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BUMBLE BEE (*BOMBUS IMPATIENS*) POLLINATION
OF GREENHOUSE TOMATOES

by

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ABSTRACT

Bumble bee (*Bombus impatiens* Cresson) pollination of commercial greenhouse tomatoes has become common in Ontario, but little research has been done on this system. The purpose of this study was to address problems associated with bumble bee pollination of greenhouse tomatoes, such as loss of bees, low levels of bee activity, inadequate levels of pollination and poor tomato quality. Separate experiments examined relationships among, 1. bee loss and activity, and greenhouse temperature and plastic type; 2. bee activity and pollination; and 3. pollination level and tomato quality. The degree of anther cone bruising resulting from buzz pollination by bumble bees was categorized into levels of pollination in commercial greenhouses and in laboratory settings. Activity was 94% greater and colony sizes were 111% greater under UV transmitting plastics than in commercial greenhouses with plastic that transmitted low levels of UV. Bee activity was weakly correlated with internal greenhouse temperature ($r^2=0.18$). Pollination decreased with distance from colonies. Tomato quality improved with greater pollination in a diminishing returns relationship.

KEYWORDS: *Bombus impatiens*, *Lycopersicon esculentum*, bumble bee, tomato, pollination, ultraviolet, activity, greenhouse, stigmatic pollen loads, fruit set, fruit quality.

CO-AUTHORSHIP

CHAPTER 2. Co-authored by L.A. Morandin, T.M. Laverty, P.G. Kevan, L. Shipp and S. Khosla. All writing was done by L.A. Morandin with editing from T.M. Laverty and P.G. Kevan. All laboratory work and data analysis was performed by L.A. Morandin.

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CHAPTER 1

1.1 GENERAL INTRODUCTION

1.1.1 Overview

Buzz pollinated crops require vibration in order to release their pollen (Buchmann, 1983), and traditionally, pollination of buzz pollinated greenhouse crops was accomplished through the use of hand-held electric vibrators (Verkerk, 1957). Because of expense, risk of damage to the flowers and developing fruit, and the time consuming nature of this method (Cribb, 1990), there has been interest in developing alternative methods of pollination. The use of bumble bees (*Bombus* spp.) to pollinate certain crops has become common because they are effective pollinators of several buzz pollinated crops such as blueberries (Shaw *et al.*, 1939), cranberries (Kevan and Gadawski, 1983) and tomatoes (Pinchinat *et al.*, 1982; van der Sande, 1990; van Ravestijn, 1990; Banda and Paxton, 1991; Kevan *et al.*, 1991; Pressman *et al.*, 1999). Studies in Europe, and more recently in Ontario, have shown that bumble bees are at least as effective as hand pollinating greenhouse tomato crops (Banda and Paxton, 1991; Kevan *et al.*, 1991; van Ravestijn and van der Sande, 1991; Straver and Plowright, 1991; Asada and Ono, 1996; Dogterom *et al.*, 1998; Pressman *et al.*, 1999). Although honey bees (*Apis mellifera* L.) have been tested as pollinators of various greenhouse crops, they have not been found to be very effective on flowers requiring buzz pollination (Banda and Paxton, 1991; Kevan *et al.*, 1991).

The farm value of greenhouse tomato crops in southern Ontario was estimated at \$77.4 million in 1998 (Horticulture crop statistics, Ontario Ministry of

Food and Rural Affairs, 1998). Before 1992, virtually all pollination of tomatoes in greenhouses in Ontario was done by hand vibration. As of 1994, all commercial growers in southern Ontario began to use bumble bees (*Bombus impatiens* Cresson) as their sole means of pollination, creating a new multi-million dollar supply business (S. Khosla, pers. comm.). Although the use of bumble bees as greenhouse pollinators has spread rapidly, very little basic research has been conducted to answer many important questions. Some of the problems that bee suppliers, crop scientists and growers are having include; low bee activity in some greenhouses and loss of bees through the ventilation systems. Both of these problems may contribute to inadequate levels of pollination in some greenhouses. In addition, little research has been done to determine how different levels of bumble bee pollination affect tomato quality.

1.1.2 Bee activity and loss

Anecdotal reports from greenhouse growers and bee suppliers suggest that there is variation among greenhouses in the level of bee activity outside the colonies, and in bee loss through the ventilation systems. Because different types of greenhouse plastic have different transmission spectra, and because bees see within the UV range, variation in plastic type among greenhouses may be responsible for some of the variation in bee activity and loss.

1.1.2.1 Bee vision

Most Hymenoptera, including bumble bees, have trichromatic colour vision (e.g. Mazokhin-Porshniakov, 1969; Menzel and Backhaus, 1991; Peitsch et al., 1992). The three colour receptors are stimulated by ultraviolet, blue and green

wavelengths, with the peak sensitivities at 345 nm, 440 nm, and 550 nm respectively (Kevan and Backhaus, 1998; similar to Peitsch et al., 1992). Trichromatic colour vision spans about 300 nm of natural daylight starting at about 310nm (Kevan and Backhaus, 1998). It has been shown that sensitivity to UV in honeybees and other insects is 4 to 6 times higher than to other parts of the spectrum (Kevan, 1970, 1979, 1983; Laughlin, 1976; Menzel and Backhaus, 1991), suggesting that UV is an important component of insect vision.

Various studies have shown that ultraviolet light produces a positive phototactic response in many insects, including honeybees (Bertholf, 1931; Kaiser et al., 1977; Menzel, 1979; Menzel and Greggers, 1985), fruit flies (Fischbach, 1979), and Diptera (Kevan, 1979). It is believed that insects use UV light as an indicator of an open environment (von Hess, 1913; Laughlin, 1976— dragonflies; Menzel and Greggers, 1985).

In addition, UV light may be an important component of bumble bee orientation. As light passes through the atmosphere, the rays become scattered, resulting in partial polarization of the light. The result is a symmetrical ray pattern that is fixed with respect to the solar meridian (Brines and Gould, 1982). The polarization pattern of the sky may be used as a celestial compass even under cloudy conditions (Brines and Gould, 1982). von Frisch's work (see von Frisch, 1965 for review) showed that honey bees use polarized light as a means of orientation and navigation. With increasing wavelengths, the polarized pattern becomes subject to atmospheric disturbance (Rozenberg, 1966), consequently, it is believed that bees primarily use polarized light within the UV range for

orientation. Von Helversen and Edrich (1974) tested the sensitivity of honeybees to different wavelengths of polarized light, and found a maximum sensitivity at 345nm. They determined that polarized light was detected exclusively by the UV receptors. Similar results were found by Duelli and Wehner (1973) for the desert ant *Cataglyphis bicolor*.

All three colour receptors in honey bees have been found to be important for large area movements (Kaiser and Liske, 1974; Kaiser *et al.*, 1977), as well as foraging behaviour (Thompson, 1995). Because bee activity has been found to be affected by various wavelengths of light, we examined the effects of plastic type on bee activity and loss.

1.1.2.2 Plastic types (Chapter 2 and 3)

In southern Ontario, various greenhouse plastics that differ in intensity and spectrum of light transmission are used by commercial tomato growers. Because of the expense of bee colonies, it is important to determine how plastic type affects bee activity and bee loss. Because bees use UV light, plastics that transmit more of the UV range may result in greater levels of bee activity. A large spectrum of UV light within a greenhouse may attract more bees to leave the colony, or enable the bees to better orient and navigate, resulting in shorter, more efficient foraging trips. Variation in intensity of transmission and wavelengths transmitted by the plastics may also cause differences in internal temperature, which may affect bee activity, because bee activity increases with increasing temperatures (Lundberg, 1980—bumble bees; Abrol, 1990—alfalfa-

pollinating bees; Armbruster and McCormick, 1990—euglossine bees; Corbet et al. 1993—honey bees and bumble bees; Abrol, 1998—alfalfa-pollinating bees).

Loss of bees through ventilation systems may also be affected by type of greenhouse plastic. The majority of tomato greenhouses in the study area were vented using 'gutter vents', which are hinged openings that extend the length of the roof of the greenhouse. Vent operation is computer controlled, being triggered when the internal greenhouse humidity or temperature reaches a set value. In most of the gutter vented greenhouses, the vents are not screened, so that bees are able to exit the greenhouse when the vents are open. In greenhouses with plastics that transmit large amounts of UV light, there is little visual contrast between the open vents and the rest of the roof. In greenhouses with UV-blocking plastics, there is a large visual contrast between the light coming through the open vents and the light coming through the rest of the roof. The difference in contrast with daylight may be a factor in explaining why bee loss is a serious problem in some greenhouses but not others.

1.1.3 Pollination levels (Chapter 4)

Various methods have been used to assess levels of pollination of greenhouse tomatoes by bumble bees. Bin and Sorressi (1973) observed that pollination of tomato flowers by bumble bees caused necrotic spots on the anther cone. In European greenhouses, van Ravestijn and van der Sande (1991) assessed the number of tomato flowers that had been pollinated by bumble bees (*Bombus terrestris*) by observing the brown discolouration of the anther cone caused by pollination visits. By following bumble bees and recording flower

handling time, they also estimated that a single active worker could pollinate at least 500 tomato plants, or 250m² of greenhouse tomatoes per day. Using this information, they suggested that 10 to 15 colonies per hectare was more than enough for sufficient pollination. Some additional European recommendations include:

- Maintaining a population of 500 to 600 bumble bees per hectare.
- Monitoring bee activity by looking for 'bite' marks on the anther cone, flight of bees, and consumption of sugar solution.
- Arranging the hives in an even distribution throughout the greenhouse.

Currently, some of the bumble bee suppliers for Ontario greenhouses check for sufficient levels of pollination by sampling a few hundred flowers and estimating the percentage of flowers that have brown discolourations on the anther cone. The suppliers are aiming to see that 100% of the flowers have brown discolourations, that is, that all of the flowers on any given day have been pollinated.

Straver and Plowright (1991) recommended that more North American studies were required before standard procedures could be created for North American greenhouses. They suggested that, in the interim, growers could use the European guidelines. However, these recommendations were based on *B. terrestris* rather than *B. impatiens*, and they did not take into account how the number of pollination visits affects tomato quality.

1.1.4 Tomato quality (Chapter 5)

Tomatoes that bumble bees pollinate have a higher percent fruit set,

larger size and heavier fruit, and more seeds per fruit than those pollinated manually or by honey bees (Banda and Paxton, 1991; Kevan et al., 1991; van Ravestijn and van der Sande, 1991). The quality of a tomato is also a result of the number of pollinator visits to the flower. Too few visits by bees may result in a low number of ovules being fertilized, and substandard tomatoes (e.g., Verkerk, 1957; Straver and Plowright, 1991, van Ravestijn and van der Sande, 1991; Pressman et al., 1998). Too much bee activity on a flower may cause damage to the reproductive organs, premature loss of the flower, and no fruit development (Cribb, 1990). Fletcher and Gregg (1907) showed that within limits, the size of the tomato was positively correlated with how much pollen was transferred to the stigma. Verkerk (1957) found that the more often flowers were manually pollinated—up to a total of four times—the greater percent fruit set and the higher the number of seeds per fruit. He also found that more pollen on the stigma resulted in faster fruit development and therefore an earlier and more profitable crop.

1.1.5 Objectives

The purpose of this study was to address bumble bee pollination of greenhouse tomatoes. More specifically, I examined how different greenhouse conditions and plastic types affect colony activity levels and bee loss levels, and resulting pollination levels. I also determined the relationship between levels of bumble bee pollination and tomato quality measures. The information from these studies can be used by crop scientists, growers and bee suppliers as a guide for the use of bumble bees as pollinators of commercial greenhouse tomatoes.

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CHAPTER 2

Bumble bee activity and loss in commercial tomato greenhouses

2.1 INTRODUCTION

Buzz pollinated crops require vibration in order to release their pollen (Buchmann, 1983), and traditionally, pollination of buzz pollinated greenhouse crops was accomplished through the use of hand held electronic vibrators (Verkerk, 1957). Because of the expense, risk of damage to the flowers and developing fruit, and the time consuming nature of this method of pollination (Cribb, 1990), there has been interest in developing alternative methods of pollination. The use of bumble bees to pollinate certain crops has become common because they are effective pollinators of several buzz pollinated crops such as blueberries (Shaw *et al.*, 1939), cranberries (Kevan and Gadawski, 1983) and tomatoes (Pinchinat *et al.*, 1982; van der Sande, 1990; van Ravestijn, 1990; Banda and Paxton, 1991; Kevan *et al.*, 1991; Pressman *et al.*, 1999). Studies in Europe, and more recently in Ontario, have shown that bumble bees are at least as effective as hand pollinating for greenhouse tomato crops (Banda and Paxton, 1991; Kevan *et al.*, 1991; van Ravestijn and van der Sande, 1991; Straver and Plowright, 1991; Asada and Ono, 1996; Dogterom *et al.*, 1998; Pressman *et al.*, 1999). Although honey bees (*Apis mellifera* L.) have been evaluated as pollinators of various greenhouse crops, they are not effective pollinators of flowers requiring buzz pollination (Banda and Paxton, 1991; Kevan *et al.*, 1991).

The farm value of greenhouse tomato crops in southern Ontario was estimated at \$77.4 million in 1998 (Horticulture crop statistics, Ontario Ministry of Food and Rural Affairs, 1998). Before 1992, pollination of greenhouse tomato crops in Ontario was done by hand vibration. As of 1994, all commercial growers in southern Ontario began to use bumble bees as their sole means of pollination, creating a new multi-million dollar supply business (S. Khosla, *pers. comm.*). Although the use of bumble bees as greenhouse pollinators has spread rapidly, very little basic research has been conducted to answer many important questions. Some of the problems that bee suppliers, crop scientists and growers have include, loss of bees through the ventilation systems, and variation in levels of bee activity.

Anecdotal reports from growers and bee suppliers suggest that variation in colony size occurs among greenhouses as a result of loss of bees through cooling vents. Because of the expense of this problem and the effect on pollination levels, it is important to determine what causes the substantial loss of bees in some greenhouses.

Greenhouse growers and suppliers have also observed variation in the level of bee activity among greenhouses, leading them to question what factors may be responsible for the variation. Factors that may be responsible for varying levels of bee activity in different greenhouses include greenhouse plastic type, internal greenhouse temperature and humidity, and internal light levels.

Various plastic types are used for the different greenhouses. The plastic types have unique transmission spectra, and differ in the amount of UV intensity of light transmitted.

2.1.2 Bee vision

Most Hymenoptera, including bumble bees, have trichromatic colour vision (eg. Mazokhin-Porshniakov, 1969; Menzel and Backhaus, 1991; Peitsch et al., 1992). The three colour receptors are stimulated by ultraviolet, blue and green wavelengths, with the peak sensitivities at 345nm, 440nm, and 550nm respectively (Kevan and Backhaus, 1998; similar to Peitsch et al., 1992). Trichromatic colour vision spans about 300nm of natural daylight starting at about 310nm (Kevan and Backhaus, 1998) (Fig. 2.1). It has been shown that sensitivity to UV in honey bees and other insects is 4 to 6 times stronger than to other parts of the spectrum, suggesting that UV is an important component of insect vision (Kevan, 1970, 1978, 1983; Laughlin, 1976; Menzel and Backhaus, 1991).

Various studies have shown that ultraviolet light produces a positive phototactic response in many insects, including honeybees (Berthoff, 1931; Kaiser et al., 1977; Menzel, 1979; Menzel and Greggers, 1985), fruit flies (Fischbach, 1979), and Diptera (Kevan, 1979). It is believed that this high responsiveness to UV light results from the insects' taking it as indicator of an open environment (von Hess, 1913; Laughlin, 1976—dragonflies; Menzel and Greggers, 1985).

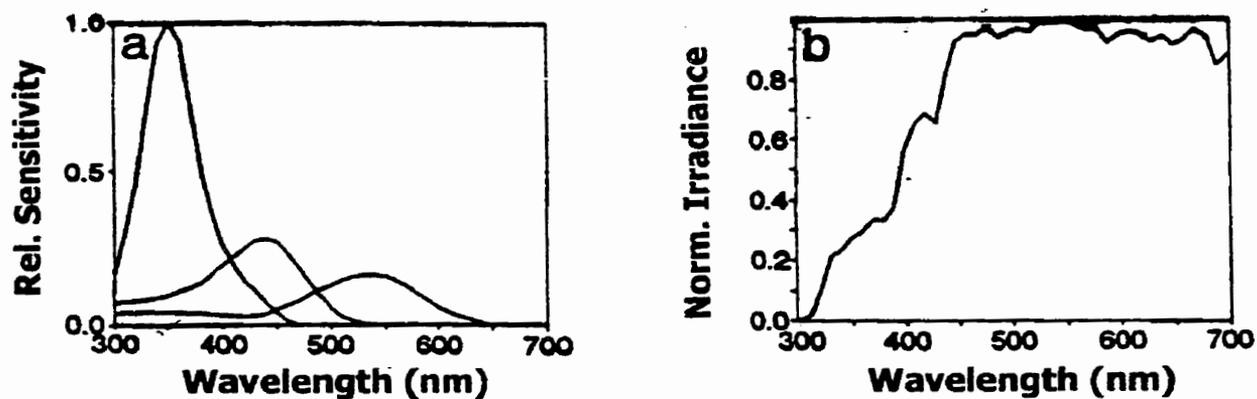


Figure 2.1. Colour vision in honey bees (similar to bumble bees). Relative spectral sensitivity of the three photoreceptors in the honey bee (after Neumeyer, 1980 and Lunau and Maier, 1995). b. Spectral distribution of daylight based on 6500 K (after Henderson, 1970). Irradiance (photons/cm²/s) in proportion of the maximum at 550 nm.

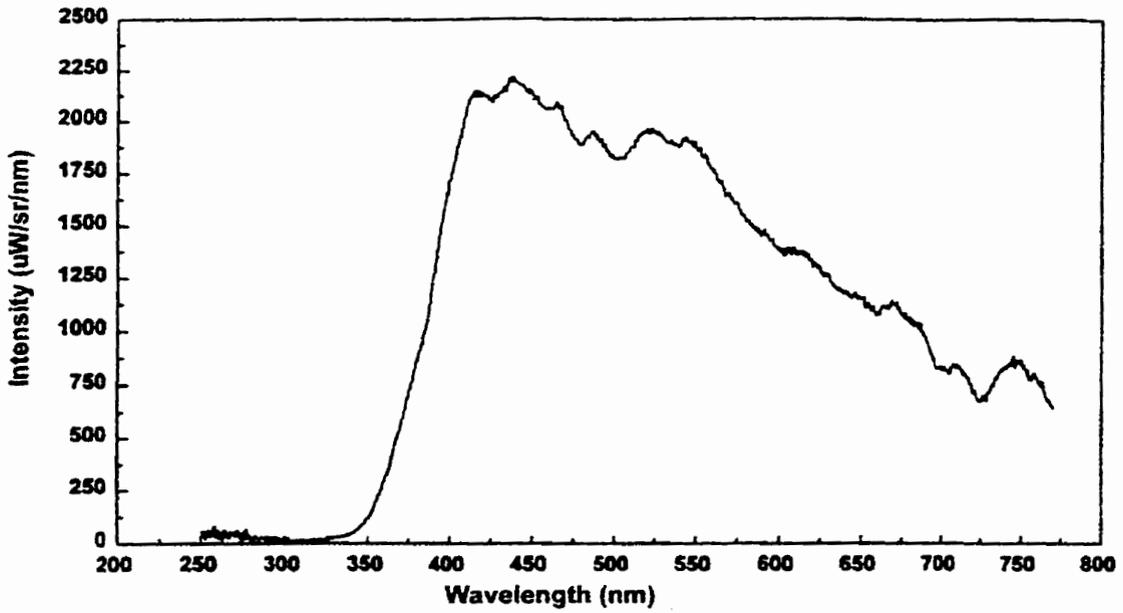
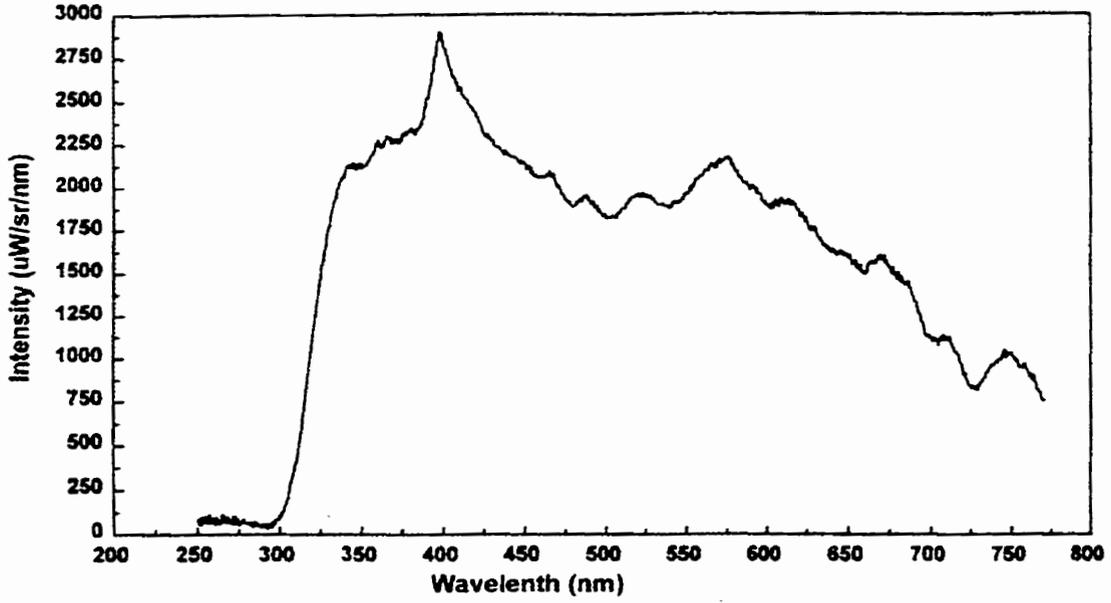
In addition, UV light may be an important component of bumble bee orientation. As light passes through the atmosphere, the rays become scattered, resulting in partial polarization of the light. The result is a symmetrical ray pattern that is fixed with respect to the solar meridian (Brines and Gould, 1982). The polarization pattern of the sky may be used as a celestial compass even under cloudy conditions (Brines and Gould, 1982). von Frisch (summarized; 1965) showed that honeybees use polarized light as a means of orientation and navigation. With increasing wavelengths, the polarized pattern becomes subject to atmospheric disturbance (Rozenberg, 1966), and consequently, it is believed that bees primarily use polarized light within the UV range for orientation. Von Helversen and Edrich (1974) tested the sensitivity of honeybees to different wavelengths of polarized light, and found a maximum sensitivity at 345nm. They determined that polarized light was detected exclusively by the UV receptors.

