepovirus groups (Harrison 1977). In early studies of nepoviruses, Callahan (1957) reported that elm mosaic virus (a strain of cherry leaf roll virus) was transmitted to elm seedlings (*Ulmus* spp.) via pollen. Lister and Murant (1967) observed transmission of raspberry ringspot and tomato black ring viruses to raspberry, *Rubus idaeus* L., mother plants and seedlings via the pollen from infected plants. Raspberry bushy dwarf virus was shown to be transmitted by hand pollination from raspberry to raspberry, and the virus was shown to spread in the field using "infector" plants (Murant *et al.* 1974). Gilmer and Way (1960) provided conclusive evidence that prune dwarf ilarvirus and prunus necrotic ringspot (PNRSV) ilarvirus were transmitted by pollen to seeds produced on healthy sour cherry trees (*Prunus cerasus* L.). The transmission of virus from tree to tree via pollen was documented by George and Davidson (1964) and Davidson (1976) in studies involving bee and hand pollination of flowers. Cherry leaf roll virus from walnut was shown to be transmitted to nuts and seedlings of English walnut (*Juglans regia* L.) by hand pollination with diseased

pollen (Mircetich *et al.* 1982). Cherry leaf roll virus was also shown to infect birch (*Betula pendula*) embryos as a result of hand pollination with infected pollen (Cooper *et al.* 1984). Virus movement from plant to plant via pollen may have a devastating effect in a perennial crop in which all plants could become infected over time.

In studying the virus-pollen interaction, plant virologists have focused mainly on associating a particular virus with pollen using electron microscopy or pollen washes, followed by enzyme-linked immunosorbent assay (ELISA). Little information is available on the spread of virus via pollen from plant to plant. Two exceptions were studies by Mink (1983) and Childress and Ramsdell (1986 and 1987). The study by Mink investigated stored pollen in honey bee colonies and the long-distance spread of PNRSV from California to Washington sweet cherries as a result of interstate movement of hives. He found that 20 of 40 hives tested contained PNRSV in infectivity tests on *Chenopodium quinoa* Willd. with pollen that had been stored in hive cells for 3 days. In the same study, a hive from a California almond and cherry orchard containing infected trees was caged with a healthy cherry tree in Washington within 24 hours after leaving the California orchard. No apparent transmission of PNRSV occurred.

In the first Childress and Ramsdell study (1986) that examined virus spread from plant to plant via pollen, they detected BBLMV antigen in the pollen from 13 of 15 infected mature 'Jersey' bushes. They tested the infectivity of the pollen by rub-inoculating the pollen onto *C. quinoa* host plants. Fourteen days after inoculation, 43% of the inoculated plants showed leaf symptoms typical of BBLMV infection. All of the pollen causing infection tested ELISA-positive for the virus.

The second study by Childress and Ramsdell (1987) demonstrated that honey bees were one causal agent in the transfer of BBLMV-infected pollen from diseased to healthy bushes . They placed virus-free 2-year-old potted bushes next to commercially planted healthy and infected bushes during bloom. They also placed potted bushes within cages containing infected or healthy commercial bushes with or without a colony of honey bees. Highest rates of infection occurred among the healthy potted bushes when they were caged with an infected bush and bees. No infection occurred when the potted bushes were caged with bees and a healthy bush. In the same study, 51.4% of the pollen loads collected from foragers working a heavily BBLMV-infected field tested ELISA-positive for the virus.

Blueberry leaf mottle virus (BBLMV) was classified as a nepovirus (Ramsdell and Stace-Smith 1981 and 1983). Unlike most nepoviruses, whose primary vectors are nematodes, BBLMV has no known nematode vector (Ramsdell and Stace-Smith 1983 , Childress and Ramsdell 1986). Virus transmission through pollen and seed is usually considered of secondary importance for nepoviruses. However, it does appear to be the primary means of transmission for some viruses (Mink 1983).

BBLMV causes a devastating disease to at least six cultivars of highbush blueberry ('Jersey', 'Rubel', 'Elliot', 'Spartan', 'Bluecrop', and 'Blueray') (Sandoval Briones 1992). The symptoms are most severe in 'Rubel'. Stems are killed to the ground. Sparse regrowth from the crown exhibits leaf distortion and mottling. Yield is nil. In 'Jersey', stem-kill occurs, but leaf symptoms are very mild. The regrowth from the crown exhibits shortened internodes and rosetting of leaves which are small and yellow-green instead of deep green. The symptoms in the other

susceptible cultivars are similar to 'Jersey'. 'Jersey' is the most important cultivar in Michigan because it makes up 45% of the total acreage. The disease causes extreme reduction of the bearing surface of bushes, yield is greatly reduced with up to a 75-100% crop loss, and eventual death of the bush results. The causal virus has been detected and serologically diagnosed in over 27 fields in the state (D. C. Ramsdell, unpublished data).

To understand certain behavioral aspects of honey bee colonies, e.g.; number of foragers in a colony, the origins of bees found foraging in fields, and drifting behavior (the movement of bees from their own colony to adjacent colonies), it is useful to have techniques that allow for distinguishing individual bees, or groups, of bees from one another. In the apiculture literature, many studies have been reported that address these behavioral characteristics of honey bee colonies. These studies used a variety of methods to ascertain the origins of foraging bees and the number of foragers from a particular colony. Gary *et al.* (1972 and 1978) used a magnetic retrieval system to study the distribution of foraging bees used to pollinate alfalfa and almond. With this system, a row of magnets is placed at the hive entrance in a manner that all incoming bees must come in contact with it. Individual foraging honey bees are anesthetized in the field and a small numbered metal disc is glued to the dorsal surface of their abdomen. The disc is located in a manner that does not interfere with their wings and is light enough so as not to disrupt flight. Under ideal conditions a recovery rate of ca. 90% is expected with this system (Gary 1971).

Mass marking of honey bees has been accomplished in a variety of fashions including the use of radioisotopes, genetic markers, and fluorescent powders. Levin (1961) fed radioactive P^{32} labeled sugar syrup to genetically marked Caucasian (black) bees. All of the Caucasian bees that were captured in an alfalfa field were found to be labeled with P^{32}. He concluded that there were no differences in using either geneticaly marked or isotope labelled bees. Robinson (1966) used genetically marked bees to examine the foraging range of honey bees in citrus. Peer (1956) used bees with the cordovan color to study the multiple mating of honey bee queens.

Fluorescent pigment has been used by a number of researchers to mark honey bees for identification purposes. Smith and Townsend (1951) developed a hive entrance block, coated with fluorescent pigment, that bees were forced to walk over as they left the colony. They determined that the entire foraging force of the colony was marked in about 90 minutes. They determined the origins of foragers in the field by examining the captured bees with an ultraviolet light. Johansson (1959) also used a "walk through" method, bees were marked not only at the colony entrance, by walking over fluorescent pigment, but also marked at select cotton flowers that were coated with fluorescent pigment. Bees and flowers were examined with ultraviolet light to determine whether they were visited by bees that were marked. Another method of marking bees with fluorescent pigment was developed by Frankie (1973). He collected bees from flowers and placed the bees in a nylon net bag. The bag was placed in a container that had a thin layer of fluorescent powder on the bottom. A hand pump was fitted to a small hole at the bottom of the container, the container was sealed, and air was pumped into the

container to circulate the powder. He observed that the powder could be detected on bees for up to 6 days after application. Dhaliwal and Sharma (1972) developed a pigment dispenser that marked the thorax of bees as they entered or left the colony. Duryea (1986) used a modified version of the dispenser to mark foragers and concluded that the pigment he used had no toxic effect on the marked bees.

Various techniques have been used to provide information about the foraging force of a colony. Many of the techniques provide useful information about the foraging force at a given time. Gary's (1967) flight cones provide data on the rate of foragers leaving the colony. An inverted hardware cloth cone, with a small opening (at the small end) from which bees can exit, is placed at the colonies' entrance with the cone opening closed for a given period of time. Foraging bees accumulate in the cone and the number of bees is recorded. This technique provides means of determining when peak foraging occurs. Burrill and Dietz (1973) developed a photoelectric counting device to record the number of incoming and outgoing bees. They concluded that with the use of their device a continuum of honey flight behavior and activity can be accurately assessed. Other methods for estimating foraging populations were also based on the flight activity at the colony entrance (Lundie 1925, Spangler 1969, Erickson *et al.* 1973, and Szabo 1980). These methods all use entrance counts and are useful for determining the number of foraging flights at a specific time.

Various methods have been described for estimating the foraging populations of individual colonies. These methods use trapping devices that capture returning foragers. Danka and Gary'(1987) describe a method that consists of 24 access tubes in the lid of a box-like trap

attached to the hive entrance. To trap bees the tubes are coated with paraffin oil. The oil causes the return foragers to slip into a mesh bag attached to the lid under the trap. The number of bees found in the trap is estimated by weighing the trapped bees. The traps are left in place for 45 minutes. Szabo's (1989) device collects returning foragers in a trap that is part of the hive. The trap is activated by closing a sliding entrance gate and is left in this position for 45 minutes. After this time the collected foragers are weighed to determine their number.

Drifting behavior of honey bees, the movement of bees from their own colony to adjacent colonies, is a common phenomenon. The majority of drifting bees are assumed to be young bees performing orientation flights where it is estimated that up to 50% of these bees may drift. Drifting may also be extensive when colonies are established from packages or colonies are moved to new locations (Corkins 1933). Researchers have reported methods that may be used to help prevent drifting within an apiary. These methods include painting the colonies different colors (Free 1958) or arranging the colonies in a serpentine or in a circular pattern (Jay 1971). Although methods to prevent drifting are known, little information on the degree of the actual number of bees that drift is available. One exception was study by Robinson (1979) who used the magnetic retrieval system of Gary (1971) to determine the number of tagged bees that drifted from their original colony. He tagged 150 bees per colony in 10 similar colonies arranged in a straight row. Three days later he placed the magnets at the hive entrances to collect the discs from the tagged bees. He recovered 73% of the discs and found that little drift occurred from colonies at the ends of the row (1 and 2%, respectivly, of the tagged bees from these colonies drifted) but that an

average of 21% of the tagged bees drifted from colonies that were within the row. He concluded that end locations served as landmarks for the foraging bees.

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A SELF-MARKING SYSTEM TO DETERMINE FORAGING POPULATIONS OF HONEY BEES (*Apis mellifera* L) (HYMENOPTERA: APIDAE)

ABSTRACT

The number of foragers within a colony was evaluated using four honey bee colonies. The colonies were fitted with pigment dispensers that marked the thorax of each bee as it entered or exited the colony. In this study 99.7% of the bees were marked. Individual frames were photographed and the number of marked (foragers) and unmarked bees (house bees) were counted. The percentage of foragers ranged from 11.7 to 42.2% of the colonies' population.

INTRODUCTION

Understanding pollination and bee foraging behavior requires knowing the number of foraging bees in a colony. Information about a colony's foraging force is essential when recommending the number of colonies to use for pollination services; too few colonies may result in poor fruit set, too many adds an extra cost to the producer. In bee foraging behavior studies, knowing the number of foragers in a colony would provide the information required to determine the percentage of foragers that were performing a specific task (e.g., observing marked bees foraging different plants).

Various techniques have been used to provide information about the foraging force of a colony. Gary's (1967) flight cones provide data on the rate of foragers leaving the colony, but the number of total foragers is not addressed. Other methods for estimating foraging populations were also based on the flight activity at the colony entrance (Lundie 1925, Spangler 1969, Burrill and Dietz 1973, Erickson *et al.* 1973, and Szabo 1980). These methods were useful for determining the number of foraging flights at a specific time, but they did not accurately estimate the total number of foragers.

Danka and Gary (1987) and Szabo (1989) have described methods for estimating the foraging populations of individual colonies. These methods use trapping devices that capture returning foragers. During the trapping period, the colony was closed off to prevent house bees (non-

foraging bees that have never left the colony) from entering the trap. This closing also prevented foragers (those still in the colony) from leaving the colony. During trapping (approximately 45 minutes) normal hive activities were interrupted. The disruption of normal hive activities should be considered when these methods are used repeatedly. Due to the dynamic nature of foraging behavior (not all foragers leave the colony at the same time) it is impossible to determine the total number of foragers with either of these methods.

In this paper we describe a method for determining the total number of foragers in individual honey bee colonies. Pigment dispensers (similar to those of Dhaliwal and Sharma (1972) modified by Duryea [1986]) were used to mark bees as they entered, or left, the colony. For this study we define a forager as any bee that leaves the colony. The number and proportion of foraging bees in a colony was determined by counting the number of unmarked (house bees) and marked (foragers) bees in photographs taken of the colony.

MATERIALS AND METHODS

Pigment Dispensers. The dispenser was constructed of wood (Fig. 1). Nylon bridal veiling (ca. 50 openings per cm^2) was stretched tightly over the bottom of the compartment opening and stapled to the dispenser. The dispenser was filled with Day-Glo™ fluorescent pigment (Day-Glo Color Corp, Cleveland, OH 44103) and the top of the dispenser was covered with a piece of wood to prevent bees from entering the dispenser. The dispenser was placed over the colony entrance, forcing foragers to

pass through the 19.3 cm long by 0.6 cm high opening. The 0.6 cm high opening was an important dimension. This size opening ensured that contact was made between the thorax of the bee and the dispenser. As a forager entered or exited the colony its thorax rubbed against the bridal veiling and was marked with pigment.

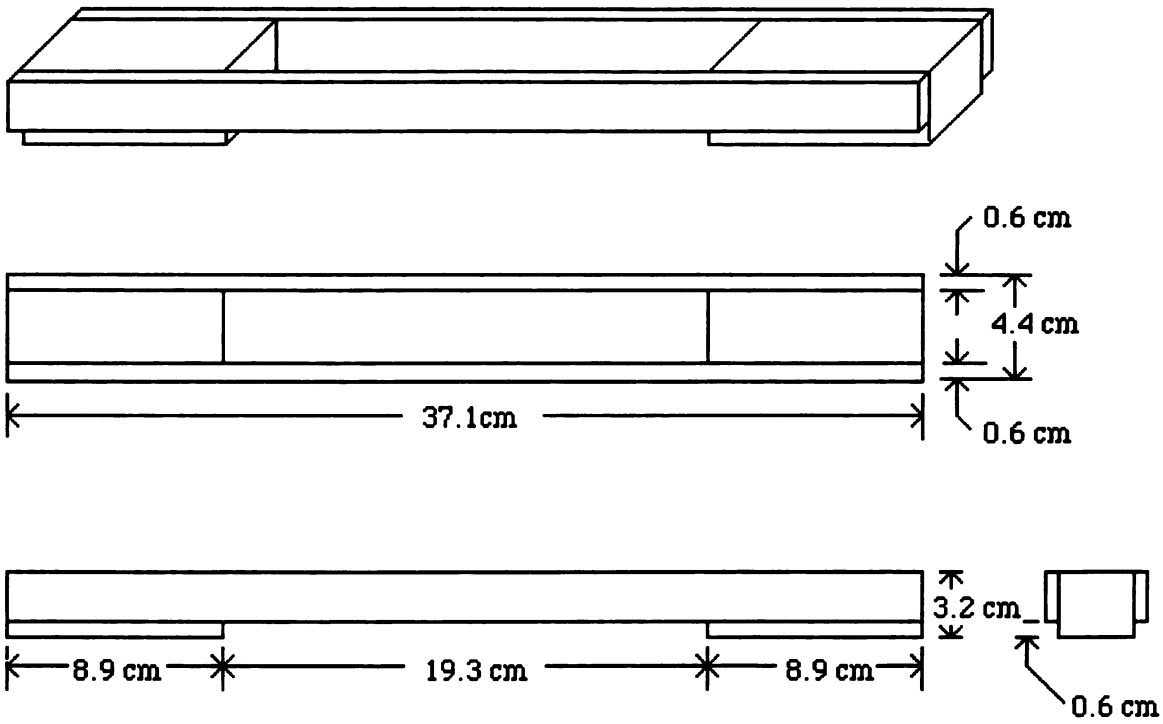

Figure 1. An isometric and orthographic perspective of the pigment dispenser used to mark foraging bees as they enter or exit the colony. The dispenser is constructed of stock lumber finished to the stated dimensions. Nylon bridal veiling (ca. 50 openings per square cm) is stretched tightly over the bottom and the dispenser is filled with pigment.

Effectiveness of Dispenser. Three colonies at the Michigan State University Apiary, East Lansing, MI, were fitted with pigment dispensers 5 days prior to data collection to allow bees to orientate to the reduced entrance. Pigment was added to the dispenser and 3,000 individual bees from three colonies (1,000 bees per colony) were observed as they left their colony. The number of marked and unmarked bees was recorded.