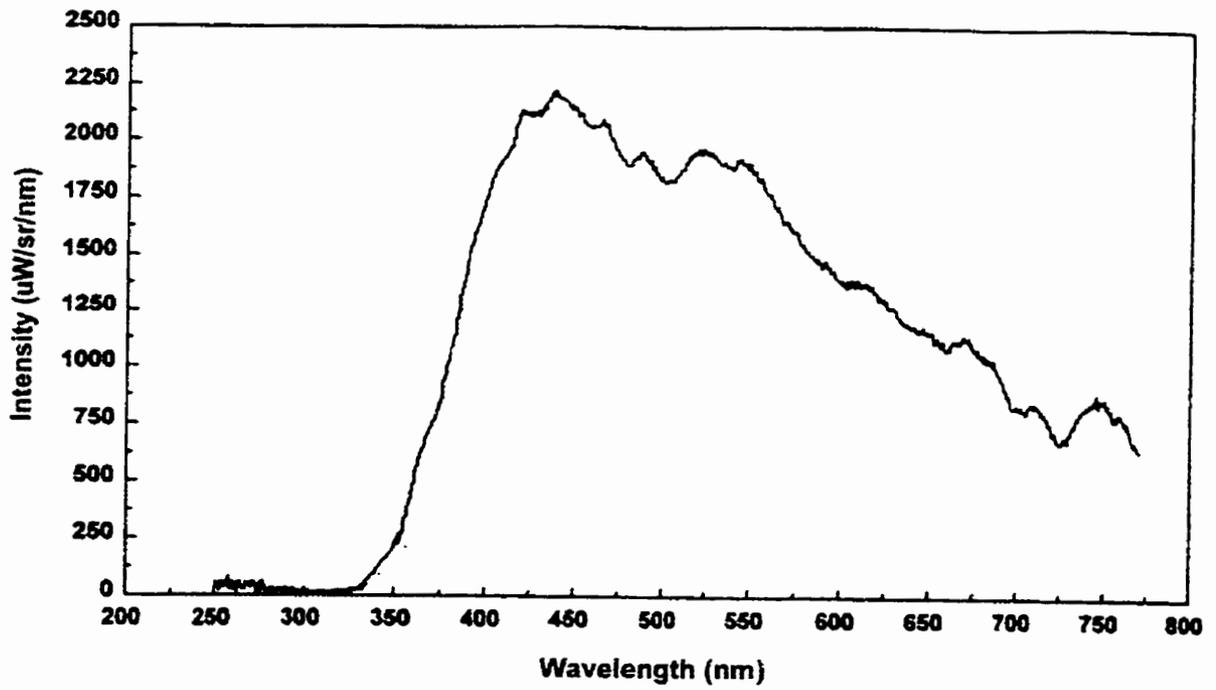
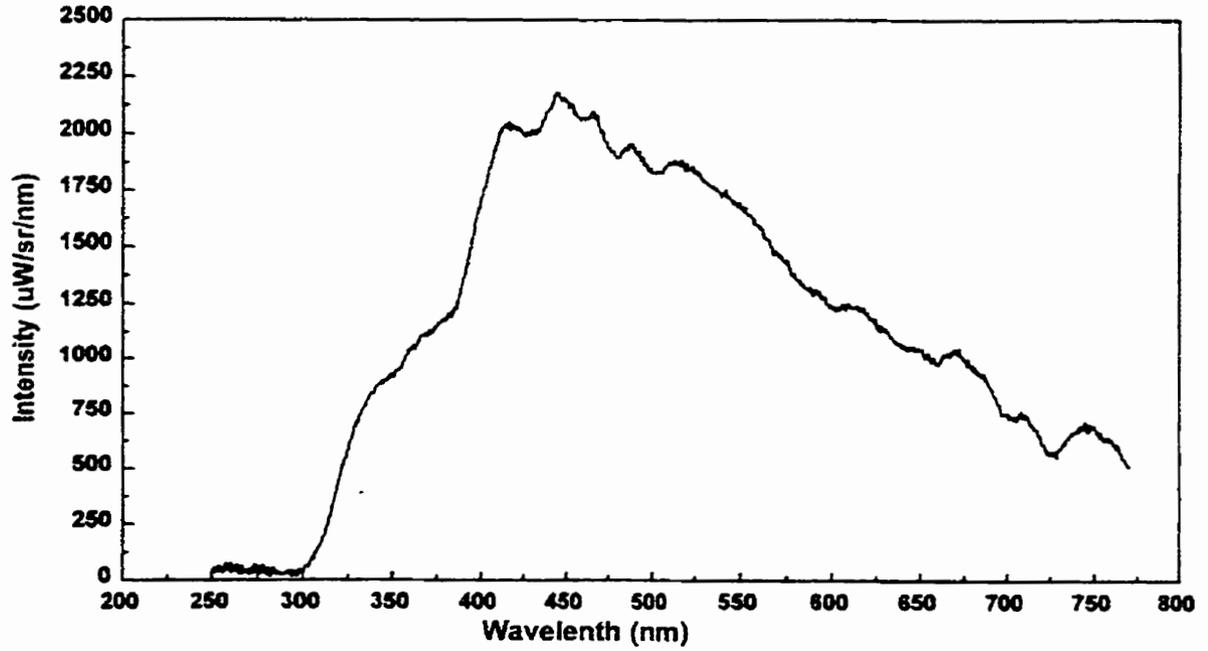
All three colour receptors in honey bees have been found to be important for large area movements (Kaiser and Liske, 1974; Kaiser *et al.*, 1977), as well as foraging behaviour (Thompson, 1995).

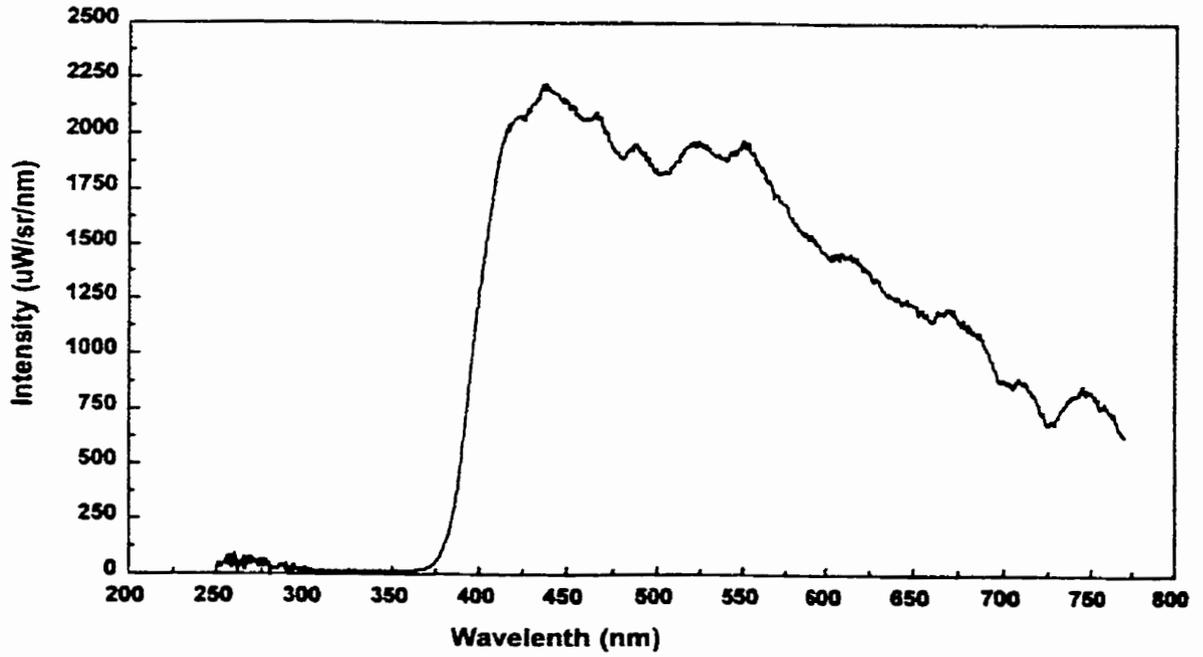
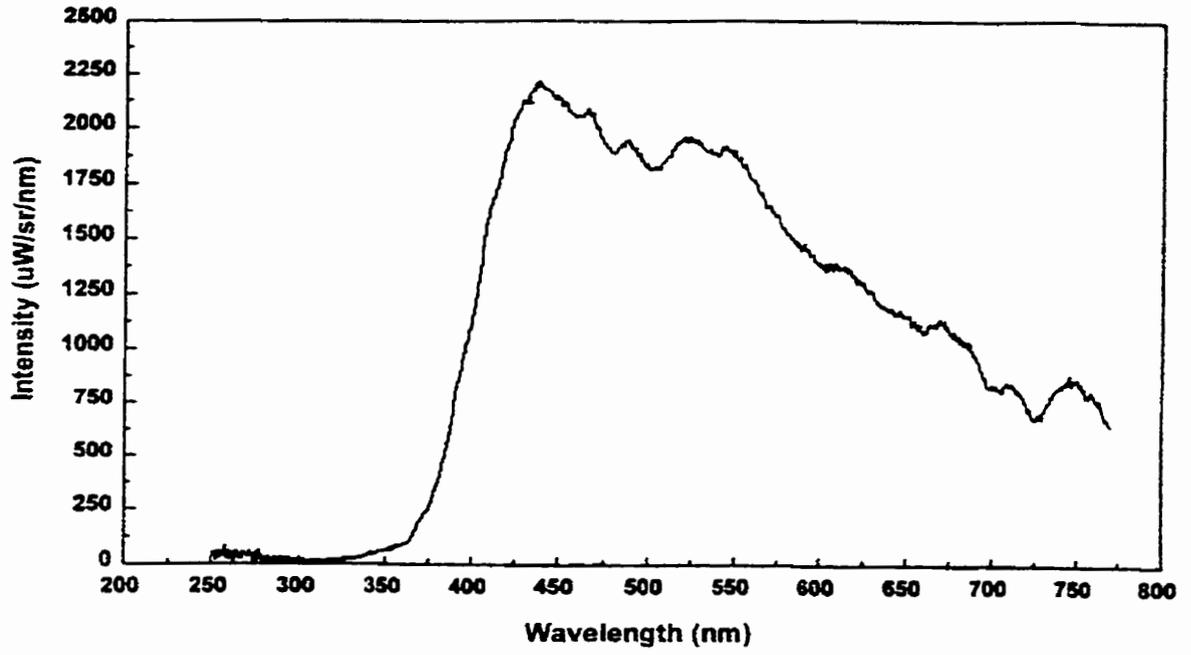
2.1.3 Types of plastic

In southern Ontario, various greenhouse plastics are used by commercial tomato growers, that differ in intensity and spectrum of light transmission (Fig. 2.2). Because of the expense of bee colonies, it is important to determine how plastic type affects bee activity and bee loss. Because bees use UV light,

Figure 2.2. a. Spectral distribution of daylight. b.-f. spectral distribution of light transmitted through five commercial greenhouse plastic types in the Leamington, Ontario area. b. AT Bee plastic, c. CT plastic, d. De Klerk plastic, e. Patilux plastic, f. Dura 3 plastic. Spectral distributions were recorded using an LI-1800 portable spectroradiometer.







plastics that transmit more of the UV range may result in greater levels of activity. High levels of UV light within a greenhouse may attract more bees to leave the colony, or enable the bees to better orient and navigate, resulting in shorter, more efficient foraging trips. Variation in intensity of transmission and wavelengths transmitted by the plastics may also cause differences in internal temperature and humidity, which may affect bee activity, because bee activity increases with increasing temperatures (Lundberg, 1980—bumble bees; Abrol, 1990—alfalfa-pollinating bees; Armbruster and McCormick, 1990—euglossine bees; Corbet et al. 1993—honey bees and bumble bees; Abrol, 1998—alfalfa-pollinating bees).

Loss of bees through ventilation systems may also be affected by greenhouse plastics. In greenhouses with plastics that transmit large amounts of UV light, there will be little visual contrast between the open vents and the rest of the roof. In greenhouses with UV blocking plastics, there will be a large visual contrast between the light coming through the open vents and the light coming through the rest of the roof.

The purpose of our experiments was to assess how plastic type affects loss of bees, and how plastic type and other internal and external greenhouse conditions affect the activity of bees. Factors that were investigated in relation to average daily activity per bee included, external light levels, greenhouse temperature and humidity, and greenhouse plastic type. Because bees see within the ultraviolet range, it was expected that plastics that transmit high amounts of UV light would be associated with greater bee activity levels. It was

also believed that daily ambient sunlight levels and internal greenhouse temperature would be positively correlated with bee activity. Because UV light acts as a positive phototaxis for bees (Berthoff, 1931; Kaiser et al., 1977; Menzel, 1979; Menzel and Greggers, 1985), it was expected that there would be greater loss of bees under plastics that transmitted less UV light. Daily activity patterns within greenhouses were also assessed.

2.2 MATERIALS AND METHODS

2.2.1 Study area

The greenhouses used in this study were chosen in conjunction with Shalin Khosla, the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) greenhouse crop advisor at the Harrow Research Station. All greenhouses in this study were in the Leamington, Ontario area and were devoted to growing tomatoes. Environmental conditions, such as temperature and humidity, were kept within standards optimal for tomato growth and were vented using 'gutter venting'. Gutter vents are hinged openings that extend the length of the roof of the greenhouse. Vent operation was computer controlled, being triggered when the internal greenhouse humidity or temperature reached a specific set value. None of the greenhouse vents were screened, and as a result, bees were able to exit the greenhouse when vents were open. The greenhouses were constructed of various plastic types and ranged in size from 6.4 to 32.1 hectares.

Beefsteak tomatoes (*Lycopersicon esculentum* Mill. (Solanaceae)) were grown in all greenhouses, with Trust and Grace cultivars being the most common. *Bombus impatiens* was used exclusively in all greenhouses and colonies were located along the center walkways within all of them.

2.2.2 Colonies

Colonies of *B. impatiens* were obtained from two supply companies, Koppert Biological Systems Canada, and Biobest Canada Ltd. Colonies from both suppliers were housed in plastic containers approximately 20 x 28 x 18 cm, surrounded by an outer cardboard casing. Upon delivery, each colony contained approximately 60 to 90 workers and one queen. Colonies were used for no more than two months, after which they began to produce male bees and new queens. Queens and males or, 'reproductives' do not forage for the colony; consequently, colonies at this stage are no longer useful for greenhouse pollination. Because tomato flowers do not produce nectar, colonies were equipped with a bag containing a nectar substitute. Foragers moved in and out of the colony through a hole, 1.5 cm in diameter. Experimental colonies were fitted with an electric powered photodiode tunnel and monitor (herein referred to as a 'bee counter') designed to count bee movements in and out of the colony (Fig. 2.3). Bee counters were designed and constructed for this experiment by E. Praetzel.

2.2.3 Commercial greenhouse experiments

Within the commercial greenhouses three response variables were examined: average daily activity (mean number of trips per bee per day), daily activity patterns (mean number of trips per bee per period of day), and bee loss.

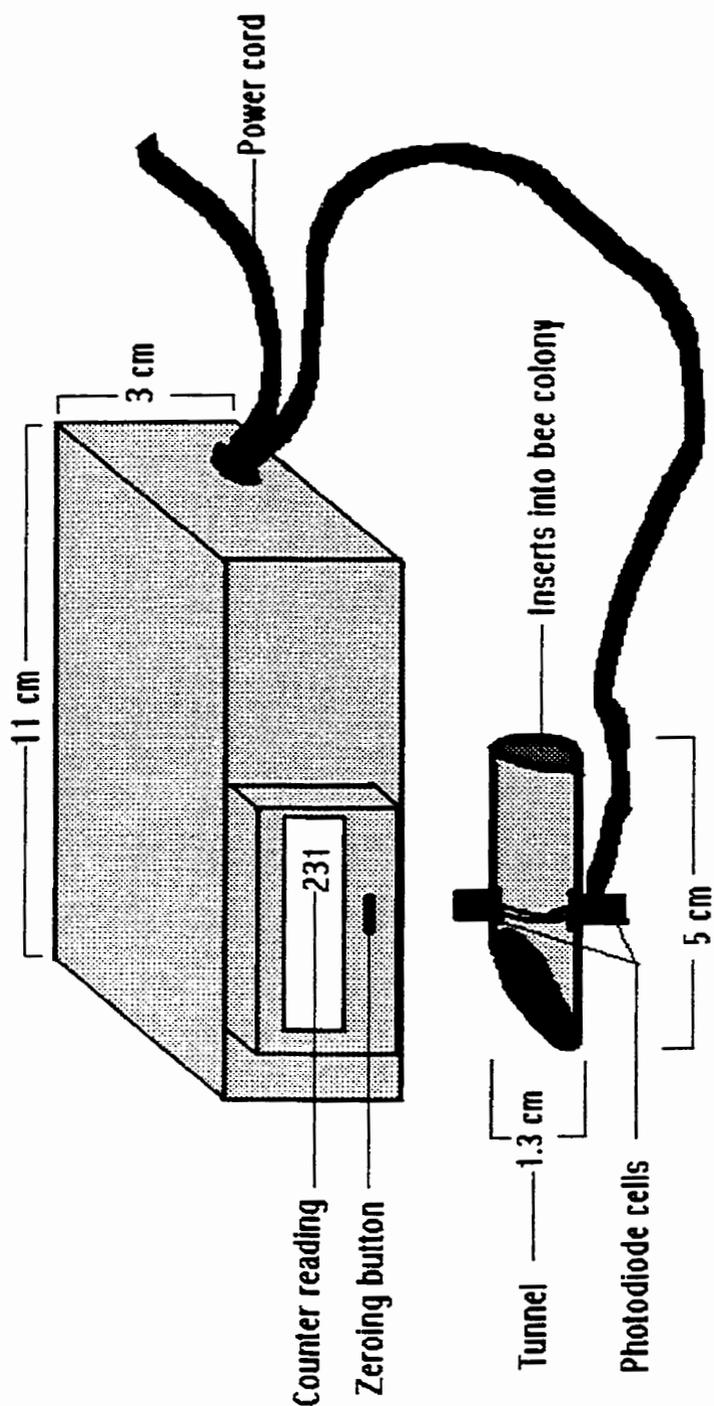


Figure 2.3. A 'bee counter' consisting of an activity monitoring unit which records the number of bee movements across photodiode cells. The photodiode cells are embedded within a tunnel which is inserted into the bumble bee colony entrance.

Average daily activity was assessed in relation to four predictor variables; plastic type, ambient sunlight, internal temperature, and internal humidity. Daily activity patterns were assessed in relation to the time of the day. Bee loss was measured in relation to type of plastic.

2.2.3.1 General experimental setup

Plastic types were grouped into four categories: 1. Patilux (Pati Corporation, Armin Films), 2. AT Bee Poly (AT Plastics) and De Klerk (The Klerk's Group), 3. AT Duratherm 3 (AT Plastics), and, 4. CT (Huntsman Films). AT Bee Poly and De Klerk. AT Bee Poly and De Klerk were grouped together based on similar light transmission spectra. Four plastic types were chosen because they are among the most common types of plastic used in the Leamington area. CT plastic was chosen because of its high UV transmittance and verbal reports of high bee activity under this type of plastic. Five bumble bee colonies were monitored in each of the four greenhouses, at one time. All greenhouses oriented in a north-south direction. Only greenhouses that were greater than 6.4 hectares were used, and with the exception of one greenhouse, all used only one type of plastic. In the case of the first set of four greenhouses, the ATBee plastic and the Dura 3 plastic were in the same greenhouses, although the monitored colonies for the two different plastic types were separated by 200 m. CT plastic was used in only one greenhouse in the study area, so all CT activity readings are from the same site. We placed experimental colonies into different sections of the CT greenhouse for each of the three sets of experiments.