Determining Forager Populations. On June 1, 1987, pigment dispensers were placed at the entrances of four colonies at the Michigan State University Apiary, East Lansing, MI. The dispensers contained no pigment at this time. This allowed the bees to adjust to the reduced entrance size of the colony. Pigment was added to the dispensers 24 hours before each data collection period. The following morning (prior to first flights) each comb of the individual colonies was photographed (Kodachrome 64, 35 mm slide film) using a Minolta Maxxum 7000 auto focus camera fitted with a 28-mm wide-angle lens and cable release. Each hive body was separated from the colony and covered to prevent bee flight before the individual combs were photographed. The combs were photographed in a systematic fashion starting with the most western facing comb. Both sides of each frame were photograpged. The combs were then returned to an empty hive body in the same order in which they were found prior to photographing. This procedure reduced the posibility of photographing bees more than once. The bottom board, inner cover and the sides of the hive bodies were also photographed to ensure accurate counts of all bees in the colony.

The processed slides were projected onto a grid and the number of marked and unmarked bees on each comb was recorded. Colonies were photographed on June 11, June 25 and July 2, 1987. One honey bee

colony (without dispenser) was placed on a scale and the weight of this colony was recorded on the dates the experimental colonies were photographed. The colony weight data served as an indicator of the honey flow in the foraging area.

RESULTS AND DISCUSSION

Effectiveness of Dispenser. Of the 3,000 bees that were observed leaving the colony, 2,999 were marked (99.97%). It should be noted that only those bees leaving the colony were recorded; the dispenser marks bees as they enter or exit the colony, thus the lone unmarked bee would probably have been marked when it returned to the colony.

For the first several minutes after the pigment-containing dispensers were in place, activity at the hive entrance was frantic. There was an accumulation of pigment blocking the entrance and preventing bees from entering or exiting the colony. The pigment dislodges from the veiling quite easily when the dispensers are jarred. After the bees removed the pigment from the hive entrance, the dispensers did not appear to interfere with normal hive activity.

Forager Populations. The proportion of foragers ranged from 11.7 to 42.2% of the total colony population on the three dates the colonies were photographed. The proportion of foragers was highest on June 10 (Fig. 2). A possible reason for the decline after June 10, was the availability of forage on those particular dates. During the first data collection period, basswood (*Tilia spp.*) was in bloom. Our scale colony on June 11, 1987 weighed 41.7 kg (during basswood bloom) and leveled off at 48.1 kg by

June 25, increasing to 48.5 kg on July 2. Apparently little forage was available from June 11 to June 25. This information suggests that more bees were recruited to forage when the rewards were abundant (June 11 in Fig. 2) and that fewer bees leave the colony when there is a nectar dearth (July 2 and 7 in Fig. 2).

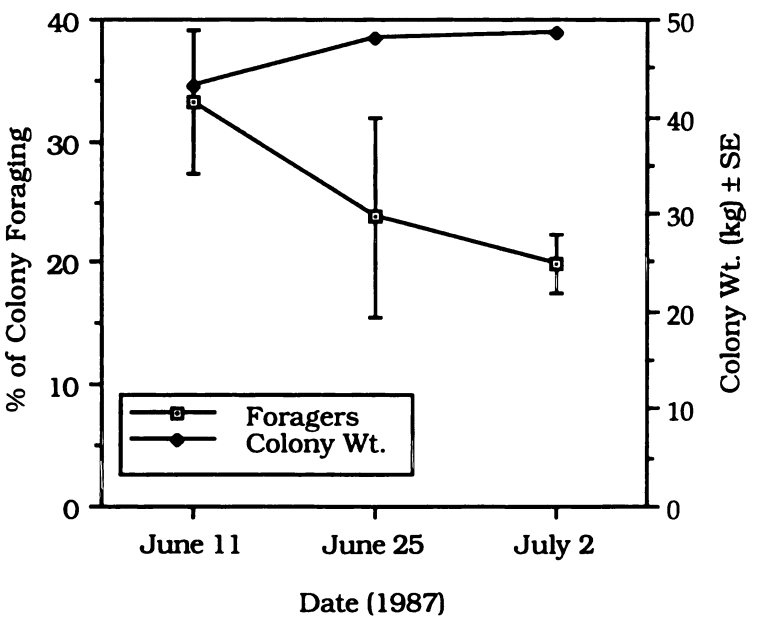

Figure 2. The average percent of foragers per colony and the weight of the scale colony on the three days of data collection. Bars equal the standard error of the mean foraging population for each

No significant correlation between the number of foragers and population was found (Fig. 3). This is in disagreement with Farrar (1937) who suggested that large colonies with a high bee-to-brood ratio have a greater number of foragers than smaller colonies with a low bee-to-brood ratio. It should be noted that our colony populations were much lower (15,000 to 26,000 bees) than Farrar's.

The pigment dispenser, along with photographs of marked and unmarked bees, provide a means for determining the total number of foragers in an individual colony. The dispensers do not seem to interfere with normal foraging behavior. Although the colony is disrupted during photographing, it is not disrupted when the colony is in its foraging mode.

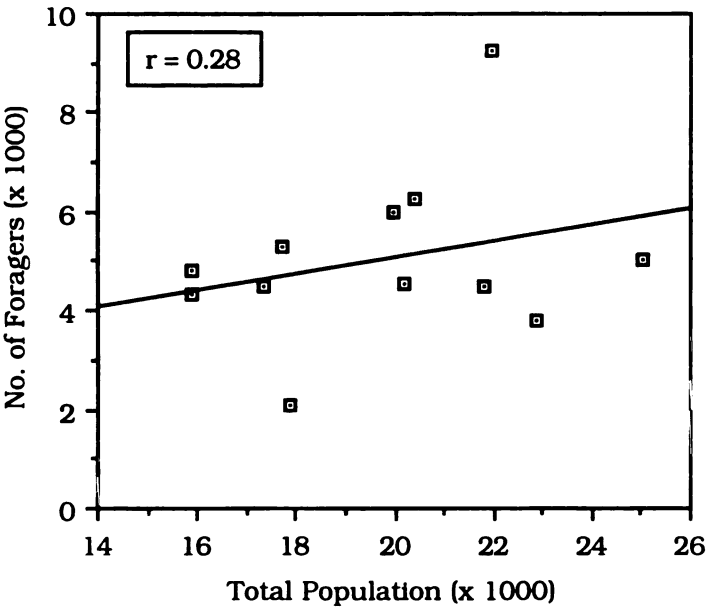

Figure 3. The number of foragers per colony plotted against the total colony population. The data points represent the four colonies that were photographed on June 11, 25 and July 2, 1987.

The pigment dispensers can serve as a means of identifying bees from a particular colony (five colors, distinguishable in the field, are available). This technique should prove useful to researchers

investigating the many facets of honey bee behavior that require knowledge of the origin and dispersal of individual honey bees from individual colonies.

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HONEYBEE FORAGING BEHAVIOR, IN-HIVE SURVIVAL OF INFECTIOUS, POLLEN-BORNE BLUEBERRY LEAF MOTTLE VIRUS AND TRANSMISSION OF THE VIRUS IN HIGHBUSH BLUEBERRRY

ABSTRACT

Factors associated with honey bees that facilitate the spread of pollen-borne blueberry leaf mottle nepovirus (BBLMV) to highbush blueberries were examined. These factors included the longevity of infectious BBLMV in the honey bee colony and in-hive virus transfer within and between colonies. Blueberry leaf mottle virus remained infectious for at least 10 days within colonies of the honey bee, which is the primary pollinator. Flowering blueberry plants were caged for various periods with colonies of honey bees derived from a blueberry farm where a high percentage of bushes were infected by BBLMV. Twelve of 84 plants later tested positive for BBLMV by enzyme-linked immunosorbent assay. Counts of pollen grains washed from "house bees" (bees that have never left the colony) resulted in a average of 5,149 pollen grains per bee, indicating that in-hive pollen-virus transfer occurs and can be a source of spread of virus-contaminated pollen within a colony. Evidence of pollen transfer between colonies and to a lesser extent between apiaries was also documented by observing the drifting (wandering far away from the bee's hive and/or visiting other hives) behavior of honey bees. Only 42.6% of the total foragers of a colony originated from their own colony and 2.4% of the foragers were from colonies located 600 m apart. This drifting phenomenon could contribute to the maximum spread of BBLMV by bee-to-bee contact and multiple colony visits.

INTRODUCTION

Associations between plant viruses and pollen have been known for over fifty years (Cooper *et al.* 1988). Since that time, the virus-pollen interaction has been established for at least 46 different viruses (Cooper *et al.* 1988). The two virus groups most often utilizing this biological means of transmission are those belonging to the ilarvirus (Fulton 1983) and nepovirus groups (Harrison 1977). In early studies of nepoviruses, Callahan (1957) reported that elm mosaic virus (a strain of cherry leaf roll virus) was transmitted to elm seedlings (*Ulmus* spp.) via pollen. Lister and Murant (1967) observed transmission of raspberry ringspot and tomato black ring viruses to raspberry, *Rubus idaeus* L., mother plants and seedlings via the pollen from infected plants. Raspberry bushy dwarf virus was shown to be transmitted by hand pollination from raspberry to raspberry, and the virus was shown to spread in the field using "infector" plants (Murant *et al.* 1974). Gilmer and Way (1960) provided conclusive evidence that prune dwarf ilarvirus and prunus necrotic ringspot (PNRSV) ilarvirus were transmitted by pollen to seeds produced on healthy sour cherry trees (*Prunus cerasus* L.). The transmission of virus from tree to tree via pollen was documented by George and Davidson (1964) and Davidson (1976) in studies involving bee and hand pollination of flowers. Cherry leaf roll virus from walnut was shown to be transmitted to nuts and seedlings of English walnut (*Juglans regia* L.) by hand pollination with diseased pollen (Mircetich *et*

al. 1982). Cherry leaf roll virus was also shown to infect birch (*Betula pendula*) embryos as a result of hand pollination with infected pollen (Cooper *et al.* 1984). Virus movement from plant to plant via pollen may have a devastating effect in a perennial crop in which all plants could become infected over time.

In studying the virus-pollen interaction, plant virologists have focused mainly on associating a particular virus with pollen using electron microscopy or pollen washes, follwed by enzyme-linked immunosorbent assay (ELISA). Little information is available on the spread of virus via pollen from plant to plant. One exception is a study by Mink (1983) that investigated stored pollen in honey bee colonies and the long-distAnce spread of PRNSV from California to Washington sweet cherries as a result of interstate movement of hives.

In the apiculture literature, many studies pertaining to honey bee foraging behavior and pollination have been reported. These studies have utilized several techniques to ascertain the origins of bees foraging on particular plants. Gary *et al.* (1978) used a magnetic retrieval system to study the distribution of honey bees foraging almonds during pollination. They concluded that the distance honey bees flew to forage was dependent on the foraging competition within the orchard. Bees foraged at a greater distance from their colony when the number of colonies at their apiary was high. Other researchers have used radioisotopes (Levin 1961), genetic markers (Robinson 1966) and fluorescent pigment (Johansson 1959) to study the foraging behavior of honey bees in a variety of pollinating systems. The drifting of bees has been studied between adjacent colonies in an apiary (Free 1958).

However, according to an extensive literature search, no studies have involved an infectious plant virus associated with pollen.

Blueberry leaf mottle virus (BBLMV) is classified as a nepovirus (Ramsdell and Stace-Smith 1981, 1983). Infective virus was associated with pollen collected from diseased blueberry, and BBLMV antigen was detected in triturated pollen (Childress and Ramsdell 1986). It has been shown that the honey bee (*Apis mellifera* L.) can transmit BBLMV from blueberry to blueberry during pollination (Childress and Ramsdell 1987). BBLMV causes a devastating disease to at least three cultivars of highbush blueberry ('Jersey', 'Rubel', and 'Blueray'). The symptoms are most severe in 'Rubel'. Stems are killed to the ground. Sparse regrowth from the crown exhibitss leaf distortion and mottling. Yield is nil. In 'Jersey', stem-kill occurs, but leaf symptoms are very mild. The regrowth from the crown exhibits shortened internodes and rosetting of leaves which are small and yellow-green instead of deep green. Yield is greatly reduced. The symptoms in 'Blueray' are similar to 'Jersey'. 'Blueray' is of minor importance in Michigan. 'Jersey' is the most important cultivar in Michigan because it makes up 45% of the total acreage. This disease causes extreme reduction of the bearing surface of bushes, 75-100% crop loss, and eventual bush death. The causal virus has been detected and serologically diagnosed in over 27 fields in the state (D. C. Ramsdell, *unpublished data*).

The importance of the honey bee as a pollinator for highbush blueberries has been well documented (Dorr and Martin 1966). Michigan blueberry growers utilize from 2.5 to 10 colonies per hectare to ensure adequate pollination (McGregor 1976). Due to the necessity of the honey bee for pollination and proper fruit development, it is impossible to

remove the vector from the blueberry system. Therefore, we must understand the factors that promote the spread of BBLMV, so possible means of decreasing the spread of the virus can be devised.

The factors associated with the honey bee colony and the foraging bee that contribute to the spread of BBLMV include, but are not limited to: Longevity of infectious BBLMV within the colony; the in-hive transfer of virus and/or pollen from bee-to-bee; and the transfer of virus between colonies via honey bee drifting (movement of foragers from their own colony to adjacent colonies). This study was initiated to examine the role of the honey bee colonies and foraging bees in the spread of BBLMV to highbush blueberry (*Vaccinium corymbosum* L.) under field conditions and to develop strategies to minimize the spread of virus in spite of the necessary widespread use of honey bees in commercial blueberry culture.

MATERIALS AND METHODS

BBLMV longevity and transmission. At the start of bloom, three honey bee colonies were placed at an Ottawa Co., MI, blueberry farm near West Olive known to have a large number of cv. Jersey blueberry bushes infected with BBLMV. The colonies were kept at this location until petal fall (for 2 weeks), at which time they were returned to East Lansing, MI, and individually placed in cages with seven healthy flowering 2-yr-old potted Jersey blueberry bushes. Seven additional flowering bushes were introduced to each cage on days 3, 6, and 10 of the study. All plants were kept in the cage until petal fall, a period of 2 weeks. Additionally, a control cage containing a colony that had not

been used for blueberry pollination was established at the same time. Four healthy flowering plants were placed in this cage following the same time schedule as noted above. A total of 100 potted, healthy, virus-tested plants were used in the experiment. Eighty-four bushes were caged with colonies from the infected field, and 16 bushes were placed in the control cage.

At the petal fall stage the plants were removed from the cages and placed in a greenhouse until leaf drop in the autumn (a period of 4 months), at which time they were placed in cold storage for vernalization for 8 weeks at 4° C. On their removal from cold storage, (approximately 10 months after exposure to bees), all plants were allowed to develop leaves and were tested for BBLMV using an established ELISA protocol (Childress and Ramsdell 1986) with the following alteration: The leaf tissue was homogenized in extraction buffer, filtered through cheese cloth, and the filtered extract was collected and kept at 5° C overnight before plating. This procedure does not alter the ELISA absorbance (A_{405nm}) values of the healthy controls (compared to samples directly plated after filtering) but did produce higher absorbance values from the infected samples. These higher absorbance values may be due to the release of virus particles that may be sequestered or bound to plant material. Leaf tissue from plants that tested ELISA-positive for BBLMV were mechanically inoculated onto *Chenopodium quinoa* Willd. for verification of virus infection. The infected blueberry tissue was ground with a mortar and pestle in a 0.01M phosphate buffer (pH 7.2, containing 2% nicotine [v/v]) and rubbed to Carborundum-dusted plants. Plants that developed symptoms typical of BBLMV infection 7-10 days after inoculation were tested by ELISA.

In-hive pollen transfer. One drone bee and four house bees (nonforaging bees) were collected from colonies used for blueberry pollination in a commercial field near Douglas, MI, as described later. To distinguish house bees from foragers, colonies were fitted with fluorescent pigment dispensers that marked bees as they enter or left the colony. The dispensers are a modification of those previously described (Dhaliwal and Sharma 1972). Bees that were not marked with pigment were considered to be house bees. The bees were individually stored in vials and kept frozen until pollen counts were made.

The pollen was rinsed from the bees by adding 20 ml of 50% ethanol to the vial containing the bee. The vial was then gently agitated so that the bee was washed with ethanol. Then, the bee was removed and the ethanol-pollen mix was filtered through a 0.45-um filter. Increasing concentrations of ethanol (75, 95 and 100%) were passed through the filter to dehydrate the pollen and prepare the sample for scanning electron microscopy (SEM). After the last 100% ethanol treatment the filters were fixed to 10 mm aluminum stub with a "Double Stick" tab and placed in a desiccator to dry. The stubs were gold coated for three minutes at 20 mA (about 21 nm of gold layer) and examined using a JEOL 35CF SEM (15 kV, magnification of X400). The stubs were scanned and all pollen was counted. Blueberry pollen was readily identified by the fact that it is in tetrads, which have a mean diameter of 33.4 um and a diameter range of 24-41 um (Megalos and Ballington 1987). Pollen exine patterns can be used to determine a particular cultivar if one has access to a scanning electron microscope.