Bee counters recorded all movements within the tunnel, including activity of 'guard bees' at the nest entrance. Thus, it was necessary to calibrate the counters for the actual number of foraging trips. A regression line was generated relating counter values to actual observed bee entrances and exits by observing 23 colonies for 10 minute intervals in various commercial tomato greenhouses, and regressing the actual number of bee entrances and exits on the counter number. The counter values for each day were then transformed using the regression equation to estimate the actual number of entrances and exits per colony, per day. This value was then divided by two, in order to estimate trips per colony per day. The number of trips per colony per hour was divided by the number of bees per colony, giving activity in mean trips per bee per day.

2.2.3.2 Activity

a. Plastic type

Three sets of four greenhouses, one from each of the plastic groups, were monitored for four days each. This was replicated three times, for a total of three rounds of collection, resulting in a balanced design with a total of 36 activity readings for each plastic group.

To begin monitoring, five unopened colonies were placed within each of the four greenhouses. The number of bumble bees per colony was recorded prior to opening, and all 20 colonies were fitted with a bee counter and opened on the same day.

After opening, colonies were left for a three to 10 day acclimation period. After sunset, in the evening prior to the monitoring day, number of bees per

colony was visually estimated and, bee counter tunnels were cleaned and set to zero. The following evening, after sunset, counter numbers were recorded.

New colonies were put into the greenhouses after the second round of data collection, using the same procedure as when the initial colonies were introduced. Repeated measures ANOVA was used to determine if activity was different among the four plastic groups, followed by Tukey's pairwise comparison test. Interactions between day, plastic and experimental round were assessed. In all experiments, significant differences among means were detected using $\alpha < 0.05$.

b. Other predictor variables

Bee activity was also assessed in relation to factors other than type of plastic. The average daily number of trips per bee from the above experiment was regressed on total daily radiation external radiation (mJ/m^2) recorded at the Harrow weather station. Because all greenhouses were within the Leamington Ontario area, one daily radiation value was used for all of the greenhouses.

The temperature and humidity within a CT and a Patilux greenhouse was recorded by the growers, at 5 minute intervals, and the mean daily temperature and humidity was calculated from sunrise to sunset over a two month period. Multiple regression and ordinary least squares regression was used to assess the relationship between bee activity and both temperature and humidity.

2.2.3.3 Daily activity

This experiment was designed to assess activity patterns throughout the day. The average number of trips per bee per period of the day was calculated

for the morning (~6:00am – 10:30am), midday (~10:30am – 3:00pm), and evening (3:00pm – 7:30pm). The experimental design was the same as when average trips per bee per day was estimated, but in this case, mean trips per bee per period of the day was calculated. For the duration of this study, March 24 to May 1, 6am to 7:30pm was an approximation of sunrise to sunset. Time periods were adjusted slightly over the five weeks to compensate for increasing day length. The three time periods were always of the same length on the same day. Average number of trips per bee per period was log transformed, and ANOVA was used to test for significant differences among means.

2.2.3.4 Loss of bees

The purpose of this experiment was to quantify bee loss in relation to type of plastic. The actual number of bees lost through the ventilation systems could not be directly assessed, hence, colony size was used as an indicator of the number of bees lost. Bee loss is only a problem during the winter months when temperatures are too cold for the bees to survive. In the summer, bees escaping through the vents are able to return to the colonies. Data was collected for the bee loss experiment from March 7 to March 31, 1998. By April, temperatures were warm enough for bees to survive outside.

The number of bees in each colony was estimated using a visual count prior to the colonies being opened. Colony size was then monitored periodically throughout the activity experiment. Seven bee counts were made in two sets of greenhouses over two, 10 day periods. Mean proportion of bees remaining in the five colonies was arcsine square root transformed and repeated measures

ANOVA was used to test for differences among plastic types, followed by Tukey's pairwise comparison test.

2.3 RESULTS

2.3.1 Activity

The counter value and the actual number of bee entrances and exits were highly correlated ($r^2 = 0.85$; $F_{24} = 129.75$; $P < 0.0001$). The regression equation used to transform the counter numbers into actual entrances and exits was, $Y = 2.704 + 0.2896x$ (Fig. 2.4).

a. Plastic type

Repeated measures ANOVA showed that the mean number of trips per bee per day differed among the four plastic types ($F_{3,20} = 5.28$, $P = 0.008$), and among experiments (greenhouse groups) ($F_{2,20} = 5.86$, $P = 0.01$). Other main effects and interactions were not significant. Pairwise comparisons between plastic types showed that bees in plastic 4 (CT) took on average 94.0% more trips per day than bees under the other 3 plastic types (Fig. 2.5). There was no difference in activity among the other 3 plastic types.

b. Other predictor variables

There was no correlation between average daily trips per bee and total daily radiation ($r^2 = 0.002$, $df = 107$, $P = 0.59$) (Fig. 2.6).

Multiple regression showed that activity was positively correlated with both temperature and humidity in the two greenhouses ($F_{2,27} = 5.16$, $P = 0.013$). There was only a very weak relationship between bee activity and humidity ($r^2 = 0.02$,

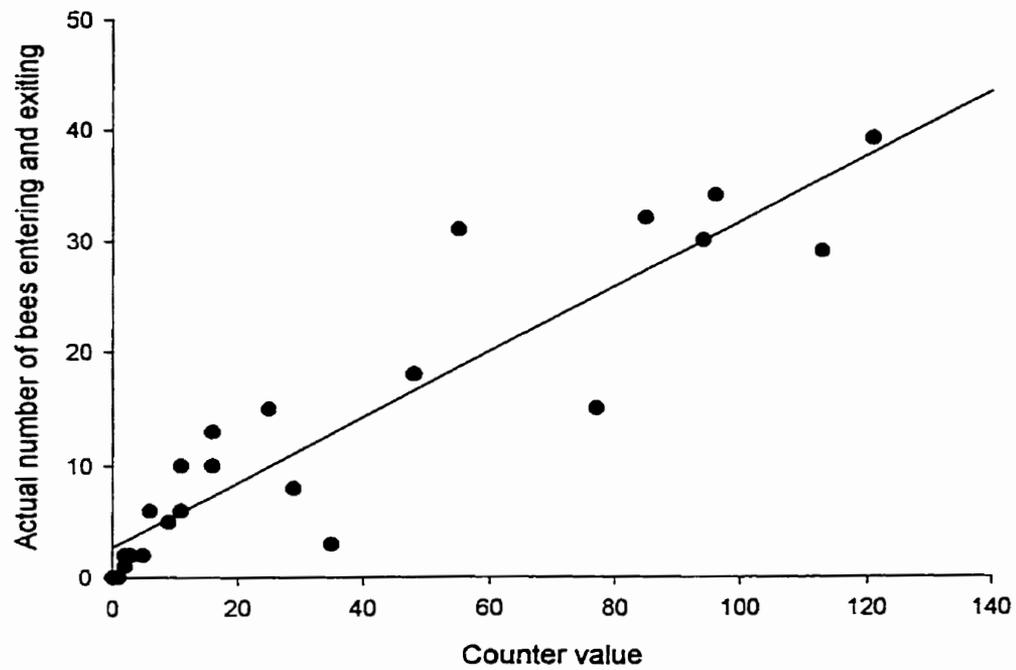


Figure 2.4. The number of bee entrances and exits from bumble bee colonies in commercial tomato greenhouses regressed on the counter value recorded by a bee counter used to monitor activity.

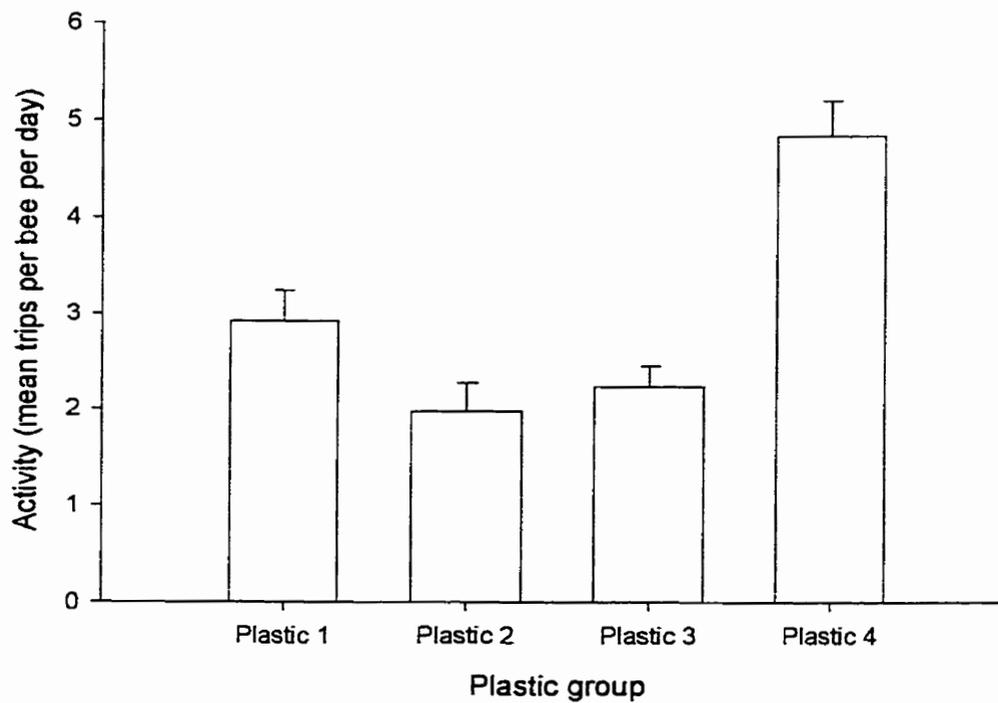


Figure 2.5. Mean bumble bee activity measured at the hive entrance of 5 colonies under four different commercial greenhouse plastic groups 1. Patilux, 2. AT Bee and De Klerk, 3. Dura 3, and 4. CT (n = 35).

$F_{1,28} = 0.51$, $P=0.481$) (Fig. 2.7). Bee activity was positively correlated with average daily internal greenhouse temperature in the two greenhouses ($F_{1,28} = 5.98$, $P=0.021$, $r^2 = 0.18$) (Fig. 2.8). Within the temperature ranges observed for these analyses (20°C to 28°C), the best fit relationship was a linear model, with no apparent decrease in activity at the upper temperature ranges. For the days used in the analysis, April 28 to May 26, the average daily temperature in the CT greenhouse was 6.3% higher than the Patilux greenhouse ($F_{1,28} = 6.79$, $P=0.015$). Activity was 129.3% higher in the CT greenhouse over the same time period ($F_{1,28} = 14.92$, $P=0.001$).

2.3.2 Daily activity

No difference in bee activity was found between the three periods of the day ($F_{3,156} = 0.10$, $P=0.977$) (Fig. 2.9), and there was no interaction between time period and plastic type ($F_{6,156} = P=0.774$).

2.3.3 Loss of bees

There was a difference in bee loss among plastic types ($F_{3,4}=16.29$, $P=0.010$). The CT plastic experienced less bee loss than the other 3 plastic groups ($P=0.004$, $P=0.004$, $P=0.010$ for CT compared to plastic types 1, 2 and 3 respectively). The mean number of bees remaining after 10 days in the CT plastic group was 91.3 bees per colony, 111% greater than the mean number remaining in the other three plastic groups.

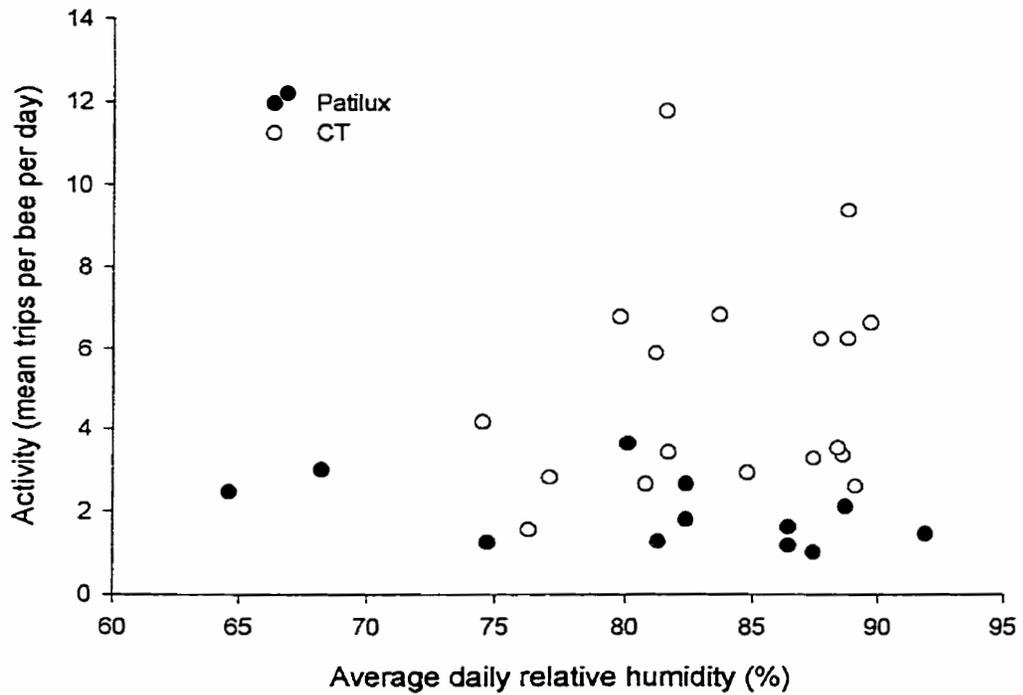


Figure 2.7. Mean daily bumble bee activity measured at the hive entrance of five colonies in two commercial tomato greenhouses in relation to the mean internal greenhouse daily relative humidity (n = 30).

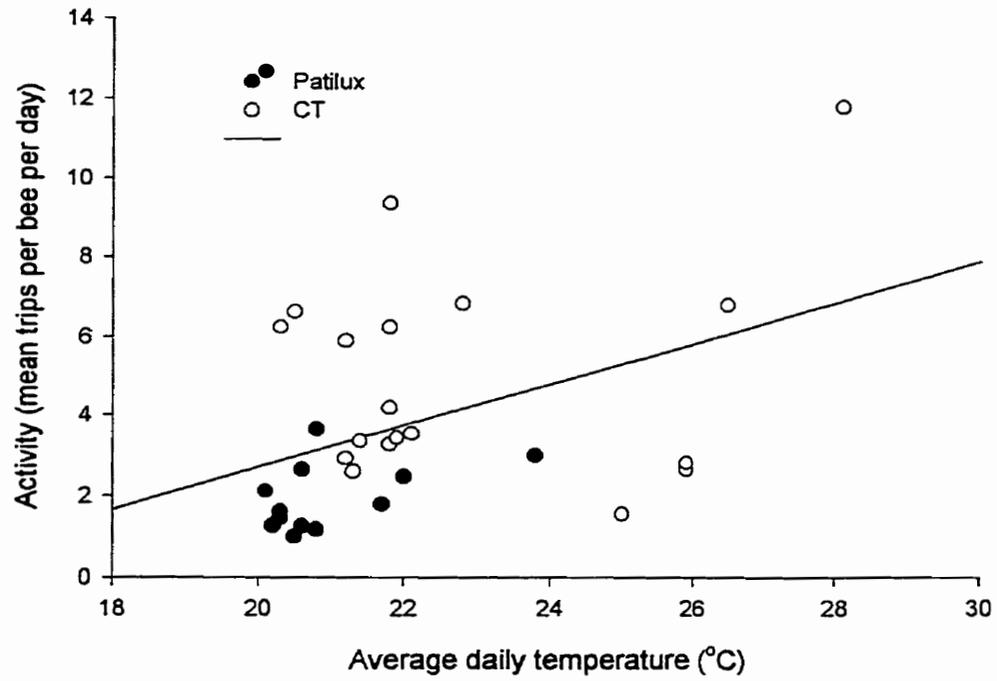


Figure 2.8. Mean bumble bee activity measured at the hive entrance of five colonies in two commercial tomato greenhouses in relation to the internal greenhouse daily mean temperature ($n = 30$).

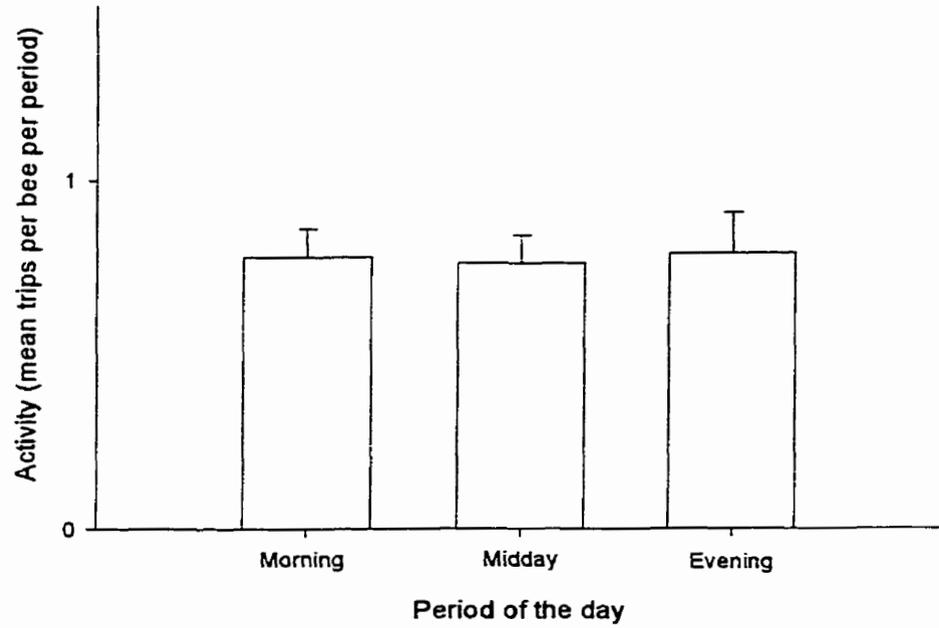


Figure 2.9. The mean bumble bee activity under all plastic types measured at the hive entrance of five colonies over three, four day periods during three periods of the day; morning (~6:00am – 10:30pm), midday (~10:30am – 3:00pm), and evening (~3:00pm – 7:30pm) (n = 48).

2.4 DISCUSSION

This study did not support the findings of previous research showing that bee activity is positively correlated with light intensity (Lundberg, 1980—bumble bees; Abrol, 1990—alfalfa-pollinating bees; Armbruster and McCormick, 1990—euglossine bees; Abrol, 1998—alfalfa-pollinating bees). It may be that during the daytime hours of this study, light intensity within the greenhouse was adequate for flight, regardless of the external conditions. The low activity with low ambient light levels found in other studies may have been caused in part by a positive correlation between light levels and temperature because these studies were conducted outside.

In all greenhouses, temperatures were kept consistently above 19°C, well above the 5°C required for bumble bee flight (Heinrich, 1979). Within the greenhouses, light levels were adequate for flight soon after sunrise (personal observation). Throughout the day, pollen was assumed to be in excess because of the large numbers of open tomato flowers that remained unpollinated. The combined effect of consistently high temperatures and adequate light levels throughout the day, along with excess pollen, may have resulted in consistent levels of bumble bee activity throughout the three periods of the day.

Extensive studies by Williams (1940; 1961) and Williams and Osman (1960), on insect activity using trap catches showed that activity increased gradually to a maximum at 29°C; further temperature increases to 34°C resulted in a negative correlation with activity. Many studies have found that bees become more active with increasing temperatures (Lundberg, 1980—bumble

bees; Abrol, 1990—alfalfa-pollinating bees; Armbruster and McCormick, 1990—euglossine bees; Corbet et al. 1993—honeybees and bumble bees; Abrol, 1998—alfalfa-pollinating bees). A positive correlation between temperature and activity may be a result of decreased thermoregulation costs as temperature increases from 5 to 25°C (Heinrich, 1979). Although few studies have looked at levels of bee activity under very high temperatures, it is believed that they may cease flight (Chappell, 1982—carpenter bees), or counter overheating through evaporative cooling (Heinrich, 1979—honeybees). Chen and Hsieh (1996), examined bumble bee activity in tomato greenhouses, and found that pollination activity was reduced in the summer as a result of extreme temperatures (up to 40°C). In commercial greenhouses, the highest average daytime greenhouse temperature was 28°C. Because there was no decreased activity up to this point, the observed greenhouse temperatures were apparently within the bees' acceptable temperature range for foraging.