Honey bee drift within and between apiaries. Two apiaries (six colonies at each location) were established in a single row facing south (two meter spacing between colonies) at an Allegan Co., Michigan blueberry farm (Fig. 4) near Douglas. One apiary (colonies labeled 1-6) was located at the northern 2 hectare planting made up equally of cvs. 'Rubel' and 'Jersey'. The other apiary (colonies labeled 7-12) was located 600 m away at the southern 1.5 hectare planting and also included equal amounts of cvs. 'Rubel' and 'Jersey'. The two plantings are separated by a stream and wooded area. The odd numbered colonies were fitted with dispensers that marked the thorax of foraging bees with fluorescent colored pigment as the bees entered or left the colony. Each marker colony's dispenser marked the bees with a colored pigment that was unique for that particular colony, allowing for the determination of the forager's origin. A forager is defined as any bee that flew from the colony. The even numbered colonies were also fitted with dispensers but contained no pigment. Three 1-minute counts recording the number and color of marked foragers as well the number of unmarked foragers entering or leaving each even-numbered colonies (unmarked colonies) at the two sites were made on 6 days of the 12-day blueberry flowering period. A mean of 54.4 bees were counted and classified at each observation.

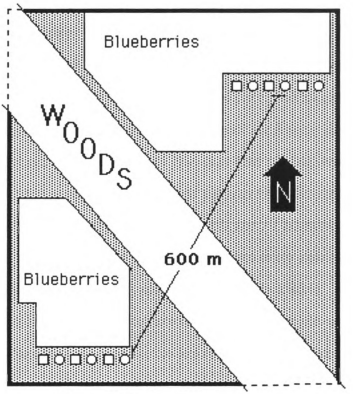

Figure 4. Arrangement of north (top) and south (bottom) apiaries used for examining the drift of honey bees within and between apiaries. The north apiary colonies are numbered 1-6 from left to right. The south apiary colonies are numbered 7-12 from left to right. Squares represent marker colonies (odd-numbered colonies) and the circles represent the unmarked colonies (even-numbered colonies). Colonies are separated by 2 m within the apiary and 600 m between apiaries.

RESULTS

BBLMV longevity and transmission. ELISA tests indicated that twelve of the 84 healthy Jersey plants that were caged with colonies previously exposed to BBLMV became infected with the virus. Figure 5 shows the percentage of the total number of infected plants corresponding to the day the plant was introduced into the cage. There is no apparent trend with respect to the day the plants were caged with the infected colonies and the number of plants that became infected. None of the blueberry plants showed symptoms of blueberry leaf mottle disease. This is not unexpected, because plant may remain symptomless for as long as 4 years (Ramsdell and Stace-Smith 1981). The results demonstrate that BBLMV was infectious and accessible to bees for transmission to healthy plants for at least 10 days in the colony.

The ELISA absorbance values of the 12 infected blueberry bushes, the day the bushes were placed in the cage, and the results of the mechanical inoculation of leaf tissue from BBLMV-infected blueberry to *C. quinoa* are shown in Table 1. Only six of the 12 *C. quinoa* plants, which were mechanically inocculated with plant tissue from blueberries that tested ELISA-positive for BBLMV, were infected. High summertime greenhouse temperatures (up to 34^{o} C) may have interfered with the maximum transmission of BBLMV to *C. quinoa*. None of the 16 plants that were caged with the control colony were infected.

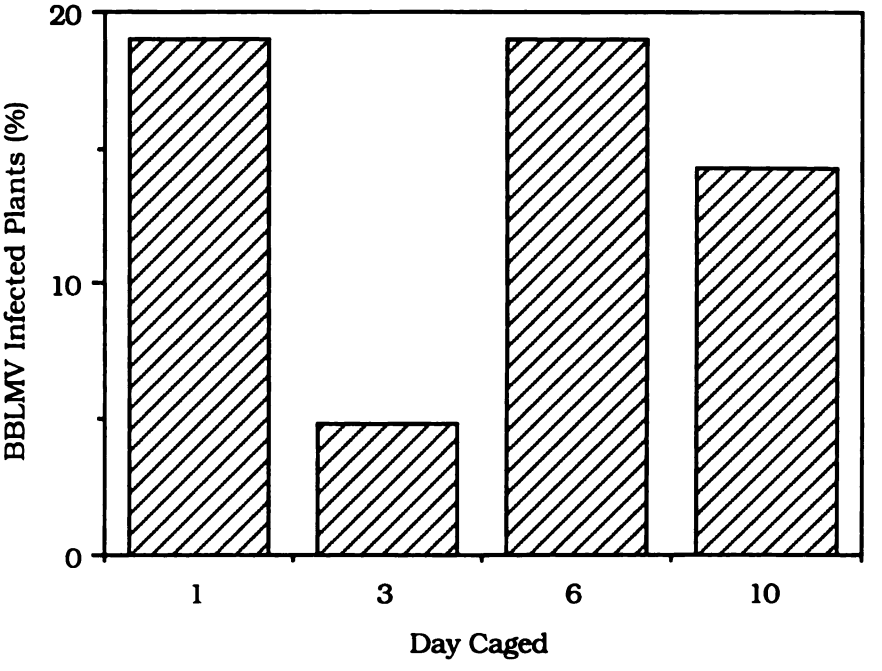

Figure 5. Percentage of the total number of caged plants (21 per time period) that tested ELISA-positive for blueberry leaf mottle nepovirus (BBLMV). Flowering Jersey plants were caged 1, 3, 6, and 10 days after cages containing colonies and BBLMV-infected pollen were established.

TABLE 1. Infection results of healthy potted Jersey blueberry bushes that were caged over time with bees and a bee hive obtained from a blueberry leaf mottle nepovirus (BBLMV)-infected blueberry field and presumed to contain BBLMV-infected pollen

Day[a]	ELISA values ($A_{405\ nm}$)		Infectivity on *Chenopodium quinoa*[d]
	Healthy controls[b]	Caged plants exposed to infected pollen[c]	
1	0.108	0.169	–
1	0.108	0.200	+
1	0.253	O.538	+
1	0.253	0.613	+
3	0.330	0.378	–
6	0 253	0 683	+
6	0.108	0.168	–
6	0.113	0.432	–
6	0.330	0.372	–
10	0.253	0.536	+
10	0.330	0.394	–
10	0.253	0.570	+

[a] Healthy 2-yr-old potted Jersey plants (three, seven-bush replicates/time period) were placed in mesh cages with a hive containing bees and BBLMV-infected pollen at day indicated after the infected pollen source was placed in the cage. Plants shown here are the 12 bushes that developed infections based on ELISA (enzyme-linked immunosorbent assay).

[b] Mean $A_{405\ nm}$ value + 3 SD of four healthy control plants placed in a cage with bees and a hive containing uninfected pollen.

[c] Mean $A_{405\ nm}$ value (duplicate wells) of leaf extracts from each test plant after exposure to bees carrying BBLMV-infected pollen, followed by incubation in isolation for at least 6 mo.

[d] Results of mechanical inoculation with triturated plant tissue from ELISA positive test bushes to *Chenopodium quinoa*. Infectivity was based on development of terminal leaf mottle/collapse symptoms characteristic of BBLMV in *C. quinoa*, followed by confirmatory tests by ELISA.

In-hive pollen transfer. The house bee pollen counts from four individuals resulted in counts of 6,034, 5,872, 5,631, and 3,059, or a mean count of 5,149 (*sd* = 1,403) pollen grains per bee. A nonworker drone bee also had a pollen count of 7,024 grains. These bees were not marked with fluorescent pigment and had not left the colony. Thus, any pollen found on these bees was the result of in-hive pollen transfer. More than 90% of the pollen found on these bees was blueberry pollen.

Honey bee drift within and between apiaries. A total of 3,115 drifting foragers were observed on the 6 days of data collection. Figure 6 shows the percentage of marked and unmarked foragers found at the entrances of the unmarked colonies within each apiary location. The data are means of all counts made during the flowering period. The colonies placed in the row (colonies 2 and 4 at the north apiary and colonies 8 and 10 at the south apiary) had 62.6 and 57.4%, respectively, of their foragers originating from colonies other than the unmarked colonies. The colonies at the end of the row (colony 6 and 12) had greatest number of unmarked foragers. However, 46% of the foragers from colony 2 and 32.4% of those from colony 12 originated from neighboring colonies.

The drifting of bees was not confined to the apiary where the colonies were located. Figure 7 shows the percentage of bees that originated from one apiary and were part of the foraging force observed at the unmarked colonies in the second apiary 600 m away. The results are a mean of all counts made during the study. The number of bees that drifted between apiaries declined over the duration of the experiment. However, bees that originated 600 m away were found in all colonies, except one, 12 days after the colonies were established.

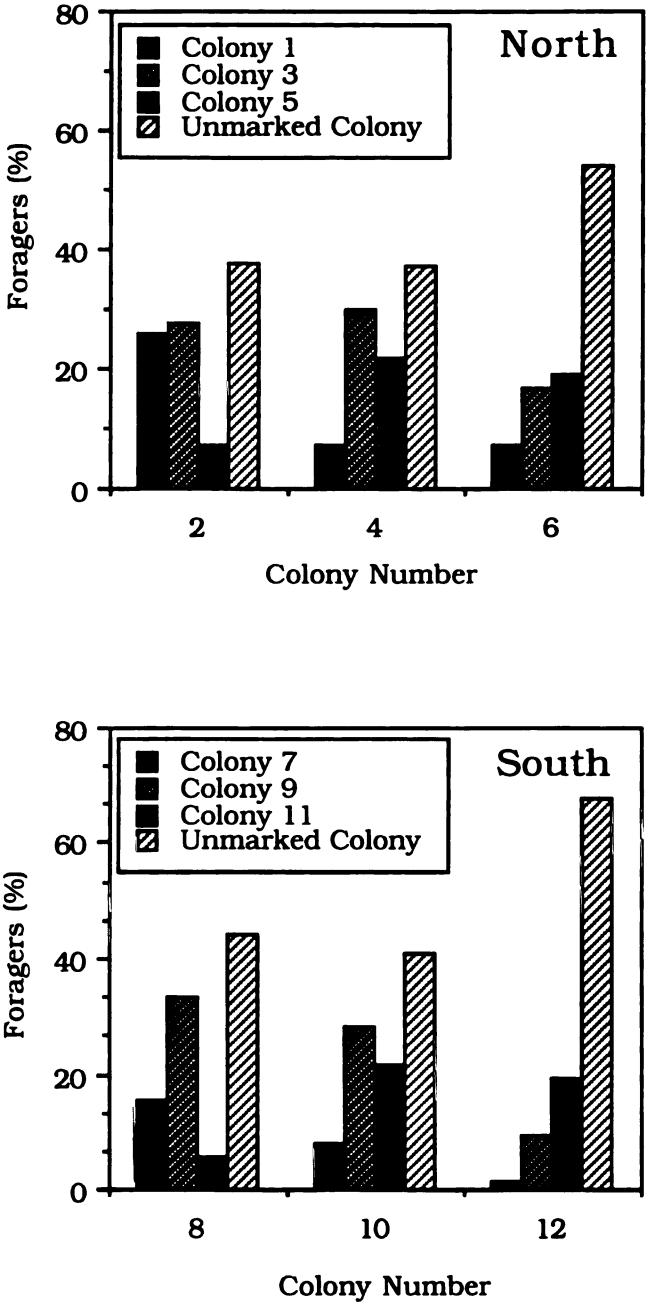

Figure 6. Percentage of marked foragers (from odd-numbered colonies) within north and south apiaries found at entrances of the even-numbered unmarked colonies (See Fig. 1 for diagram of apiaries and colonies within them). Apiaries were located 600 m apart. Results are the mean of six, 3-min counts made over 12 days of the blueberry flowering period. "Unmarked colony" refers to bees that either were entering their own unmarked colony or bees from a nearby unmarked colony.

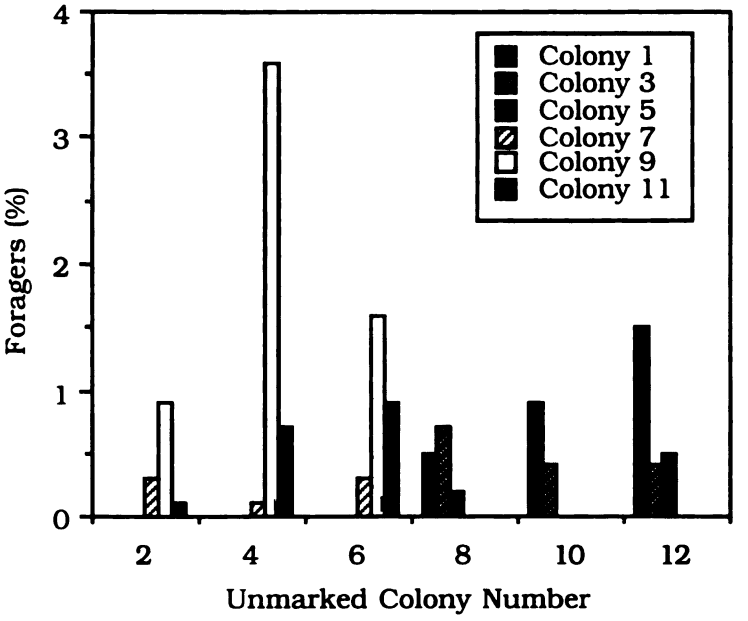

Figure 7. Percentage of foreign foragres (marked honey bees) originating 600 m away that were found entering unmarked colonies. Colonies 1-6 were located at the northern apiary and colonies 7-12 were located at the southern apiary.

DISCUSSION

Michigan is a major producer of highbush blueberries (7,300 hectares) with a high concentration of farms in the southwest region of the state. Many of the farms border one another, and the spread of a pollen-borne virus via honey bee colonies used for pollination is probable. The results presented in this paper provide information on the behavior of the honey bee colony and foraging bees that could facilitate the spread of BBLMV.

The phenomenon of bee-to-bee spread of virus-infected pollen, coupled with visits by bees to several colonies, some as far as 600 meters, could lead to rapid spread of BBLMV from one or more infection foci in a plantation.

The longevity of infectious BBLMV within a honey bee colony would allow for the long distance spread of the virus. Infected colonies used for pollination in one area of the state and then moved to a farm in a later blooming area could transfer the virus over 100 miles. In a previously reported study by Mink (1983), the long distance transfer of PNRSV from California to Washington was examined. He found that 20 of 40 hives tested contained PNRSV in infectivity tests on *C. quinoa* with pollen that had been stored in hive cells for 3 days. In the same study, a hive from a California almond and cherry orchard containing infected trees was caged with a healthy cherry tree in Washington within 24 hours after leaving the California orchard. No apparent transmission of PNRSV occurred. In the present study, stored pollen was not tested for virus, because pollen stored in the cells of the hive is inaccessible to bees for possible virus spread. Stored pollen is used as a protein source for the larvae of the colony (Gojmerac 1980). Instead, the BBLMV-infected colonies were allowed to pollinate healthy plants in a cage. Plants placed in the cage up to 10 days after the colnies were removed from the virus field source became infected with BBLMV. These results indicate that the colony serves as a reservoir of virus; bees inadvertently move virus-infected pollen form insect to insect in the hive. The result is the spread of BBLMV from the hive to previously healthy bushes.

Our results are in agreement with those of Free and Williams (1972), who conducted extensive studies determining the number of pollen grains on nonforaging bees. The transfer of pollen within the hive from bee-to-bee has also been documented in the apple orchard system (DeGrandi *et al.*, 1986). Because virus is associated with pollen and pollen exchange does occur between bees, the spread of virus from one plant to any plant in the foraging range of the colony is possible. The foraging range of honey bees is dependent on many environmental factors and conditions within the colony but may be as great as 4 km (Free and Williams, 1972). Thus, virus transferred between bees foraging in opposite directions could spread 8 km from the infected plant.

The large number of drifting foragers observed during this study would suggest that virus transfer occurs not only within a single colony, but between all colonies within an apiary. The drift of foragers between apiaries would also promote the spread of BBLMV to adjacent blueberry fields.

It is not known whether BBLMV was vectored by bees that injured blossom tissues during pollination, thus allowing direct inoculation by deposition of infected pollen in wounds, (pollen bears the virus both externally and internally [Childress and Ramsdell 1986]), or by ovule infection following fertilization by infected sperm.

The current recommendations for the control of BBLMV is to rogue out all infected plants as soon as the virus is detected visually or by ELISA. This study indicates that additional steps can be taken to minimize the spread of BBLMV to highbush blueberries. Honey bee drift can be minimized by arranging colonies of an apiary in a serpentine pattern, or in a circle, with all colony's entrances facing different

directions (Jay 1971). This arrangement may give bees a better reference as to the locationof their own hive. However, no sure method of controlling long range drifting is known. The prior location of the colonies used for pollination must be known and colonies used previously in blueberries or located near blueberry plantations should be avoided. The most effective means of controlling the spread of BBLMV is to identify all infected plants by ELISA tests, kill and remove them, and replant with a cultivar resistant to BBLMV. Presently, the only cultivars known to be susceptible to BBLMV are Jersey, Rubel, and Blueray. Studies are underway to identify suitable virus-resistant replacement cultivars.