Greater bee activity in the CT greenhouse supports the hypothesis that bees are most active when exposed to an extended range of ultraviolet light. Although the hypothesis is supported, the higher internal temperatures in the CT greenhouse may have been partially responsible for the greater activity observed. However, it is unlikely that the 6% higher temperature in the CT greenhouse could have been solely responsible for the 4-fold increase in activity in the CT greenhouse over the Patilux greenhouse during the temperature and humidity experiment. The higher temperature along with greater amounts of UV

light may both have been factors resulting in the greater activity observed in this greenhouse.

As predicted, CT plastic had the smallest bee loss, as evident through consistently large colony sizes. Under this type of plastic, bees may be unable to visually detect the open vents because of the relatively small contrast between the transmitted light and the light through the vents. Because of this, fewer bees fly out of these greenhouses and die during the winter months, and as a result colonies remain large.

Although greenhouses with plastic coverings that transmit more light within the UV range result in greater bee activity and less bee loss, growers must balance various factors when considering which kinds of plastics to install. Increased grey mold (*Botrytis*) spore germination has been associated with plastics that transmit greater levels of ultraviolet (Pearson *et al.*, 1997), making the use of high UV plastics less desirable. Also, greater transmission across the spectrum results in temperatures that are above optimal for tomato growth within the greenhouse during the summer months.

Maximizing bee activity and minimizing bee loss has important economic consequences on bumble bee pollination in greenhouses. Currently, bumble bee supply companies service tomato growers on a supply basis. Greenhouses that experience high bee loss and/or low bee activity require many more colonies than was originally estimated. Consequently, bee suppliers have lost money in some situations, and have changed their billing to a fee per colony basis. For many growers this has resulted in increases in the price for crop pollination. In

order to reduce costs for suppliers and growers, greenhouse environments should facilitate crop pollination as well as crop growth and health. This study indicates that UV transmitting plastics and high average daily temperatures promote colony activity. In addition, UV transmitting plastics result in minimal loss of bees through ventilation systems.

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CHAPTER 3

Bumble bee activity in experimental greenhouses and orientation in a Y-maze

3.1 INTRODUCTION

Bumble bees have become common pollinators of commercial greenhouse tomato crops in southern Ontario (S. Khosla, pers. comm.). Although they are effective pollinators of tomatoes (Pinchinat et al., 1982; van der Sande, 1990; van Ravestijn, 1990; Banda and Paxton, 1991; Kevan et al., 1991; Pressman et al., 1999), some growers and bee suppliers are experiencing problems with loss of bees through ventilation systems and insufficient levels of pollination. Variation among greenhouses in the effectiveness of pollination by bumble bees has lead bee suppliers and crop scientists to the question the causes of these differences. Commercial greenhouses are constructed from various plastic types that differ in light transmission spectra, particularly in the levels of ultraviolet (UV) light that are transmitted. Because bees see within UV range, different types of plastic may be responsible for some of the observed variation in activity and loss of bees among greenhouses.

3.1.1 Bee vision

Bumble bees have trichromatic colour vision (e.g. Mazokhin-Porshniakov, 1969; Menzel and Backhaus, 1991; Peitsch et al., 1992) with ultraviolet, blue and green receptors with peak sensitivities of 345 nm, 440 nm, and 550 nm respectively (Kevan and Backhaus, 1998; similar to Peitsch et al., 1992). Trichromatic colour vision spans about 300 nm of natural daylight starting at about 310nm (Kevan and Backhaus, 1998). It has been shown that sensitivity to UV in honeybees and other insects is 4 to 6 times stronger than to other parts of

the spectrum, suggesting that UV is an important component of insect vision (Kevan, 1970, 1979, 1983; Laughlin, 1976; Menzel and Backhaus, 1991).

Various studies have shown that ultraviolet light produces a positive phototactic response in honeybees (Bertholf, 1931; Kaiser et al., 1977; Menzel, 1979; Menzel and Greggers, 1985) and Diptera (Fischbach, 1979; Kevan, 1979). It is believed that insects use UV light as an indicator of an open environment (von Hess, 1913; Laughlin, 1976—dragonflies; Menzel and Greggers, 1985).

In addition, UV light may be an important component of bumble bee orientation. As light passes through the atmosphere, the rays become scattered, resulting in partial polarization of the light. The result is a symmetrical ray pattern that is fixed with respect to the solar meridian (Brines and Gould, 1982). The polarization pattern of the sky may be used as a celestial compass even under cloudy conditions (Brines and Gould, 1982). Frisch's work (see von Frisch, 1965 for review) showed that honey bees use polarized light as a means of orientation and navigation. With increasing wavelengths, the polarized pattern becomes subject to atmospheric disturbance (Rozenberg, 1966), and consequently, it is believed that bees primarily use polarized light within the UV range for orientation. Von Helversen and Edrich (1974) tested the sensitivity of honeybees to different wavelengths of polarized light, and found a maximum sensitivity at 345 nm, and determined that polarized light was detected exclusively by the UV receptors.

3.1.2 Types of plastic

Because UV light is used for orientation, navigation, and elicits an escape phototaxis in bees, plastics that transmit more of the UV range may result in greater levels of bee activity. High levels of UV light within a greenhouse may attract more bees to leave the colony, or enable the bees to better orient and navigate, resulting in shorter, more efficient foraging trips. Variation in intensity of transmission and wavelengths transmitted by the plastics may also cause differences in internal greenhouse temperature, which may affect bee activity, because bee activity increases with increasing temperatures (Lundberg, 1980—bumble bees; Abrol, 1990—alfalfa-pollinating bees; Armbruster and McCormick, 1990—euglossine bees; Corbet et al. 1993—honey bees and bumble bees; Abrol, 1998—alfalfa-pollinating bees).

The escape response caused by UV light may also contribute the large levels of bee loss through greenhouse ventilation systems. The majority of tomato greenhouses in the Leamington, Ontario area are vented using 'gutter vents', which are hinged openings that extend the length of the roof of the greenhouse. Vent operation is computer controlled, being triggered when the internal greenhouse humidity or temperature reaches a set value. In most of the gutter vented greenhouses, the vents are not screened, so that, bees are able to exit the greenhouse when vents are open. In greenhouses with UV-transmitting plastics, there is little visual contrast between the open vents and the rest of the roof. In greenhouses with UV-blocking plastics, there is a large visual contrast between the light coming through the open vents and the light coming through

the rest of the roof. This difference in contrast with daylight may be a factor in explaining why bee loss is a serious problem in some greenhouses but not others.

The purpose of this study was to examine the effect of different types of greenhouse plastic on bee activity and loss in a controlled setting. To do this, we conducted two experiments. In the first experiment, we constructed miniature greenhouses from four different types of greenhouse plastic to quantify bumble bee activity based on plastic type. Because bees see within the ultraviolet range, we hypothesized that UV-transmitting plastics would be associated with greater activity levels of bees at the hive entrance. The second experiment was designed to simulate a greenhouse setting, in which the bumble bees have the choice of either orienting towards (and going out) open vents, or, of staying within the greenhouse. Because UV light acts as a positive phototactic cue for bees (Berthoff, 1931; Kaiser et al., 1977; Menzel, 1979; Menzel and Greggers, 1985), it was expected that greenhouses with UV-blocking plastics would experience more loss of bees than UV-transmitting plastics. The purpose of this experiment was to test the response of bees to a situation where they could either move towards an area with direct light, or towards an area with light that had been transmitted through a greenhouse plastic. We expected that the choice of bees would be different for UV-blocking plastics and UV transmitting plastics. We hypothesized that for UV-blocking plastics, bumble bees would be attracted to direct light more often than towards an area where the light was transmitted through plastic. For UV-transmitting plastics, we expected that bees would

choose areas with light transmitted through plastic and areas with direct light at equal frequencies. If this were so, it would help to explain some of the variation in bee loss among greenhouses with gutter ventilation systems.

3.2 MATERIALS AND METHODS

3.2.1 Colonies

Colonies of *B. impatiens* were obtained from two supply companies, Koppert Biological Systems Canada, and Biobest Canada Ltd. Colonies from both suppliers were housed in plastic containers approximately 20 x 28 x 18 cm, surrounded by an outer cardboard casing. Each colony contained approximately 60 to 90 workers and one queen. Because tomato flowers do not produce nectar, a required food for bees, colonies were equipped with a bag containing a nectar substitute. For our experiments, we removed the nectar bags, so that colonies would forage on sugar solution. Pollen, a required food for bee larvae was put directly into the colonies daily.

3.2.2 Miniature greenhouse experiment

Four 2.4 x 1.2 x 1.8 m experimental greenhouses with peaked roofs were constructed, each covered with a different type of plastic attached to a wooden frame (Fig. 3.1). The greenhouses had hinged, north-facing doors,

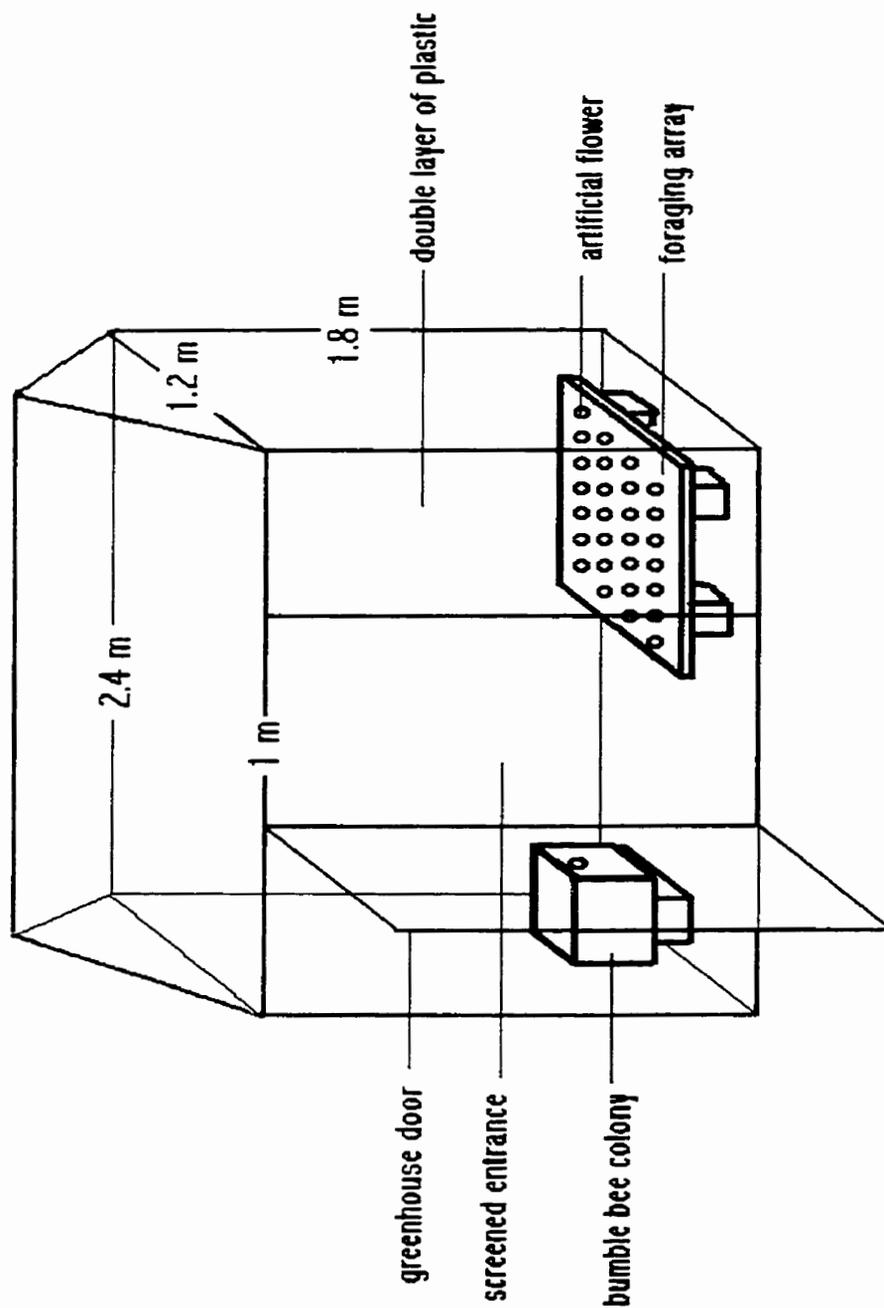


Figure 3.1. Miniature greenhouse design housing a bumble bee (*Bombus impatiens*) colony and artificial foraging array. Bumble bees foraged on artificial flowers made from centrifuge tubes. There were approximately 80 artificial flowers on each array. The greenhouse frame was constructed of wood, and covered with a double layer of greenhouse plastic.

approximately 1 m across. In addition to the plastic, door openings were covered with 2 mm mesh screening in order to reduce loss of bees when doors were open. The greenhouses were aligned east to west, and were arranged such that they did not shade one another. The four types of greenhouse plastic used were; ATBee, Deklerk, CT and Patilux (Transmission spectra shown in Chapter 2, Fig. 2.2). All greenhouses had a double layer of their respective plastic types, as is standard in commercial greenhouses.

One artificial floral array was placed on the floor of each greenhouse. Each array contained 80 artificial flowers constructed from 1.5 ml clear centrifuge tubes imbedded in styrofoam sheets measuring 70 cm x 70 cm. Flowers were filled daily, and as required, with 30% sucrose solution.

One *Bombus impatiens* colony was put into each greenhouse. The nectar bags were removed prior to the colonies being opened, at which time the colonies were fitted with an electric powered photodiode tunnel and monitor (herein referred to as a 'bee counter') designed to count bee movements in and out of the colony (Chapter 2, Fig. 2.3). In each greenhouse, the colony and the array were raised 30 cm off of the ground to protect the artificial flowers from ants.

The four colonies were numbered and randomly assigned to a greenhouse on each data collection day. Data were collected from October 23, 1999 to November 11, 1999. Colonies were taken out of the greenhouses each evening because of low overnight temperatures. Counter numbers were

recorded for one-hour periods, for a maximum of three periods a day. Numbers of bees per colony was visually estimated by opening the top of the outer casing of the colony each day prior to data collection. Temperatures, (nearest 0.1°C), were recorded prior to each hour of data collection using thermometers suspended in the centre of each greenhouse. On data collection days, if any of the greenhouse temperatures exceeded 25°C, all of the greenhouse doors were opened to increase air-flow and reduce the temperature. If any of the colonies started producing reproductives, all colonies were replaced with new ones.

Bee counters recorded all movements within the tunnel, including activity of 'guard bees' at the nest entrance. Thus it was necessary to calibrate the counters for the actual number of foraging trips. A regression line was generated relating counter values to actual observed bee entrances and exits by observing 23 colonies for 10 minute intervals in various commercial tomato greenhouses, and regressing the actual number of bee entrances and exits on the counter number. The counter values for each one-hour period were then transformed using the regression equation to estimate the actual number of entrances and exits per colony, per hour. This value was then divided by two, in order to get trips per colony, per hour. The number of trips per colony, per hour was divided by the number of bees per colony, giving activity in trips per bee, per colony, per hour. Activity based on plastic type was analyzed using an ANOVA. Activity was also analyzed using an ANCOVA with temperature as the covariate. Because CT plastic transmits more UV light than the other plastics, we predicted that bee activity would be greater under this plastic.

3.2.3 Y-Maze experiment

The experiment was conducted using a Y-maze, constructed from transparent, ultraviolet-transmitting Plexiglas, with two tunnels branching from a decision chamber (Fig. 3.2). An ultraviolet light and a fluorescent light were placed behind the two back panels of the maze. A black plastic covering was placed over the entire maze, except for the two back panels. Bees entered the decision chamber through an aperture (2.5 cm in diameter) connected to the hive by a short length of wire mesh tubing. There was a gating system within the tubing, which allowed bees to be tested one at a time.

The colony was trained to forage on a 30% nectar solution placed in Petri dishes at the back of the box, on both sides. Equal nectar rewards were placed on both sides of the box. After the colony had been trained to forage on both sides of the Y-maze, one of the five plastic types; AT Bee, CT, De Klerk, Patilux, or Dura 3 (Transmission spectra shown in Chapter 2, Fig. 2.2), was placed over one of the back panels. A bee was then released into the maze, and recorded as having chosen one side or the other after crossing one of the decision lines (see Fig. 3.2). The bee was then captured and kept in a separate container until the experiment was completed. The number of bees tested per experiment depended on the number of available foragers. The experiment was conducted four times for each plastic type, with between 10 and 20 bees each time. The plastic side was switched half way through each experiment in order to minimize

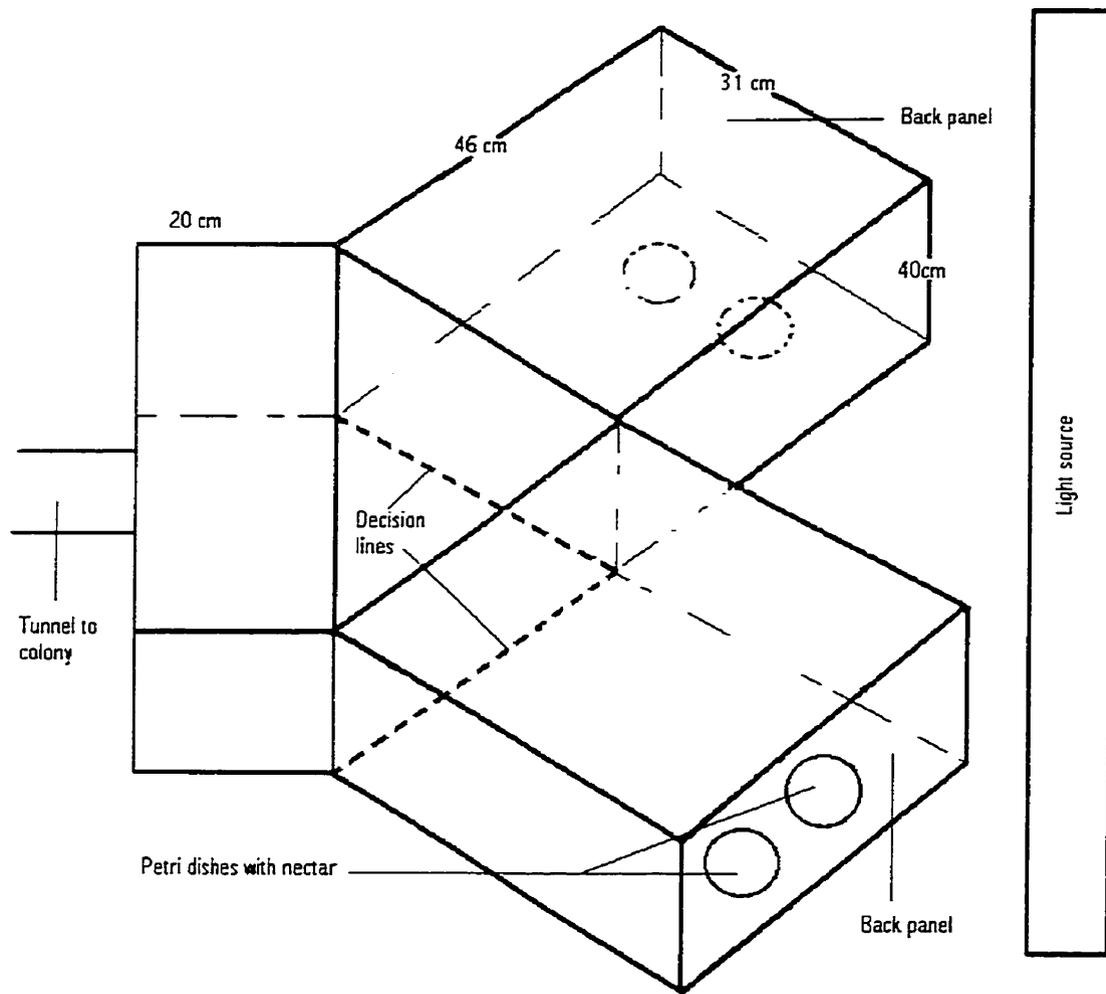


Figure 3.2. Y-Maze used in the bumble bee decision experiment. The entire box except for the two back panels was covered in black plastic.

the influence of a side bias. We tested to determine if the bees showed a bias towards the tunnel with no plastic. If they could not discriminate between the plastic tunnel, and the tunnel with no plastic, we expected a 50:50 distribution. Data from each type of plastic was analyzed using a 1 x 2 Chi-square test with an expected distribution of 50:50. Data from all types of plastic were tested against one another in a 2 x 5 Chi-square. Because the CT plastic transmitted a much larger proportion of UV light than the other plastics, we hypothesized that bee movement using this plastic would be different from the other plastics, and that it would not differ from the expected 50:50 distribution.

3.3 RESULTS

3.3.1 Miniature Greenhouse Experiment

The counter value and the actual number of bee entrances and exits were highly correlated ($r^2 = 0.85$; $F_{24} = 129.75$; $P < 0.0001$). The regression equation used to transform the counter numbers into actual entrances and exits was, $Y = 2.704 + 0.2896x$ (Chapter 2, Fig. 2.4).