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DRIFTING OF HONEY BEE FORAGERS WITHIN AND BETWEEN APIARIES POLLINATING BLUEBERRY, *VACCINIUM CORYMBOSUM*.

ABSTRACT

Two 6-colony apiaries were situated 600 m apart at similar blueberry plantings. Three colonies at each apiary were fitted with florescent pigment dispensers which marked the bees' thorax with colored pigment as they entered or left the colony. Each dispenser-fitted colony marked bees with a colored pigment that was unique for that colony. Counts at non-marking colony entrances were made by recording the number and color of foragers. The percentage of foragers originating from different colonies within the apiary ranged from 32 to 63 percent. Bees originating from colonies located 600 m away comprised 1.3 to 4.5 percent of the foraging force of the un-marked colonies.

INTRODUCTION

Drifting behavior of honey bees, the movement of bees from their own colony to adjacent colonies, is a common phenomenon. The majority of drifting bees are assumed to be young bees performing orientation flights where it is estimated that up to 50% of these bees may drift. Drifting may also be extensive when colonies are established from packages or colonies are moved to new locations (Corkins 1933). Researchers have reported methods that may be used to help prevent drifting within an apiary. These methods include painting the colonies different colors (Free 1958) or arranging the colonies in serpentine or in a circular pattern (Jay 1971). Although methods to prevent drifting are known little information on the degree of the actual number of bees that drift is available.

Michigan is a major producer of highbush blueberry (*Vaccinium corymbosum* L.). Most Michigan blueberry producers utilize from 2.5 to 10 colonies per hectare to ensure adequate pollination (Dorr and Martin 1966). In the blueberry system the drifting of honey bees has the potential for both beneficial and adverse effects on fruit production. A beneficial effect would be the mix of compatible pollen within colonies via in-hive pollen exchange similar to that found in the apple system (DeGrandi-Hoffman 1986). An adverse effect would include the possibility of transferring a pollen-borne virus (Blueberry Leaf Mottle Virus [Ramsdell and Stace-Smith 1980]) between colonies (Boylan-Pett *et*

al. 1991a). Honey bee drift also has the potential for negetive effects on the honey bee colony itself. These would include the spread of European foulbrood (Wardell 1982) or other bee diseases, and the rapid spread of parasitic mites.

In this paper we report on the drifting behavior of honey bees between colonies within an apiary established for blueberry pollination. We also report on the drifting of foraging honey bees between apiaries located 600 meters apart.

MATERIALS AND METHODS

Two apiaries (six colonies at each location) were established in a single row facing south (two meter spacing between colonies) at a Allegan Co., Michigan blueberry farm. One apiary (colonies labeled 1 through 6) was located at the southern 1.5 hectare planting and the other (colonies labeled 7 through 12) was located 600 m away at the northern 2 hectare planting. The two 'Rubel'/'Jersey' plantings are separated by a stream and wooded area.

The odd numbered colonies were fitted with dispensers which mark the thorax of a forager with fluorescent colored pigment. The dispensers are a modification of those described by Dhaliwal and Sharma (1972). Each marker colony's dispenser marked the bees with a colored pigment that was unique for that particular colony allowing for the determination of the forager's origin. A forager is defined as any bee that flies from the colony. The even numbered colonies were also fitted with dispensers but contained no pigment. Three one-minute counts recording the number

and color of marked foragers as well the number of unmarked foragers entering or leaving the even numbered colonies (unmarked colonies) were made on six days of the blueberry flowering period.

RESULTS

Drifting within an apiary. A large number of drifting foragers were observed on the six days of data collection. Figure 8 shows that two days after the colonies were established 68.2% of the foragers of the unmarked colonies originated from neighboring colonies. Those colonies that were placed in the row (colonies 2, and 4 at the south apiary and colonies 8 and 10 at the north apiary) had 78.2% and 70.0% respectively, of their foragers originating from colonies other than the unmarked colonies. The two colonies that were at the end of the rows (colony 6 and 12) had a greater number of unmarked foragers. Still 45.8% of the foragers from colony 6 and 54.9% of those from colony 12 originated from neighboring colonies.

Eleven days later the percentage of bees that originated from neighboring colonies and comprised the foraging force of the un-marked colonies had decreased to 58%. The colonies placed in the row had slightly less than 50% of the foragers originating from colonies other than the home colony (Fig. 9). The colonies located at the end of the row had 33.8% (colony 6) and 38.4% (colony 12) of the foragers originating from different colonies.

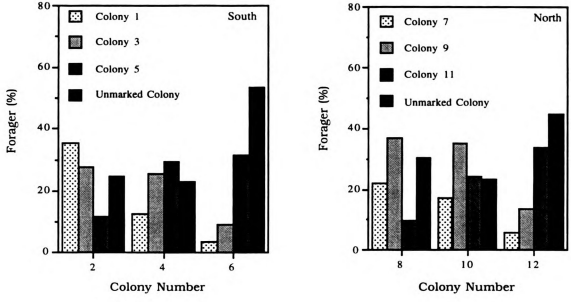

Figure 8. Percent of marked foragers (within apiary) found at the entrances of the un-marked colonies two days after establishment.

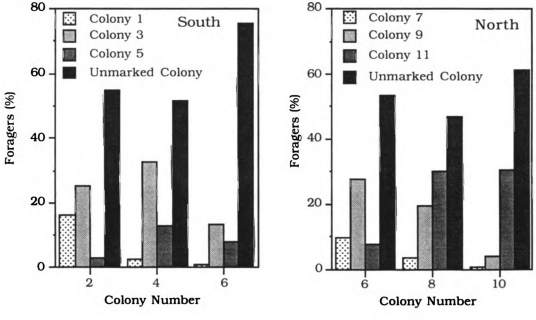

Figure 9. Percent of marked foraers (within piary) found at entrances of un-marked colonies eleven days after establishment.

Drifting between apiaries. The drifting of bees was not limited to the apiary where the colonies were located. Figure10 shows the percent of bees originating 600 m away that comprise the foraging force of the un-marked colonies the first day after the colonies were established at the planting. The number of bees that drifted between apiaries declined over the duration of the experiment. However, bees that originated 600 m away were found in all colonies, except one, 12 days after the colonies were placed into blueberries.

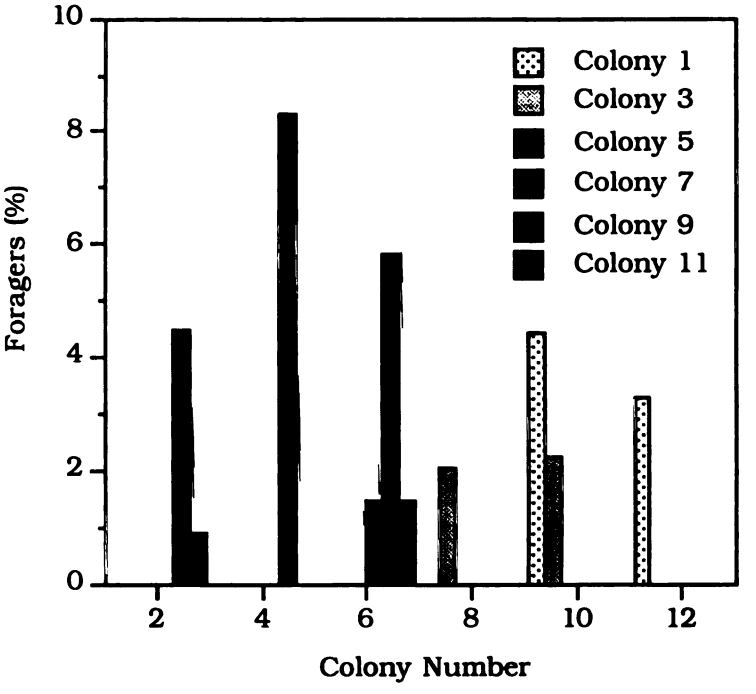

Figure 10. Percent of marked foragers (originating 600 m away) that comprise the foraging force of the un-marked colonies. Cols. 1-6 are located at the southern apiary, 7-12 at the northern apiary.

DISCUSSION

There was a large variation between colonies in the contribution to the drifting population. Unfortunately, we did not make a population, or age structure analysis of these similar-sized colonies to know if these factors had a bearing on this phenomenon.