There was no difference in activity based on type of greenhouse plastic ($F_3 = 0.28$; $P = 0.837$; Fig. 3.3). When activity was tested with temperature as a covariate, there was no difference in activity based on type of plastic ($F_3 = 0.41$; $P = 0.748$), but the covariate was significant ($F_1 = 12.61$; $P = 0.001$). There was a positive correlation between bee activity and temperature ($Y = 0.582 + 0.003x$; $r^2 = 0.13$; $F_{84} = 12.63$; $P < 0.001$; Fig. 3.4). The average temperatures within the

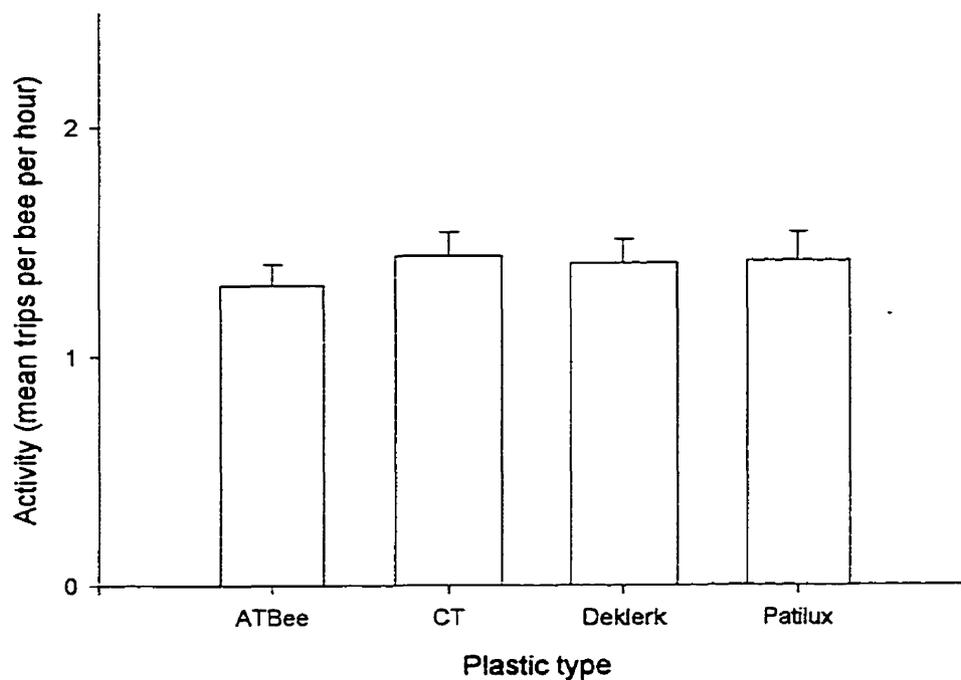


Figure 3.3. Bumble bee activity (measured in trips per bee per hour) in four miniature experimental greenhouses constructed from four different greenhouse plastic types; ATBee, CT, Deklerk, and Patilux.

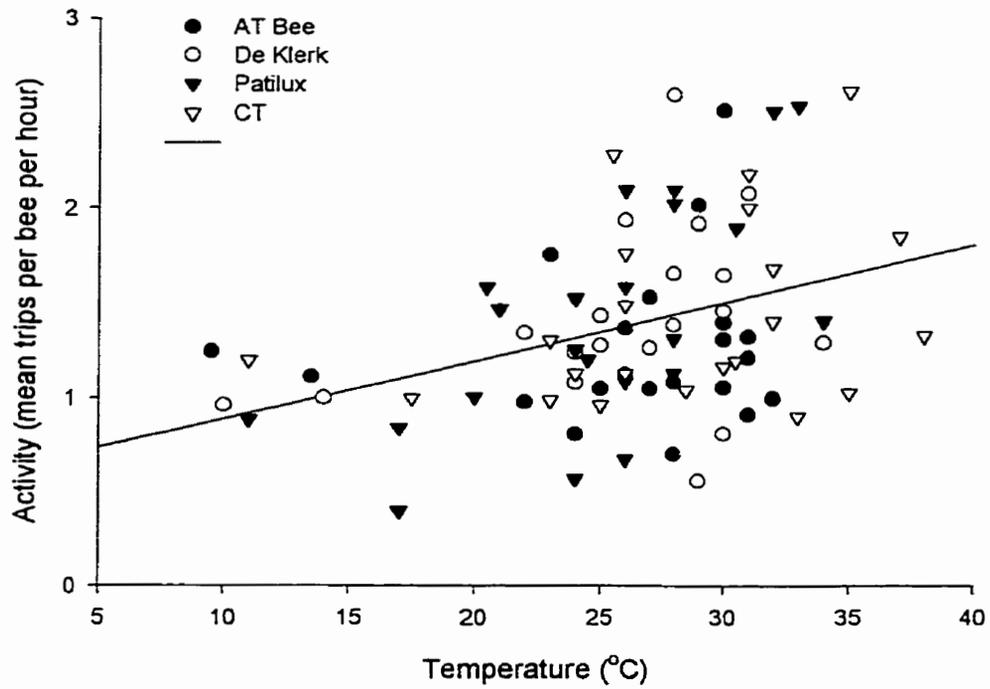


Figure 3.4. The correlation between bumble bee activity (measured as trips per bee per hour) and greenhouse temperature in miniature greenhouses. $n = 85$; $Y = 0.58 + 0.03x$; $r^2 = 0.13$; $p < 0.001$.

greenhouses, averaged across all readings \pm standard error were; ATBee, $26.41 \pm 1.24^\circ\text{C}$; CT, $28.18 \pm 1.24^\circ\text{C}$; Deklerk $26.00 \pm 1.30^\circ\text{C}$; Patilux, $24.93 \pm 1.24^\circ\text{C}$. The average temperatures were not significantly different ($F_3 = 1.19$; $P=0.318$), although the CT greenhouse consistently had the highest average temperature, possibly due to greater UV transmission.

3.3.2 Y-maze experiment

The bees chose the side of the Y-maze without the plastic more often than expected for the Patilux ($X^2_1 = 6.25$; $P=0.01$), ATBee ($X^2_1 = 11.52$; $P=0.001$), and Dura 3 ($X^2_1 = 4.55$; $P=0.035$) plastics. For the CT and the De Klerk plastics, there was no difference in choice based on the presence of plastic ($X^2_1 = 0.02$; $P=0.872$ and $X^2_1 = 3.75$; $P=0.055$ respectively) (Fig. 3.5). 52.5% of the bees chose the CT plastic side of the maze. Although not significantly different from a 50:50 distribution, only 39.0% of the bees chose the De Klerk plastic. There was a difference in the number of bees that chose the plastic side versus the non-plastic side among plastic types ($X^2_4 = 12.02$; $P=0.017$). A greater number of bees went towards the CT plastic than any of the other types of plastic ($X^2_1 = 7.38$; $P=0.007$).

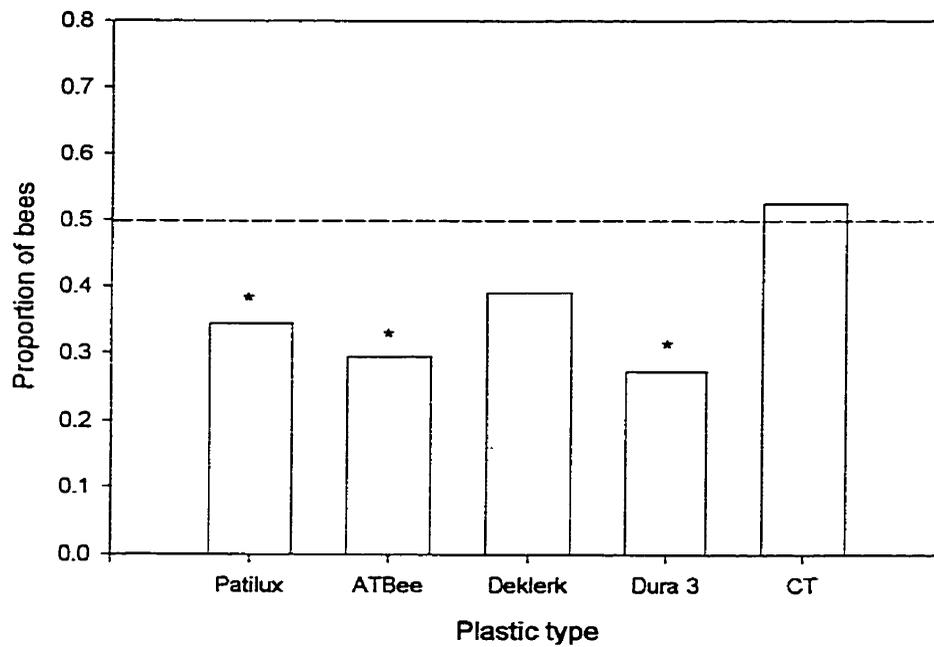


Figure 3.5. The proportion of bumble bees that chose the plastic side of a Y-Maze decision box.

*Chi-square is significant from the expected distribution of 50:50 at $p < 0.05$.

3.4 DISCUSSION

3.4.1 Miniature greenhouse experiment

The miniature greenhouse experiment did not support our hypothesis that there would be more bee activity under the plastic that transmitted the most ultraviolet light. This finding was contrary to data collected from commercial tomato greenhouses (Chapter 2), perhaps because the miniature greenhouse design was not sufficient to detect differences in bee activity based on light transmission, or, the differences in bee activity in commercial tomato greenhouses are a result of factors other than type of plastic. Within commercial greenhouses, the bees must navigate through large areas, using many different visual stimuli in order to find tomato flowers. In the miniature greenhouses, the foraging arrays were only about 100 cm from the colonies and were made up of a high density of artificial flowers, with little visual distraction among them. It would be most beneficial if further studies on the effects of plastic type on bee activity were conducted in large experimental greenhouses, using actual plants rather than foraging arrays.

We found a positive correlation between bee activity and temperature in the miniature greenhouses. Studies by Williams (1940; 1961) and Williams and Osman (1960), showed that activity based on trap catches increased gradually up to a maximum at 29°C; above the optimum, further temperature increases to 34°C resulted in a negative correlation with activity. Studies on bees have found that activity increases with increasing temperature (Lundberg, 1980—bumble bees; Abrol, 1990—alfalfa-pollinating bees; Armbruster and McCormick, 1990—

euglossine bees; Corbet et al. 1993—honeybees and bumble bees; Abrol, 1998—alfalfa-pollinating bees). A positive correlation between temperature and activity may be due to decreasing thermoregulation costs as temperature increases from 5 to 25°C (Heinrich, 1979). In our study, greenhouse temperatures were maintained below 30°C, and the activity increase up to this point followed a linear trend.

3.4.2 Y-Maze experiment

Of the two plastics that did not significantly affect bee movement, the CT plastic had a larger proportion of bees going towards the plastic side than the side with no plastic. Because CT plastic transmits more UV light than the other plastics, the prediction of fewer bees being lost from greenhouses with these plastics is supported. De Klerk plastic shows a transmission spectrum that, while similar to ATBee and Patilux, transmits slightly more UV light. This may explain why the choice of bees with De Klerk plastic in the Y-Maze was marginally close to being significantly different from the expected 50:50 distribution ($P = 0.055$). Significant chi-square analysis of plastics that transmit less UV light, verses CT plastic, indicated that bees chose the plastic side more often when CT plastic was used, than when the other plastics were used.

Within greenhouses, bees experience a situation similar to the Y-Maze simulation—they can either stay within the greenhouse where the majority of light is transmitted through the greenhouse plastic or the bees can escape through the greenhouse ventilation. In the winter, the bees choosing the outside route will likely die before they can make it back to the colony. Our results support the

prediction that plastics that transmit the most light within the UV range will result in less visual contrast between areas with transmitted light and areas with direct light and, consequently, fewer bees are lost through open vents. Various studies have found similar positive phototactic responses in insects to UV light, including honeybees (Berthoff, 1931; Kaiser et al., 1977; Menzel, 1979; Menzel and Greggers, 1985), fruit flies (Fischbach, 1979), and Diptera (Kevan, 1979). Because the high responsiveness to UV light is thought to result from the insects' taking it as indicator of an open environment (von Hess, 1913; Laughlin, 1976— dragonflies; Menzel and Greggers, 1985), greenhouse plastics that transmit more UV light will result in less bee loss through open ventilation systems.

While plastics that transmit more UV light result in less bee loss, the growers must balance various factors when considering which kinds of plastics to install. Increased grey mold (*Botrytis*) spore germination has been associated with plastics that transmit high levels of ultraviolet (Pearson *et al.*, 1997), making the use of UV transmitting plastics less desirable than UV blocking plastics. Also, greater transmission across the spectrum may result in temperatures that are above optimal for tomato growth during the summer months. We recommend that growers using UV blocking plastics should use ventilation systems that prevent bee loss, such as fan ventilation or screened gutter vents.

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CHAPTER 4

Bumble bee activity and pollination levels in commercial tomato greenhouses

4.1 INTRODUCTION

In southern Ontario, manual pollination of commercial tomato crops has been replaced by bumble bee pollination, creating a new multi-million dollar supply business (S. Khosla, pers. comm.). Although the use of bumble bees as greenhouse pollinators has spread rapidly, very little basic research has been conducted to address problems that bee suppliers and tomato growers are having, including inadequate levels of pollination in some greenhouses.

The level of bumble bee activity on a tomato flower is particularly easy to assess. Bin and Sorressi (1973) observed that pollination of tomato flowers by bumble bees caused bruising or necrotic spotting on the anther cone. In European greenhouses, van Ravestijn and van der Sande (1991) assessed the number of tomato flowers that had been pollinated by bumble bees (*Bombus terrestris*) by observing the brown discoloration of the anther cone caused by pollination visits. Although some studies have looked at fruit quality in relation to the number of pollination visits, total duration of pollination visits is a better measure because it is a more accurate indication of pollen transfer (Jarlan et al., 1997). Thus, the level of discoloration of tomato anther cones may be a good indicator of stigmatic pollen load because the intensity of discoloration corresponds to the duration of contact by bumble bees. Bin and Sorressi (1973) observed that pollination of tomato flowers by bumble bees caused necrotic spots on the anther cone. In European greenhouses, van Ravestijn and van der Sande (1991) assessed the number of tomato flowers that had been pollinated

by bumble bees (*Bombus terrestris*) by observing the brown discolouration of the anther cone caused by pollination visits. By following bumble bees and recording flower handling time, they also estimated that a single active worker could pollinate at least 500 tomato plants, or 250 m² of greenhouse tomatoes per day. Using this information, they suggested that 10 to 15 colonies per hectare was more than enough for sufficient pollination. Some additional European recommendations include:

- Maintaining a population of 500 to 600 bumble bees per hectare.
- Monitoring bee activity by looking for 'bite' marks on the anther cone, flight of bees, and sugar solution consumption.
- Arranging the hives in an even distribution throughout the greenhouse.

Straver and Plowright (1991) recommended that more North American studies were required before standard procedures could be created for North American greenhouses. They suggested that, in the interim, growers could use the European guidelines. However, these recommendations were based on *B. terrestris* rather than *B. impatiens*, are under glasshouse conditions, and do not take into account how the number of pollination visits affects tomato quality.

The spatial distribution of colonies within greenhouses may be important to ensure adequate pollination throughout the greenhouse. Osborne et al. (1999) used a harmonic radar tracking system to examine bumble bee (*B. terrestris*) movement in the field. They found that bumble bees do not necessarily forage close to their nests and that trip distances from the colony ranged from 70 to 631 m. Chen and Hsieh (1996) examined efficiency of bumble bees (*B. terrestris*) in

a 50 x 50 m tomato greenhouse and found that the percentage of flowers visited by bumble bees did not decrease with distance, up to 40 m from colonies. No studies have examined pollination patterns in relation to the proximity of bumble bee colonies within large commercial greenhouses.

We examined bumble bee (*B. impatiens*) activity and pollination of tomatoes in commercial greenhouses. The purposes of this study were to: 1. Quantify the relationship between bee activity at the hive entrance and pollination levels, 2. Determine if probability of pollination was related to the distance from the colony, and, 3. Assess what level of activity was required for adequate levels of pollination based on the relationship between pollination levels and tomato quality (Chapter 5).

For the main study on pollination in relation to activity, data were collected only on the first day of flowering. If the probability of a flower being visited was independent of age, then estimates could be made from these data on the expected level of pollination over a flower's life at a given activity level. Based on initial observations, we hypothesized that flowers that were pollinated earlier in their life would not last as long, and would set fruit sooner than those that were not pollinated until later, or received no bumble bee pollination. Based on the large foraging distances of *B. terrestris* found in the field by Osborne et al. (1999), we hypothesized that the average pollination level per flower would be the same regardless of distance from the colony. We hypothesized that higher bee activity levels at the colony entrance would be associated with greater pollination.

4.2 MATERIALS AND METHODS

4.2.1 Study area

The study was conducted in commercial greenhouses, devoted primarily to growing tomatoes, in the Leamington, Ontario area. Environmental conditions such as temperature and humidity were kept within standards optimal for tomato growth, and all were vented using 'gutter venting'. Gutter vents are hinged openings that extend the length of the roof of the greenhouse. Vent operation was computer controlled, being triggered when the internal greenhouse humidity or temperature reached a set value. In all of the greenhouses in this study, the vents were not screened, and as a result, bees were able to exit the greenhouse when the vents were open. The greenhouses were constructed of various plastic types and ranged in size from 6.4 to 32.1 hectares.

Beefsteak tomatoes (*Lycopersicon esculentum* Mill. (Solanaceae)) were grown in all of the greenhouses, with Trust and Grace cultivars being the most common. *Bombus impatiens* was used exclusively in all greenhouses, and colonies were located in the center walkways in all of them.

4.2.2 Colonies

B. impatiens colonies were obtained from Koppert Biological Systems Canada and Biobest Canada Ltd. Upon delivery, each colony contained approximately 60 to 90 workers and one queen. Colonies were used for no more than two months, after which they began to produce male bees and new queens. Queens and males or, 'reproductives' do not forage for the colony, and consequently, colonies at this stage are no longer useful for greenhouse

pollination. Because tomato flowers do not produce nectar, colonies were equipped with a bag containing a nectar substitute. Foragers moved in and out of the colony through a hole, 1.5 cm in diameter. Experimental colonies were fitted with an electric-powered photodiode tunnel and monitor (herein referred to as a 'bee counter') designed to count bee movements in and out of the colony (Chapter 2, Fig. 2.3).

4.2.3 Pollination levels

Four pollination categories were defined based on visual assessment of the degree of necrotic discoloration observed on the anther cones of tomato flowers resulting from buzz pollination by bumble bees (Fig. 4.1). Fig. 4.1, in conjunction with Table 4.1, were used as guides in assigning tomato flowers to the pollination level groups based on degree of necrotic bruising.

Flowers were examined under a microscope to determine if the level of bruising corresponded to the number of pollen grains on the stigma,. Six flowers from each category were randomly selected, dissected immediately after being picked, and the stigmas removed. The pollen grains on the stigmas were then stained with basic fuchsin jelly (see Kearns and Inouye, 1993), and the number of pollen grains on each stigma was visually estimated under a compound microscope. ANOVA was used to determine if numbers of pollen grains were different among the four pollination categories. In order to meet the ANOVA

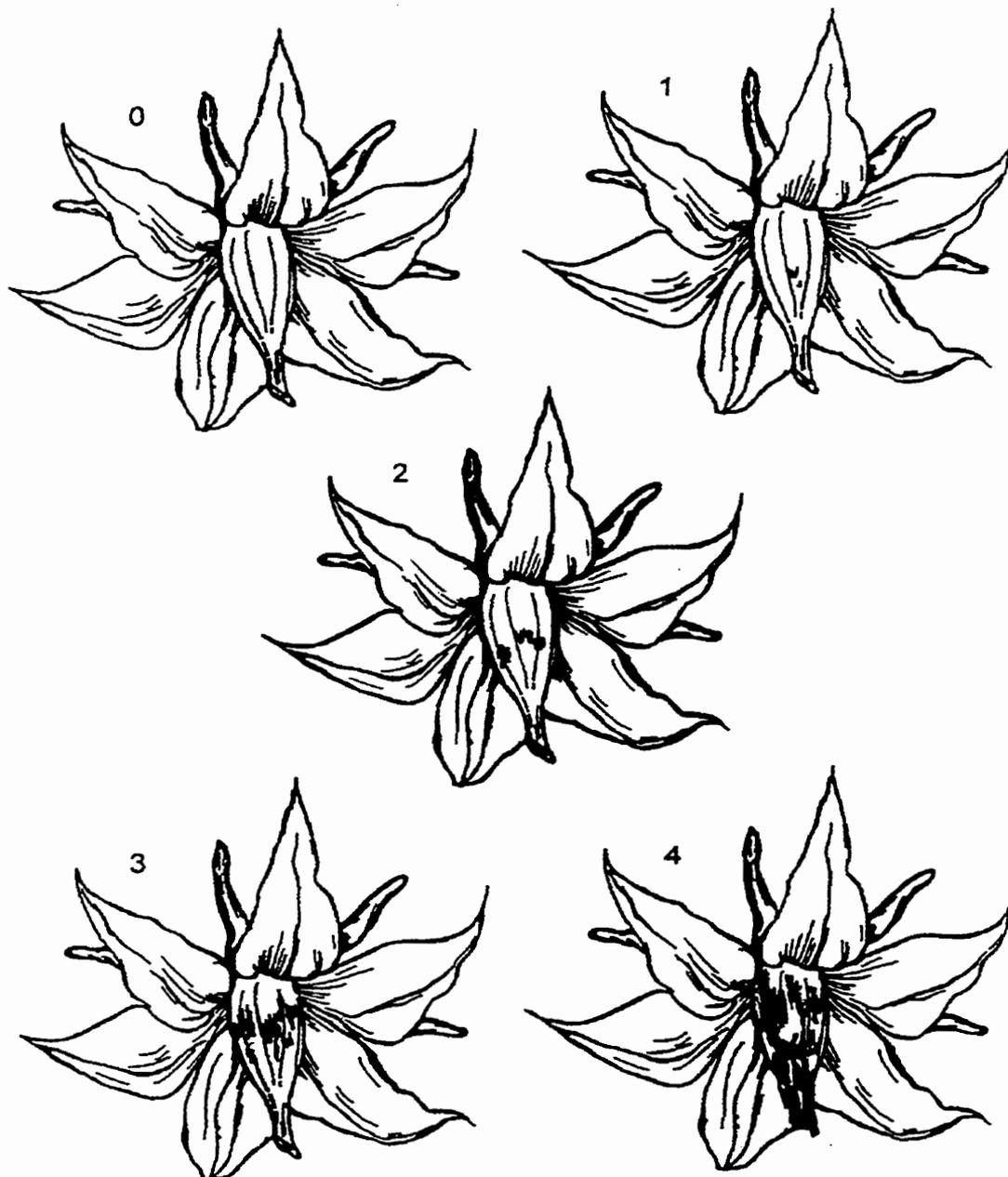


Figure 4.1. Pollination levels of tomato flowers based on necrotic bruising of the anther cone as a result of buzz pollination by bumble bees. Pollination level 0: no pollination, Pollination level 1: lightly pollinated, Pollination level 2: moderately pollinated, Pollination level 3: heavily pollinated, Pollination level 4: very heavily pollinated.

Table 4.1. Pollination level categories of tomato flowers based on levels of necrotic discolouration of the anther as a result of bumble bee (*Bombus impatiens*) buzz pollination.

Pollination level	Description	Visual assessment	Typical number of bee visits
0	No pollination	No bruising	0
1	Lightly pollinated	One or two small areas of discolouration	1
2	Moderately pollinated	Two to three small to medium size discolourations	1-2
3	Heavily pollinated	One or more large, or greater than 3 medium discolourations	2-4
4	Very heavily pollinated	Entire anther cone bruised, and anthers coming apart	>4

assumption of homogeneity of variances, the data were transformed using Taylor's Power Law (Taylor, 1961). All reported averages are from the non-transformed data. Differences among treatment groups were assessed using Tukey's pairwise comparison tests. The association between numbers of pollen grains per stigma with pollination level was examined using Spearman's correlation and regression analysis.

4.2.4 Probability of pollination and flower age

Preliminary tests in two greenhouses were made to determine if the probability of a flower being visited by a bumble bee was independent of flower age. New flowers that had not yet opened were tagged. Tagged flowers were then checked at midday the next day and then again in the evening for the presence of anther cone bruising. Using this protocol, the tagged flowers were monitored for four consecutive days, during which flowers were recorded in a hierarchy as either immature, one of the four pollination levels, or senescent. Flowers were assigned a pollination level based on the amount of anther cone bruising. Immature flowers were those that did not yet have all petals reflexed back towards the receptacle, and senescent flowers were those in which at least one of the mature petals was closing around the anther cone. Flowers could stay over the four days, or move up the hierarchy. The probability of a flower being visited on the first, second or third day of flowering was compared using a 2 x 3 chi-square contingency table. The average flowering time span was estimated using the calculated life spans of flowers from these two greenhouses. An

ANOVA was used to compare flower life span based on which day of its flowering period it was first bruised.

4.2.5 Pollination levels and activity

4.2.5.1 Activity

At the start of the first round of data collection, five unopened colonies were set up within each greenhouse. After opening, colonies were left for three to 10 days before monitoring began. After sunset, on the evening prior to the monitoring, numbers of bees per colony was recorded and, bee counter tunnels were cleaned and set to zero. Numbers were recorded the following evening, after sunset.

Bee counters recorded all movements within the tunnel, including activity of 'guard bees' at the nest entrance. Thus, it was necessary to calibrate the counters for the actual number of foraging trips. A regression line was generated relating counter values to actual observed bee entrances and exits by observing 23 colonies for 10 minute intervals in various commercial tomato greenhouses (refer to chapter 2), and regressing the actual number of bee entrances and exits on the counter number. The counter values for each day were then transformed using the regression equation to estimate actual number of entrances and exits per colony, per day. This value was then divided by two, in order to estimate trips per colony, per day. The average number of trips per colony per day was then multiplied by the number of colonies per hectare, resulting in an estimate of the average number of bee trips per hectare per day.

4.2.5.2 Pollination levels

Actual levels of pollination were collected in a manner similar to that of the preliminary pollination study, with a few modifications. Flowers were tagged and numbered from one to 50, starting at an experimental colony on the walkway. Fifty flowers were tagged per row, with number one being closest to the experimental colony, and number 50 being the farthest. Tagged flowers were about 1 m apart, resulting in a row of tagged flowers approximately 50 m in length. The level of flower pollination was recorded only after day one of flowering. This experiment was repeated four times in four commercial greenhouses, and three times in one.

In order for the data to be analyzed using parametric statistics, pollination levels were converted into number of pollen grains based on the mean numbers of pollen grains calculated for each level of pollination. Data from the 19 experiments were pooled, and correlation analysis was done to test if the level of pollination was related to distance from the colony. The number of colonies per hectare and the mean number of bee trips per hectare per day were assessed as predictors of the mean number of pollen grains per stigma per day. We expected that the number of colonies per hectare would not be a good indicator of mean pollen load, due to variation in levels of colony activity among greenhouses (Chapter 2). The average number of bee trips per hectare should be a better indicator of greenhouse pollination than the number of colonies per hectare, because bee trips per hectare took into account variation in levels of colony activity among greenhouses.

4.3 RESULTS

The mean number of pollen grains per stigma differed with respect to the degree of bruising ($F_{3,20} = 168.05$, $P < 0.0001$). Tukey's pairwise comparison test showed that the mean number of pollen grains was different among the four groups ($P < 0.0001$ for all comparisons). There was a strong positive correlation between the number of pollen grains and the pollination level ($r_s = 0.97$, $n = 24$, $P < 0.0001$), indicating that the degree of anther cone bruising is a good predictor of stigmatic pollen load.

In the preliminary study, there was no difference in the probability of a mature flower being pollinated based on flower age in either greenhouse ($X^2_2 = 1.20$; $P = 0.548$ and $X^2_2 = 4.26$; $P = 0.119$). The average lifespan of a flower, from opening to senescence, was 2.76 days \pm 0.06 S.E. ($n = 200$). The lifespan of a flower decreased if it was pollinated soon after it reached maturity ($F_{3,97} = 17.49$; $P < 0.001$; Fig 4.2). Flowers that received no bumble bee pollination survived 50.7% longer, and thus fruit set occurred later, than flowers that were pollinated on their first day of maturity.

Across the 50 m row, the average level of pollination per flower decreased with distance from the colony ($y = 285.32 - 2.93x$; $r^2 = 0.23$; $P = 0.01$; Fig. 4.3). Density of colonies in the study greenhouses ranged from 7.6 to 19.8 colonies per hectare with a mean of 11.6 ± 0.9 . Colonies per hectare was not a good predictor of mean number of pollen grains per stigma ($r^2 = 0.02$; Fig. 4.4).

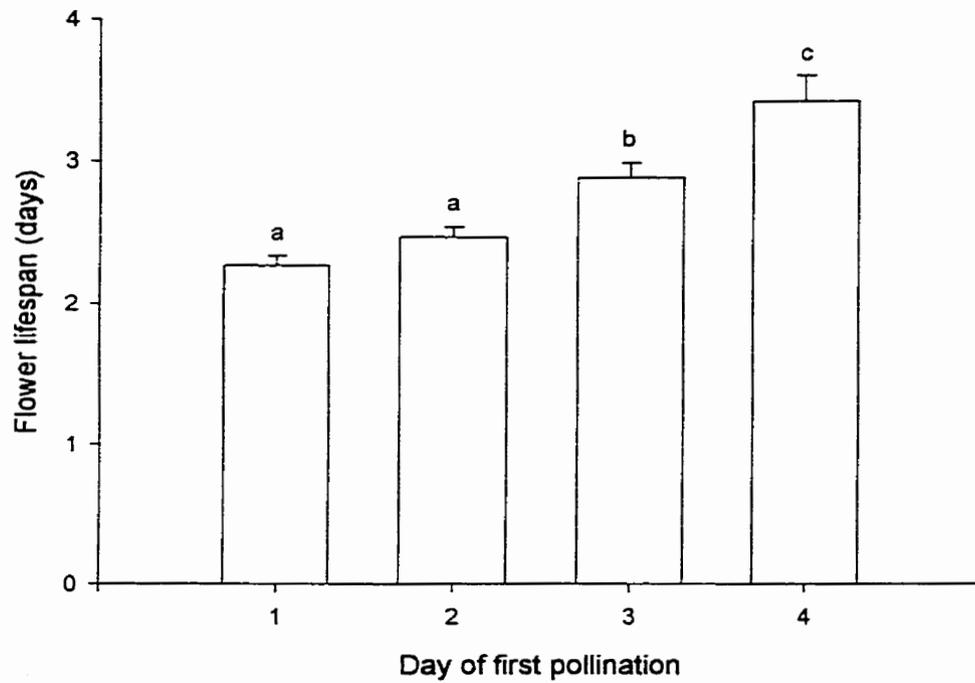


Figure 4.2. The mean lifespan of a tomato flower in relation to when it was first pollinated by bumble bees; 1 = first day of maturity, 2 = second day of maturity, 3 = third day of maturity, 4 = not pollinated. Bars with different letters are significantly different by Tukey's pairwise comparison test.

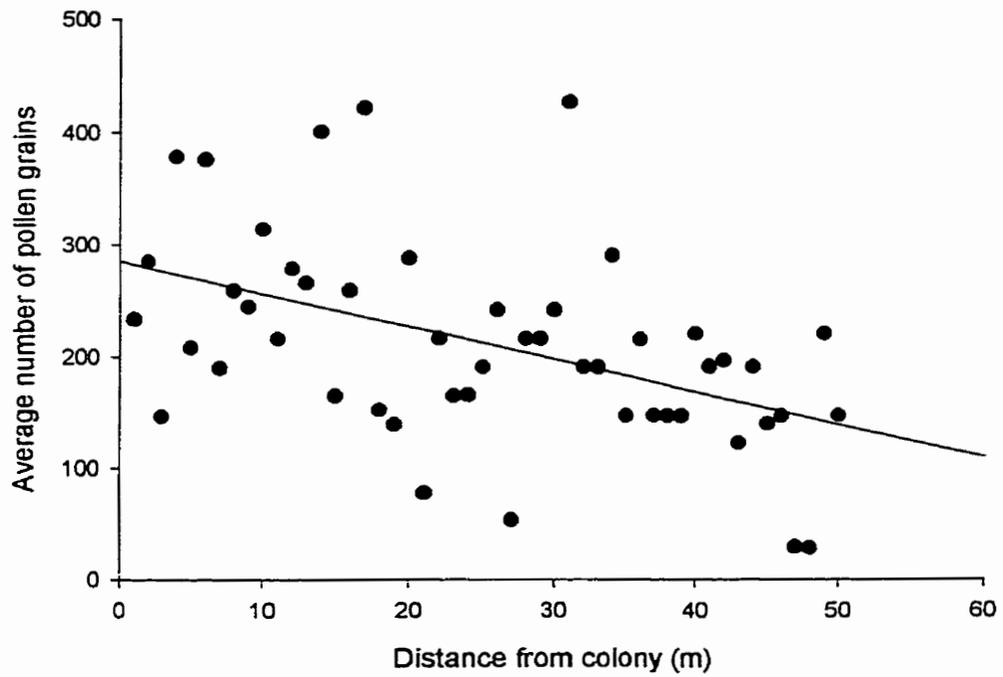


Figure 4.3. The mean number of pollen grains from 50 tomato flower stigmas in 24 commercial tomato greenhouses pollinated by bumble bees in relation to flower distance from the nearest bumble bee colony.

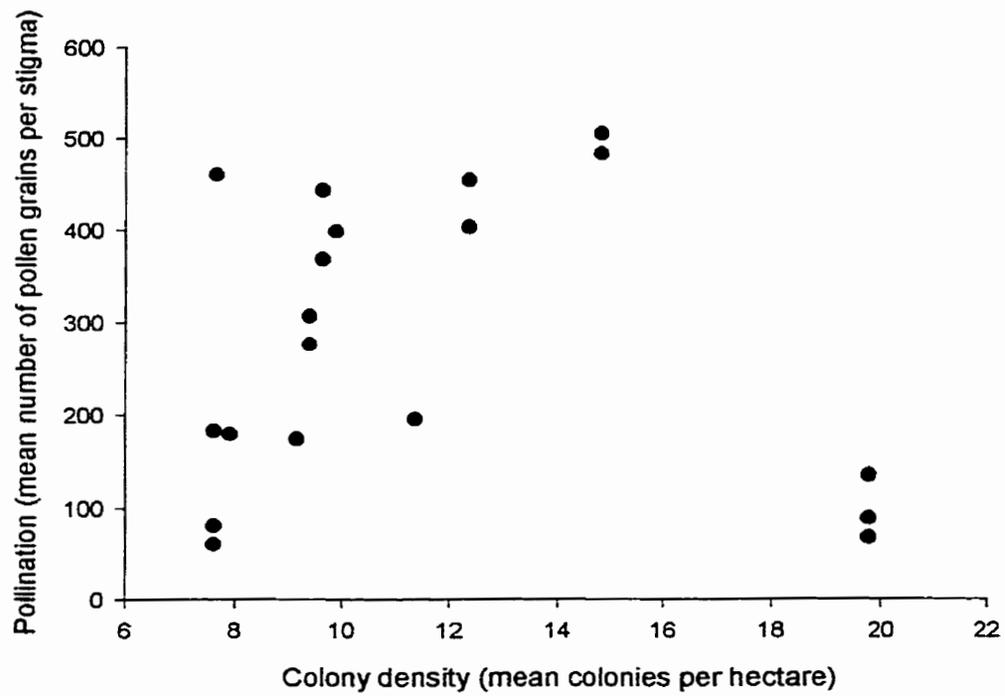


Figure 4.4. The mean number of pollen grains from 50 tomato flower stigmas pollinated by bumble bees in commercial tomato greenhouses in relation to the mean colony density.

Colony activity (mean number of bee trips per hectare per day) was the best predictor of average number of pollen grains per stigma (Fig. 4.5). An exponential rise to a maximum curve fit the data better than a linear model ($r^2 = 0.50$ versus $r^2 = 0.416$ linear regression). There was no increase in the mean number of pollen grains per stigma per day once an activity level of approximately 2000 bee trips per hectare per day was reached. The maximum mean number of pollen grains per stigma was 504, corresponding to a mean pollination level between one and two per flower per day. Greater levels of activity did not increase average pollination above this value.

Because there was great variation in activity among greenhouses (Chapter 2), the number of colonies required per hectare to achieve 2000 bee trips per hectare cannot be estimated without considering greenhouse factors (correlation between colonies per hectare and activity, $r^2 = 0.051$; Fig.4.6). We found that 2000 bee trips or greater was attained across the entire range of colony densities, from 7.6 to 19.8 colonies depending on the greenhouse. Because there was no difference in the probability of a mature flower being pollinated with respect to flower age (see p. 77), levels of pollination from the first day of flower maturity can be extrapolated to estimate expected pollination levels over the life of a flower. The average number of pollen grains per stigma when the activity was greater than 2000 bee trips per acre per day was 284 grains per stigma. Based on the average flower life expectancy of 2.76 days, the average number of pollen grains per flower stigma in a greenhouse with an activity level

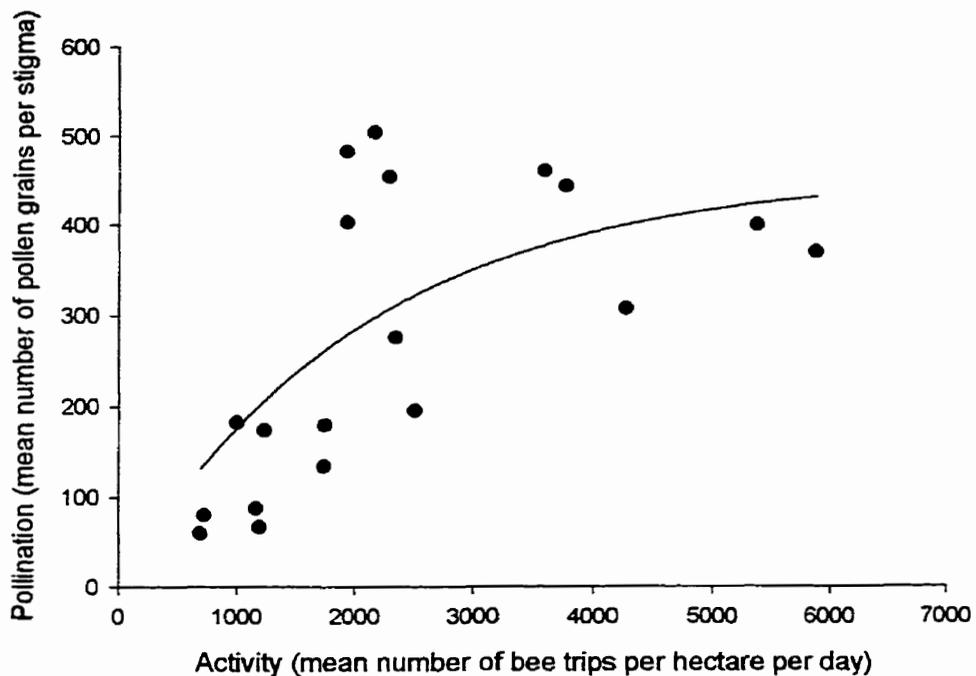


Figure 4.5. The mean number of pollen grains on tomato flower stigmas pollinated by bumble bees in five commercial tomato greenhouses in relation to mean bumble bee activity. $Y = 454.3(1 - e^{-0.000492x})$.

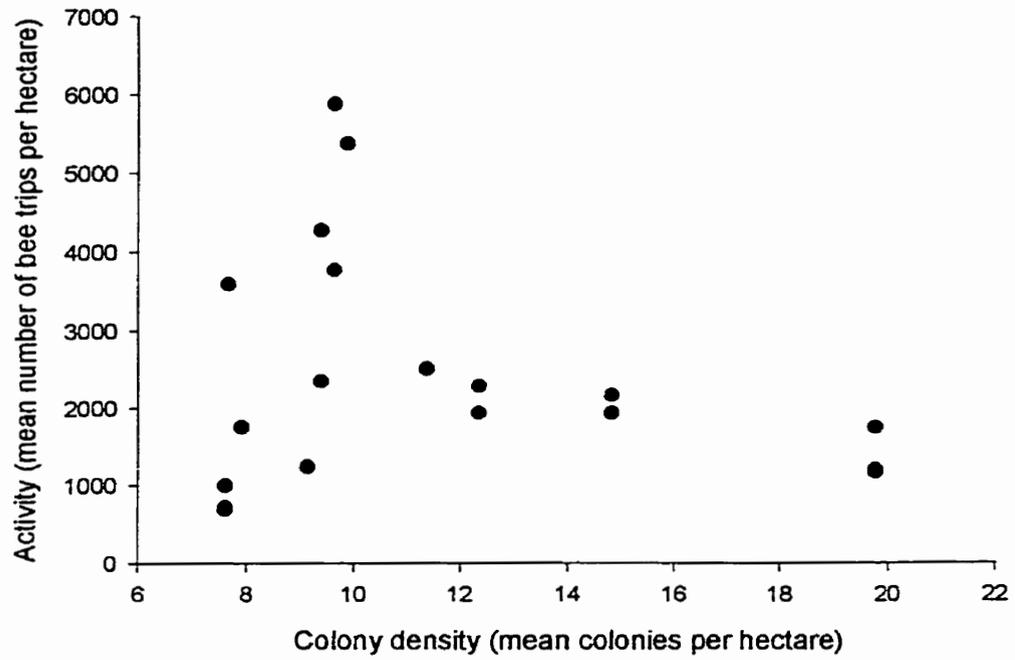


Figure 4.6. The relationship between mean bumble bee activity in commercial tomato greenhouses and bumble bee colony density.

of at least 2000 bee trips per hectare per day would be 784 pollen grains, corresponding to slightly greater than an average of a level two pollination per flower.