The differences in the amount of drifting between colonies placed in the row and those at the end of the row are in partial agreement with a previous report by Robinson (1979). In his experiments bees drifted to end colonies but there was little drift of bees from colonies at the end of the row. Our results shows that bees from colonies 1 and 7 (colonies at the opposite end of the rows) made up a considerable portion of the foraging force of the colonies placed next to them (colonies 2 and 8 respectively) (Fig. 8). However, it was also observed that a greater movement of bees between colonies occurred from colonies placed in the row (Fig. 8, bees originating from colonies 3, 5, 9, and 11). Colonies placed at the end of the row are unique in that they have no adjacent colony on one side. This distinguishing feature may serve as a landmark for returning foragers.

While the drifting within an apiary could possibly show that bees recognize placement, i.e., ends of rows versus the middle, this recognition of placement did not seem to hold when drifting was between apiaries. This was in spite of the fact that the apiaries had similar shape, orientation, and number of colonies.

Michigan has about 30,000 colonies that are annually moved into orchards and fields for pollination. These colonies are then returned to their permanent apiary sites located throughout the state. With nearly 10 colonies/hectare used in blueberry pollination, there are often

pollination apiaries that are located less than 500 meters apart. This density, and drifting between apiaries, would help explain the very rapid dissemination of diseases and mites within our honey bee population.

We do not feel that 600 meters is a remarkable distance for drifting of foragers between apiaries. This experiment was initiated because we had previously found marked foragers at colonies over 1.6 k from their parent colony.

Robbing of honey between colonies should not be a factor in this study since abundant nectar is found in the blueberry flowers.

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CONCLUSION

The association between blueberry leaf mottle virus (BBLMV) and highbush blueberry (*Vaccinium corymbosum* L.) pollen was first detected by Childress and Ramsdell (1986). This pollen/virus relationship led these researchers to speculate that the honey bee (*Apis mellifera* L.) played a vital role in the spread of the disease. Honey bees are the primary pollinator of highbush blueberries in Michigan (McGregor 1976). The role of the honey bee as a vector of BBLMV to highbush blueberries was first documented by Childress and Ramsdell (1987) and again by Boylan-Pett *et al.* (1991a).

Understanding the virus/honey bee interactions that are associated with the transmission of BBLMV allows for the development of recommendations to help prevent the spread of the disease in the field. Factors that are associated with the honey bee colony and the foraging bee that contribute to the spread of BBLMV include, but are not limited to : longevity of infectious BBLMV within a colony; the in-hive transfer of virus and/or pollen from bee to bee; and the transfer of virus between colonies via honey bee drifting (movement of foragers from their own colony to adjacent colonies) (Boylan-Pett *et al.* 1991a).

Longevity of infectious BBLMV within a colony can lead to the long range spread of the virus. Infected colonies used for pollination in one area of the state and then moved to a later blooming area could transfer the disease over 100 miles. The longevity of infectious BBLMV within a

colony can also lead to the spread of the disease within the same area between susceptible cultivars with different blooming periods. BBLMV remained infectious and accessible to honey bees for 10 days when colonies used for pollination in a known BBLMV-infected field were caged with healthy plants (Boylan-Pett *et al.* 1991a). These results indicate that the colony serves as a reservoir of virus and that bees may come in contact with the virus through in-hive virus/pollen transfer.

In-hive virus/pollen transfer (the transfer of virus-infected pollen from bee-to-bee within the hive) is a factor associated with the honey bee colony that may play a vital role in the spread of BBLMV. When honey bees foraging on BBLMV infected bushes return to their colonies' they come in contact with other returning foragers. During this contact, virus-infected pollen may be transferred from a virus vector bee to a non-infectious bee. This new virus vector leaves the colony and may infect a healthy bush in its foraging range. In-hive virus transfer is one explanation for the random and spotty distribution of infected plants found in diseased fields as described by Childress and Ramsdell (1987) . In-hive pollen transfer has been documented in the apple orchard system (DeGrandi-Hoffman *et al.* 1986).

Examination of non-foraging honey bees collected from colonies used for blueberry pollination resulted in an average count of 5,149 pollen grains per bee (Boylan-Pett *et al.* 1991a). These results are in agreement with Free and Williams (1972) who conducted extensive studies determining the number of pollen grains on non-foraging bees. Because the virus is associated with pollen and pollen transfer occurs between bees, the spread of the virus from one plant to any plant in the foraging range of the colony is possible. The foraging range of honey bees

is dependent on many environmental factors and conditions within the colony but may be as great as 4 km (Free and Williams 1972). Thus, virus transfer between bees foraging in opposite directions could spread 8 km from the infected bush.

The drifting behavior of honey bees is an important factor associated with the spread of BBLMV. Results from our experiments indicate that high levels of drifting occurred within an apiary. Two days after colonies were established 68.2% of the observed foragers had drifted from their original colonies (Boylan-Pett and Hoopingarner 1991, Boylan-Pett *et al.* 1991a). Drifting declined over the 12 days of the experiment; still, 58% of the foragers observed on the last day of the experiment had drifted from their original colony. Drifting between apiaries was also observed at an unexpected rate. The first day after the colonies were established as many as 8% of the observed foragers had drifted from colonies located 600 m away. This between apiary drifting also declined over the duration of the experiment. However, bees that had drifted 600 m were found in all colonies, except one, 12 days after the colonies were established. The observed drifting behavior together with in-hive virus/pollen transfer indicated that virus transfer could occur not only within a single colony, but between all colonies of the apiary. Long range drifting, between apiaries, promotes the spread of BBLMV to adjacent blueberry fields.

To investigate the drifting behavior of honey bees, and to differentiate foragers from non-foragers for pollen counts, a method to distinguish the origins of foragers with respect to their original colony was employed (Boylan-Pett *et al.* 1991) . This method marks the thorax of foragers with fluorescent pigment as they enter or leave the hive. The

method was 99.97% effective since 2,999 of the 3,000 bees observed leaving the colony were marked. It should be noted that only bees leaving the colony were recorded; and that the bees are marked as they enter or leave the colony, thus the lone unmarked bee would have probably been marked when she returned to the colony. This system was also used to determine the foraging populations of four honey bee colonies on three days in 1987. The foraging populations ranged from 11.7 to 42.2% of the colony's population. The highest number of foragers occurred on June 10 when basswood (*Tilia spp.*) was in bloom. No significant correlation between the number of foragers and colony population was found.

The three published papers that comprise this dissertation provide information on the factors associated with the honey bee colony and the foraging bee that contribute to the spread of BBLMV to highbush blueberry. Recommendations based on this information to prevent the spread of BBLMV include:

i. Due to the longevity of infectious virus within a colony the prior location of honey bee colonies used for blueberry pollination must be known.

ii. Avoid colonies previously used for blueberry pollination or located near blueberry plantations. No action is required, and none available, to prevent in-hive virus/pollen transfer.

iii. Due to the high occurrence of honey bee drift, within the apiary, observed in the blueberry plantation, colonies should be arranged in a serpentine or circular fashion to prevent drifting.

Many beekeepers paint their hive bodies different colors to minimize bee drift. However, it should be noted that the hive

entrances of the colonies used in my experiments all had unique colors, due to pigment dislodging from the dispensers, and drifting was high.

iv. No method to prevent long range drifting is known. However, providing some type of landmark for each colony may help minimize bee drift.

v. The most effective method to control the spread of BBLMV is to identify all infected plants by ELISA tests, kill and remove the plants, and replant with healthy cultivars that are resistant to BBLMV.

These studies not only investigate the interactions of honey bees and the spread of BBLMV, but they also provide methods and information that are useful for researchers studying various aspects of pollination biology or honey bee behavior. Techniques from these studies would be helpful in answering many of the concerns that have been raised over the release of genetically engineered plant material and the risks associated with their introduction to the environment. Of great concern is the escape of genes from transgenic plants to wild populations causing the development of new weedy plants. The most probable means of gene escape is via pollen. The studies that have examined pollen movement in the past have generally used small plots and have provided useful information on only one type of honey bee pollen movement. The research from this dissertation suggests that there are three distinct types of pollen movement associated with bees. The first type of pollen movement, would be the movement of pollen from individual bees as they move from flower-to-flower. The second type of pollen movement would

be the movement of pollen associated with in-hive pollen transfer. Pollen from one plant, located at one end of the colony's foraging range, could be distributed to a plant at the other end of the foraging range by the bee-to-bee transfer of pollen in the hive. The third type of pollen movement involves the long range movement of pollen that would be associated with the long range drifting of honey bees. Studies using the information and techniques described in this dissertation could provide information that could be used to help make better informed decisions regarding the risks associated with the release of engineered plant material.

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