4.4 DISCUSSION

Within commercial greenhouses, tomato flowers in close proximity to bumble bee colonies received, on average, twice as many pollen grains than did flowers that were 50 m away (285.32 to 138.82 pollen grains per stigma per day). Chen and Hsieh (1996) found the percentages of flowers visited by bumble bees (*B. terrestris*) did not decrease up to a distance of 40 m from the colony in a 50 x 50 m greenhouse. Our results may be different because of the much larger sizes of the study greenhouses. Although Osborne et al. (1999) found that foraging distances of *B. terrestris* averaged 275.3 ± 18.5 m in the field, they suggested that distance traveled may be related to availability of food. Availability of food within tomato greenhouses may be sufficiently plentiful close to the colony so that bumble bees do not need to travel as far as they do in a field setting to acquire food. Our results suggest that colonies should be evenly distributed within greenhouses to ensure adequate pollination of all flowers.

The exponential rise to a maximum pollination level and bumble bee activity at the hive entrance suggested that activity over a certain level would not result in greater pollination. The lowest mean number of pollen grains per stigma found in our study was approximately 59.58 pollen grains per flower stigma per day. Over the average lifespan of a tomato flower of 2.76 days, this would result

in a mean pollination of 164.45 pollen grains per stigma, corresponding to an average pollination level of approximately zero to one, over the life of the flower. In another study (Chapter 5), we found that measures of tomato quality such as fruit set, number of seeds, tomato diameter and tomato weight, did not increase after a pollination level of two. Based on this information, we recommend that growers and bee suppliers should aim for an average of a level one pollination per flower, to ensure that flowers receive a pollination level of at least two prior to flower senescence and fruit set. An average activity level of at least 2000 bee trips per hectare per day would be more than adequate to achieve this level of pollination.

Colony activity was highly variable among greenhouses, possibly as a result of greenhouse conditions such as plastic type (Chapter 2) and internal temperature (Lundberg, 1980—bumble bees; Abrol, 1990—alfalfa-pollinating bees; Armbruster and McCormick, 1990—euglossine bees; Corbet et al. 1993—honeybees and bumble bees; Abrol, 1998—alfalfa-pollinating bees; Chapter 2). Greater internal greenhouse temperatures and plastics that transmit more ultraviolet light were associated with greater bumble bee activity levels. In a greenhouse that transmitted large amounts of UV light, we found that average activity was 4.82 ± 0.37 trips per bee per day (refer to Chapter 2). For an average colony size of 60, that activity level would translate to 2024 bee trips per hectare per day with a colony density of only 7 colonies per hectare. The activity among the other plastic types studied did not differ, and averaged 2.37 ± 0.34 trips per bee per day, requiring approximately 15 colonies per hectare to reach

an activity level of at least 2000 bee trips per hectare per day. These values are comparable to the 10 to 15 *B. terrestris* colonies per hectare recommended by van Ravestijn and van der Sande (1991) for European tomato glasshouses. It should be noted that factors other than type of greenhouse plastic such as, temperature and the condition of colonies, contribute to bumble bee activity differences among greenhouses, and that our recommendations are meant only as a guide. Further studies on factors that may influence levels of bumble bee activity in commercial greenhouses are required so that more specific recommendations on required colony density can be made.

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CHAPTER 5

Effect of bumble bee pollination intensity on tomato quality

5.1 INTRODUCTION

Although the tomato (*Lycopersicon esculentum* Mill. (Solanaceae)) is largely self-fertile, the flowers require agitation of the anther cone to release pollen from the anthers (Free, 1993; Buchmann, 1983, 1986). In greenhouses, this has been achieved through the use of hand-held electric pollinators. More recently, bumble bees have been found to be at least as effective, and in many cases more effective, than manual pollination or honeybee pollination in terms of the quantity and quality of tomatoes produced (Banda and Paxton, 1991; Kevan *et al.*, 1991; van Ravestijn and van der Sande, 1991; Straver and Plowright, 1991; Dogterom *et al.*, 1998; Pressman *et al.*, 1999). By 1994, all commercial tomato greenhouses in southern Ontario were using bumble bees as their sole means of pollination (S. Khosla, pers. comm.).

Although experiments using bumble bees to pollinate tomato flowers have found that high quality fruit in terms of yield, seed set, fruit weight, and roundness are produced (Kevan *et al.*, 1991; van Ravestijn and van der Sande, 1991; Straver and Plowright, 1991), there is little information on how different levels of pollination by bumble bee affects quality. Too few visits by bees may result in a low number of ovules being fertilised, and substandard fruit (e.g., Verkerk, 1957; Straver and Plowright, 1991, van Ravestijn and van der Sande, 1991; Pressman *et al.*, 1998). Too much bee activity on a flower may cause damage to the reproductive organs, premature loss of the flower, and no fruit

development (Cribb, 1990).

Studies on various plant species such as *Oenothera fruticosa* (Silander and Primack, 1978), *Turnera ulmifolia* (Shore and Barrett, 1984), *Passiflora vitifolia* (Snow, 1982), *Polemonium viscosum* (Galen and Newport, 1988), *Delphinium nelsonii* (Waser and Price, 1991) and *Lesquerella fendleri* (Mitchell, 1997) have found that as pollen dose increases, so does seed set. There is also a large body of literature showing positive correlations among the number of seeds per fruit, fruit weight, sugar content, and fruit shape (e.g., Hasegawa and Nakajima, 1990; Patterson, 1990; Rohitha and Klinac, 1990; Hong et al., 1991; Chen, 1996; Gonzalez et al., 1998; Suzuki et al., 1998).

For the most part, more pollination results in better quality fruit (Chagnon et al., 1989; Chen, 1996; Pressman et al., 1998). For example, Chen (1996) found that the percent fruit set, and quality of cantaloupe increased with greater numbers of pollination visits by honeybees. Similarly, Pressman et al. (1998) found that in sweet peppers, greater numbers of manual pollinations per flower resulted in larger, improved shape fruit. They concluded that an increase in the number of pollinations could greatly increase size and quality of yield, thus improving farmers' income.

Few studies have examined tomato quality in relation to levels of pollination. Fletcher and Gregg (1907) showed that, within limits, the size of a tomato is dependent on how much pollen is transferred to the stigma. Verkerk (1957) found that the more often tomato flowers were manually pollinated—up to a total of four times—the greater the percent fruit set and the higher the number

of seeds per fruit. He also found that a greater number of pollen on the stigma resulted in faster fruit development and therefore an earlier and more profitable crop.

The level of bumble bee activity on a tomato flower is easy to assess. Bin and Sorressi (1973) observed that pollination of tomato flowers by bumble bees caused bruising or necrotic spotting on the anther cone. In European greenhouses, van Ravestijn and van der Sande (1991) assessed the number of tomato flowers that had been pollinated by bumble bees (*Bombus terrestris*) by observing the brown discoloration of the anther cone caused by pollination visits. Although some studies have examined fruit quality in relation to the number of pollination visits, total duration of pollination visits is a better measure because it is a more accurate indication of pollen transfer (Jarlan et al., 1997). Thus, the level of discoloration of tomato anther cones may be a good indicator of stigmatic pollen load because the intensity of discoloration corresponds to the duration of contact by bumble bees.

In southern Ontario, greenhouse growers and suppliers use the necrotic discoloration of the anther cone as an estimate of the proportion of flowers visited, and as an indication of the amount of pollination within the greenhouse. Most suppliers and growers want 80 to 100% of the flowers with heavy bruising on the anther cone (S. Khosla, pers.comm.). Currently, in southern Ontario, bumble bee (*Bombus impatiens*) colonies are added to greenhouses largely based on this method of pollination assessment. Nonetheless, there has been no research to determine if visual evaluation of the degree of necrotic bruising on

anther cones is a reliable method of assessing stigmatic pollen load, or, what degree of bruising is required for optimal tomato yield and quality.

In this study, we addressed two questions: 1. Is the degree of necrotic discolouration on tomato anther cones a good predictor of stigmatic pollen load? and 2. What is the relationship between the level of necrotic bruising on tomato flowers, and various fruit characteristics? We believed that measures of fruit quality would increase up to a level of pollination of one or two, after which, additional pollination would not result in improved quality.

5.2 MATERIALS AND METHODS

In order to determine the relationship between bruising and tomato quality, we first developed a rating scale to categorize the degree of bruising. We then assessed the relationship between the level of bruising on the anther cone, and the stigmatic pollen load. The relationship between pollination level and fruit characteristics was then evaluated.

5.2.1 Study area and organisms

The study was conducted in an experimental glasshouse at the University of Western Ontario from March 1999 to October 1999. The greenhouse was approximately 6 m wide x 6 m long x 3 m high, and temperature was regulated using screened roof vents, at 22°C in the day and 20°C at night. Approximately 100 tomato plants (*L. esculentum* CV. Trust) were grown from seed planted in sterilized potting soil. For the first month after the seeds were planted, they were given 10-52-10 Plan-Prod plant starter fertilizer every week. For the rest of the

experiment, the plants were given 20-20-20 Plant-Prod all purpose fertilizer, weekly. The plants were watered daily, or more often if needed. The experiment began shortly after the first flowers appeared.

Colonies of *B. impatiens* were obtained from two bumble bee supply companies, Koppert Biological Systems Canada, and Biobest Canada Ltd. Colonies from both suppliers were housed in plastic containers approximately 20 x 28 x 18cm, surrounded by an outer cardboard casing. Upon delivery, each colony contained approximately 30 to 60 workers and one queen. Colonies were used for no more than two months, after which they began to produce male bees and new queens. Queens and males or, 'reproductives' do not forage for the colony, and consequently, colonies at this stage are no longer useful for greenhouse pollination. Because tomato flowers do not produce nectar, a required food for bumble bees, colonies were equipped with a bag containing a nectar substitute. Foragers moved in and out of the hive through a hole in the hive casing, 1.5 cm in diameter.

5.2.2 Pollination levels

Five pollination categories were designated based on visual assessment of the degree of necrotic discolouration on tomato flower anther cones as a result of buzz pollination by bumble bees (Chapter 4, Fig. 4.1 and Table 4.1). Prior to the categorization of the degree of bruising, flowers were visually assessed in commercial tomato greenhouses in the Leamington, Ontario area as part of a larger study on bumble bee pollination of greenhouse tomatoes. The fifth pollination category, representing extremely heavy bruising and destruction of the

anther cone was added after the tomato quality experiment had begun. Because of this, no data were collected on number of pollen grains for this level of pollination. Level four pollination was never observed in the commercial greenhouses, but did occur occasionally in the small experimental greenhouse. Fig. 4.1, in conjunction with Table 4.1, were used as guides in assigning tomato flowers to the pollination level groups based on degree of necrotic bruising.

Flowers were examined using a microscope to determine if the level of bruising corresponded to the number of pollen grains on the stigma. Six flowers from each category were randomly selected, dissected immediately after being picked, and the stigmas removed. The pollen grains on the stigmas were then stained with basic fuchsin jelly (see Kearns and Inouye, 1993). The number of pollen grains on each stigma was visually estimated under a compound microscope. ANOVA was used to determine if numbers of pollen grains were different among the first four pollination categories. In order to meet the ANOVA assumption of homogeneity of variances, the data were transformed using Taylor's Power Law (Taylor, 1961). All reported averages are from the non-transformed data. Differences among treatment groups were assessed using Tukey's pairwise comparison tests. The association between the number of pollen grains per stigma and pollination level was examined using Spearman's correlation and regression analysis.

5.2.3 Tomato quality

One colony was introduced into the experimental greenhouse, and allowed to forage on the tomato flowers for two to three days. The hive's exit

hole was then covered, leaving only the entrance hole open. After all bees had returned to the colony, flowers were tagged. Each tag was labelled with a unique number, dated, and the level of pollination was assessed using the criteria outlined in Fig. 4.1 and Table 4.1. The bees were not released from the colony for at least four days, when all of the tagged flowers had either completely closed or fallen off. The colony was then reopened, and the tagging and recording was repeated every two to three weeks. The number of flowers tagged in each category was approximately the same for each tagging period.

All tomatoes were harvested at the same state of ripeness, based on visual assessment of colour. The tomatoes were considered ripe when they were a uniform orange-red colour. The number of tomato flowers that set fruit, verses the number that did not set, was compared among pollination level groups using 4 x 2 Chi-square contingency analysis (df = 3). Minimum and maximum tomato diameters were measured using digital callipers. Average diameter was calculated from the minimum and maximum diameters. Tomato roundness was calculated using a revised equation taken from Kevan *et al.* (1991) (Equation 1). The revised equation took into account minimum and maximum diameter, but left out the calyx to stigmatic scar diameter because when tomatoes are assessed for roundness in greenhouses, it is the equatorial roundness that is important for efficient packaging (S. Khosla, pers. comm.).

$$\text{Roundness} = \sum_{i=1}^2 |1 - d_i/d_{ave}|$$

$$\text{Where } d_i = \text{diameter} \\ d_{ave} = \text{average diameter} \quad [1] \\ (d_1 + d_2)/2$$

(Modified from Kevan *et al.*, 1991)

Tomatoes were weighed to 0.01 grams using a Mettler PJ300 electronic scale. The entire tomato was then homogenized in a commercial food blender, and the percent of sugars of the blended mixture was measured to one decimal place using a hand held 0 to 50% Bellingham and Stanley refractometer. The blended mixture was then rinsed through a 1 mm sieve, to separate the seeds from the liquid. The total seeds per tomato were then counted. The number of days until ripe was calculated starting from the date the flower was tagged, until the date the tomato was harvested.

The data were first analysed by MANOVA, with roundness, weight, sugars, number of seeds, minimum diameter, difference in diameter between the minimum and maximum diameter, and days until ripe as the response variables. This was followed by univariate ANOVA and Tukey's pairwise comparisons. We predicted that roundness, weight, sugars, number of seeds, average diameter, and minimum diameter would increase logarithmically with greater levels of pollination. We hypothesized that the difference between the minimum and maximum diameter, and the number of days until ripe would decrease with increasing pollination levels. The nine variables were compared using Spearman's correlation analysis.

The Agriculture Canada grades and standards for greenhouse tomatoes (1998) categorizes tomatoes into three groups: Canada number 1 grade, Canada commercial grade, and Canada number 2 grade. One of the main criteria that divides the Canada commercial grade from the Canada number 2 grade, is a minimum diameter for the commercial grade of 63 mm. Based on these criteria, if

the minimum diameters were greater than 63 mm, then we categorized the tomatoes as commercial grade. If the minimum diameters were less than 63 mm, then we categorized the tomatoes as number 2 grade. The Canadian standards outline additional factors, which we did not take into account, such as the amount of creasing and the extent of growth cracks, when tomatoes are graded. The number of tomatoes in each group in each pollination level was compared using a 2 x 5 Chi-square contingency table ($df = 4$). For all chi-square analyses, when data sets had expected values of less than five, the data were adjusted using Yates correction (Yates, 1934). We predicted that lower pollination levels would result in tomatoes with smaller diameters, and subsequently, more tomatoes in the number 2 grade.

5.3 RESULTS

5.3.1 Pollination levels

Pollination categories based on the degree of bruising differed in the mean number of pollen grains per stigma (ANOVA: $F_{3,20} = 168.05$, $P < 0.0001$). Tukey's pairwise comparison test showed that the mean number of pollen grains was different among the four groups ($P < 0.0001$ for all comparisons). There was a strong positive correlation between the number of pollen grains and the pollination level ($r_s = 0.97$, $n = 24$, $P < 0.0001$; Fig. 5.1), indicating that the degree of anther cone bruising is a good predictor of stigmatic pollen load.

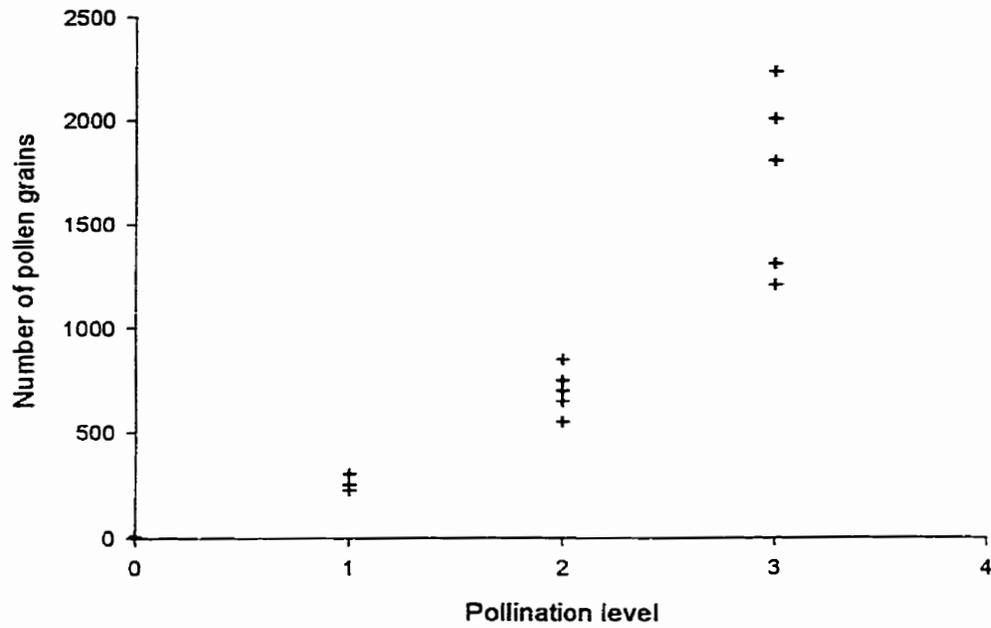


Figure 5.1. Relationship between the number of pollen grains on the stigma of tomato flowers and the pollination level resulting from bruising of the anther cone from buzz pollination by bumble bees ($r_s = 0.971$, $n = 24$; $F_{22} = 115.76$, $p < 0.001$).

5.3.2 Tomato quality

The number of tomatoes that set fruit verses the number of flowers that did not set fruit was different among pollination level groups ($\chi^2_4 = 48.24$; $P < 0.0001$; Table 5.1). The pollination treatment group contributing most to the difference was level zero, no pollination, with 13 set tomatoes, and 30 flowers that did not set fruit ($\chi^2 = 32.83$). With 30.2% of the flowers that set fruit, pollination level zero had the lowest percent fruit set. Pollination level one had a fruit set of 83.3%, level two had 84.37%, level three had 81.25%, and level four had the highest with 100% fruit set (Fig. 5.2). When pollination level zero was removed from the analysis, there was no difference in the number of flowers that set to the number that did not set fruit ($\chi^2_3 = 2.47$; $P = 0.498$). This suggests that a greater degree of pollination did not increase fruit set over levels one to four. When pollination levels one to three were pooled and compared to level four, there was no difference in fruit set ($\chi^2_1 = 2.67$; $P = 0.113$).

Multivariate analysis of variance on the five pollination levels showed a difference among pollination treatments with respect to roundness, weight, sugars, number of seeds, average diameter, difference in diameter between the minimum and maximum diameter, minimum diameter and days until ripe ($F_{28,384} = 2.60$; $P < 0.0001$, power = 1.00). Univariate analysis of variance showed that there was no difference among pollination levels with respect to the difference in diameter ($F_{4,99} = 1.59$; $P = 0.184$, power = 0.474), the number of days until the tomato was ripe ($F_{4,99} = 1.41$; $P = 0.235$, power = 0.426), tomato roundness ($F_{4,99} =$

Table 5.1. The number of tomato flowers that set and did not set tomatoes in five pollination level treatment groups resulting from different amounts of pollination by bumble bees.

	Pollination level				
	0	1	2	3	4
Set	13	20	27	26	20
Not set	30	4	5	6	0

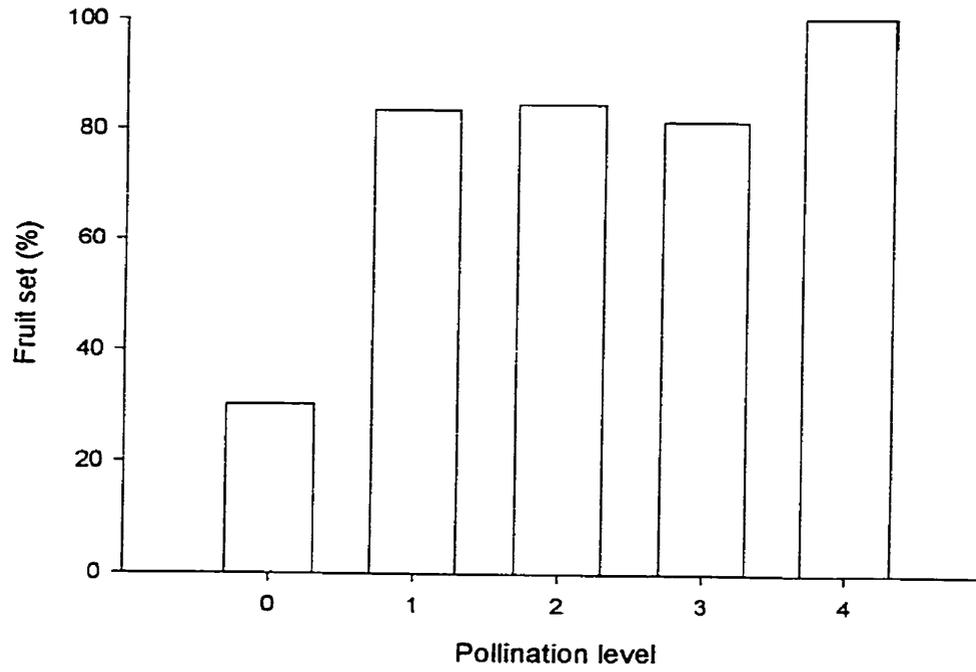


Figure 5.2. Percent of tomato flowers that set fruit in relation to the level of bumble bee pollination.

1.34; $P=0.259$, power = 0.406), and the percent sugars ($F_{4,99} = 0.60$; $P=0.665$, power = 0.191). There was a difference in the average diameter ($F_{4,99} = 4.31$, $P=0.003$, power = 0.920), minimum diameter ($F_{4,99} = 3.72$; $P=0.007$, power = 0.871), number of seeds ($F_{4,99} = 18.82$; $P<0.0001$, power = 1.00), and tomato weight ($F_{4,99} = 4.67$; $P=0.002$, power = 0.941) with respect to pollination level (Table 5.2). There was no increase in tomato weight, average diameter, or minimum diameter after a pollination level of one, and there was no increase in the number of seeds after level two pollination. Spearman's correlation showed that there were many significant relationships between the eight factors tested (Table 5.3). Number of seeds, weight and minimum diameter were strongly correlated with pollination level (Figs. 5.3 to 5.5). Because pollination level was an ordinal data set, only linear analysis was appropriate, however, the relationship between pollination level and minimum diameter, number of seeds, and weight all appear to have a logarithmic relationship. Pollination level was most highly correlated with number of seeds ($r_s = 0.63$, $P<0.0001$). The number of seeds was correlated with minimum average diameter ($r_s = 0.65$, $n=108$, $P<0.0001$; $y = 94.49 + 37.60\log x$; Fig. 5.6).

Based on the minimum diameter, the percentage of tomatoes that were commercial grade verses the number 2 grade was, 7.9%, 30.0%, 42.9%, 60.7%, and 60.0% for pollination levels 0, 1, 2, 3, and 4, respectively (Fig. 5.7). Chi-squared analysis of the number of tomatoes in the commercial grade verses the number in the number 2 grade showed that there was a difference among

Table 5.2. Comparison of seven measures of tomato quality recorded from four pollination level treatment groups.

Pollination level	Diameter difference (mm)	Minimum diameter (mm)	Days to ripen	Roundness	Number of seeds	Sugars (%)	Weight (g)
0	1.52±1.34 a	56.37±2.18 a	68.69±3.64 a	0.026±0.02 a	71.69±16.12 a	3.32±0.16 a	94.65±12.80 a
1	3.93±1.34 a	59.48±1.86 ab	58.33±3.10 a	0.061±0.02 a	138.61±13.70 b	3.12±0.14 a	120.87±10.88 ab
2	2.92±0.95 a	62.28±1.54 ab	64.27±2.58 a	0.045±0.01 a	185.69±11.40 bc	3.38±0.12 a	135.72±9.05 ab
3	4.15±0.93 a	63.12±1.52 ab	63.74±2.53 a	0.061±0.01 a	211.22±11.19 bc	3.35±0.11 a	148.03±8.88 b
4	5.49±1.08 a	66.25±1.76 b	60.70±2.94 a	0.074±0.02 a	227.05±13.00 c	3.34±0.13 a	157.89±10.32 b

Means followed by the same letter in any given column are not significantly different from one another ($\alpha = 0.05$) by Tukey's pairwise comparison test.

Table 5.3. Spearman correlation coefficients of the relationships among tomato quality measures.

	Pollination level	Diameter difference	Minimum diameter	Days to ripe	Roundness	Number of seeds	Percent sugar	Weight
Pollination level	1.00							
Diameter difference	0.167	1.00						
Minimum diameter	0.356**	0.247**	1.00					
Days to ripe	-0.060	-0.071	-0.052	1.00				
Roundness	0.132	0.991**	0.138	-0.064	1.00			
Number of seeds	0.623**	0.244*	0.682**	-0.197	0.170	1.00		
Percent sugar	0.016	-0.098	0.134	0.175	-0.109	0.038	1.00	
Weight	0.366**	0.435**	0.792**	0.351**	0.351	0.598**	0.117	1.00

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

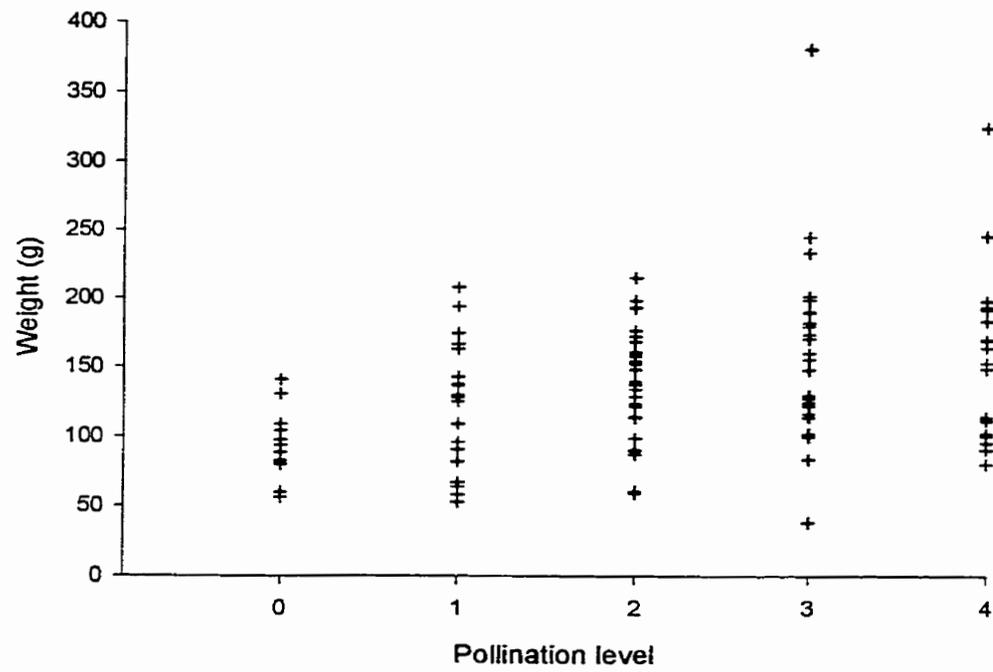


Figure 5.3. The relationship between weight and level of pollination of tomato flowers pollinated by bumble bees. $n = 108$; $r_s = 0.37$.

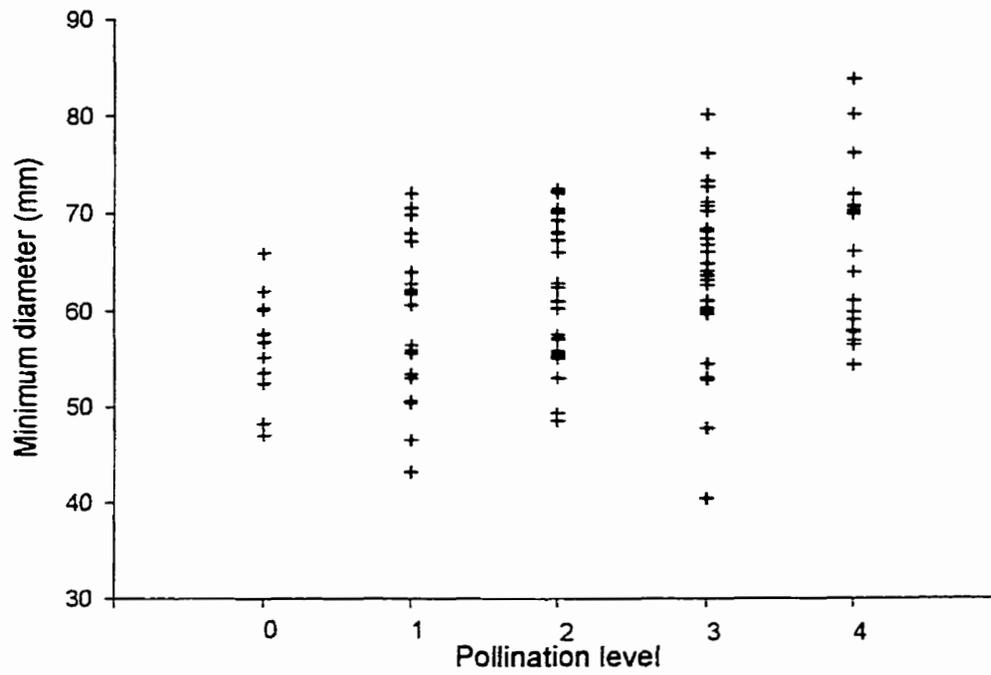


Figure 5.4. The relationship between minimum tomato diameter and pollination level of tomato flowers pollinated by bumble bees. $n = 108$; $r_s = 0.36$.

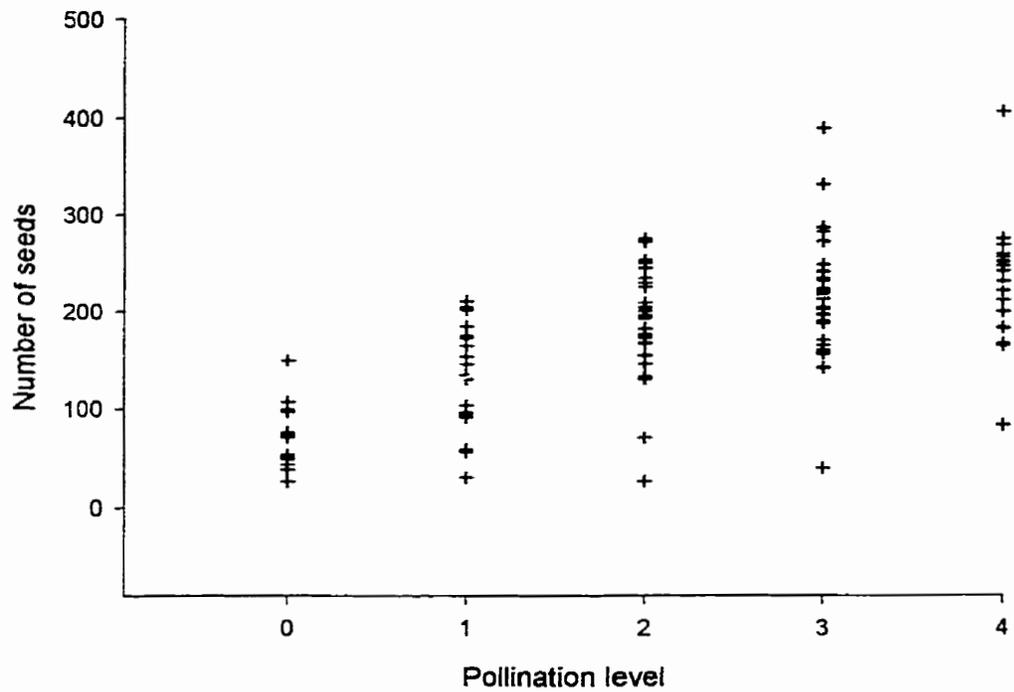


Figure 5.5. The relationship between the number of seeds and pollination level of tomato flowers pollinated by bumble bees. $n = 108$.

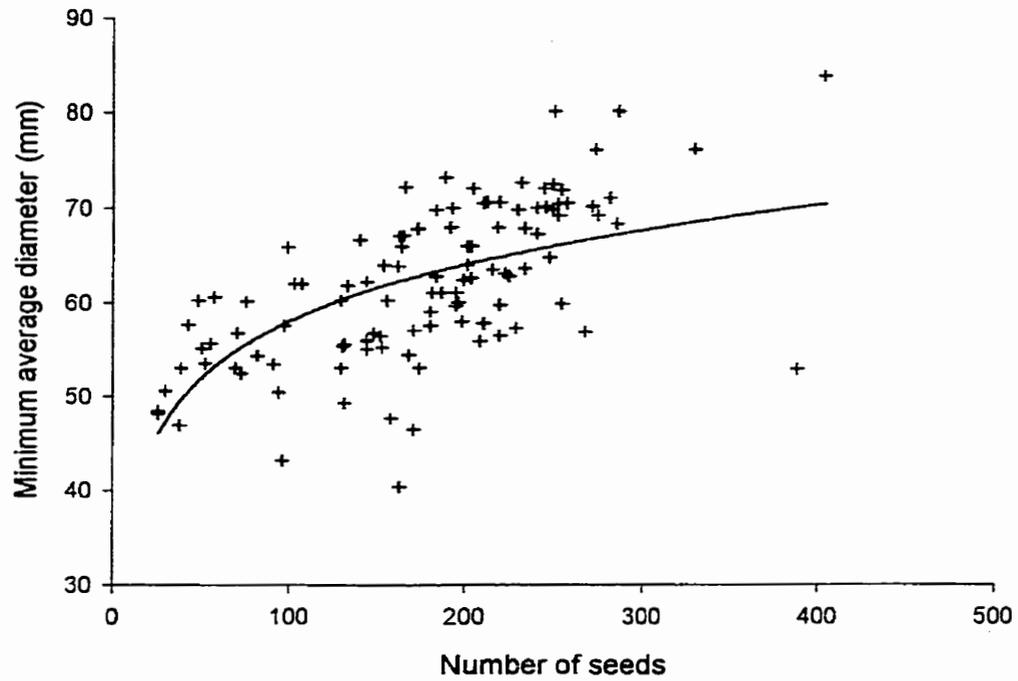


Figure 5.6. The relationship between minimum diameter and the number of seeds (\log_{10}) of beefsteak tomatoes pollinated by bumble bees. $n = 108$; $Y = 8.815 + 17.43\log x$.

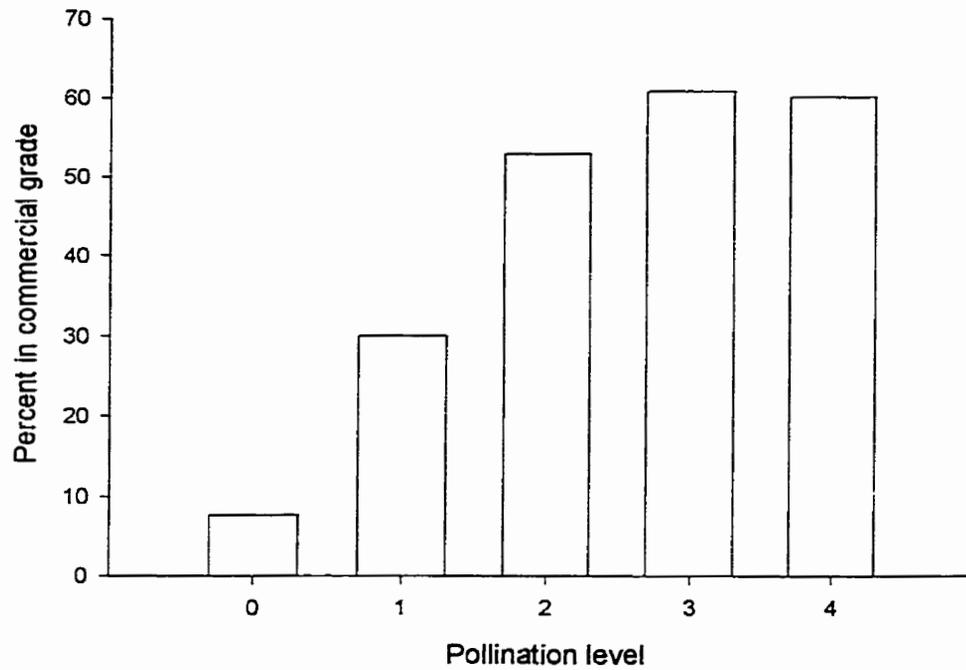


Figure 5.7. The percent of tomatoes that were graded as commercial grade versus the lower quality number 2 grade based on a minimum diameter of 63 mm.

pollination levels ($\chi^2_4 = 13.81$; $P=0.008$). When pollination level zero was removed from the analysis, there was no difference in the number of tomatoes in the commercial grade verses the number 2 grade with respect to pollination level ($\chi^2_3 = 5.82$; $P=0.121$).

5.4 DISCUSSION

Duration of bumble bee visits to tomato flowers were highly variable (*personal observation*), and as a result, the number of bee visits to a flower may not be a good indicator of pollination level. Instead, bruising of the anther cone appeared to be a better indication of the duration of contact of bumble bees with the stigma, and subsequent pollen transfer. We found that bruising of the anther cone was a good indicator of stigmatic pollen dose, and we recommend this method for assessing levels of bumble bee pollination of tomato flowers. Similarly, Jarlan et al. (1997) found that the number of drone fly, *Eristalis tenax*, pollination visits to sweet pepper flowers did not influence fruit characteristics, but instead, that total duration of contact of flies was a better indicator of pollen transfer to the stigma.

Our results indicate that any level of bumble bee pollination increases fruit set in relation to no pollination. Similar results have been found in other studies comparing bumble bee pollination of tomato flowers to no pollination (Banda and Paxton, 1991; van Ravestijn and van der Sande, 1991; Kevan et al., 1991). In our study, pollination levels greater than zero did not result in significantly greater fruit set. Chen (1996) reported similar results for cantaloupe fruit set in relation to

the number of honey bee visits. He found that fruit set was 3.1% in the absence of honey bees, and increased to 45%, 69%, and 59% for 1, 2, and 3 bee visits, respectively. His findings and ours indicate that there is a large difference in fruit set between pollinated and unpollinated flowers, but not between different levels of pollination. In our study, although there was no significant increase in fruit set between pollination levels, there was a 17% increase in fruit set between the average fruit set of levels one, two and three (83.0%) and level four (100%). However, the levels of fruit set within the experimental greenhouses may have been lower than what is experienced in the commercial greenhouses due to occasional high temperatures in the experimental greenhouses. In addition, level four pollination corresponds to the entire anther cone being bruised and damaged, and was never observed in the commercial greenhouses, indicating that realistic levels of pollination result in similar fruit set. Within commercial greenhouses, tomato clusters are pruned from seven to eight tomatoes usually down to four tomatoes, making quality measures more important than 100% fruit set.

It is well known that fruit characteristics such as seed set, size, and weight increase with greater levels of pollination (e.g. Stephenson, 1988; Chagnon et al., 1989, 1991; Chen, 1996; Mitchell, 1996; Pressman et al., 1998). We also found positive trends between the level of pollination and the number of seeds, minimum diameter, and weight, although we found diminishing return relationships. But, at what level is pollination, and consequently tomato quality, adequate? Although this is partly dependent on greenhouse growing conditions,

and individual grower preferences, some conclusions and recommendations can be made from our study.

Of the variables that increased significantly with pollination level, minimum diameter is the most important when tomatoes are graded. The average minimum diameter increased only 0.84 mm (62.38 mm to 63.12 mm) from a level two pollination to a level three pollination. The logarithmic relationship between minimum diameter with number of seeds indicated that greater levels of pollination result in lower returns in terms of size increases. Considering all factors that were significantly correlated with pollination level, we found that pollination of tomato flowers greater than a level two pollination did not result in a significant increase in tomato quality. Although levels of pollination greater than two resulted in non-significantly larger tomatoes with more seeds, the increase was non-linear, indicating diminishing returns with further pollination. Fruit quality based on our variables produced adequate quality tomatoes, based on the grades and standards outlined by Agriculture Canada.

Variation in some of the variables tested was high, resulting in low power in tests of differences among pollination levels. Specifically, the number of days for tomatoes to ripen, the percent sugars and roundness were all tested at a power of less than 0.5. These variables should be tested again with larger sample sizes to test for pollination level effects. Seed count was probably the most accurate indicator of sufficient pollination in our study, because other quality measures were influenced by environmental conditions such as plant resources and greenhouse conditions (Picken, 1984). Because no increase in fruit set,

weight, and diameter were detected after a level one pollination, and no significant increase in the number of seeds after a level two pollination, we recommend level one to two pollination as adequate to ensure sufficient fruit set and quality of greenhouse tomatoes.

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CHAPTER 6

6.1 GENERAL DISCUSSION

Although use of bumble bees to pollinate commercial tomato crops has become common, little research has addressed growers problems such as low levels of bee activity and loss of bees through gutter ventilation systems. Because of these problems, some greenhouses have been getting inadequate levels of pollination, and some growers have had to resort to manual pollination for parts of the growing season.

6.1.1 Overview

i) Bee activity and ultraviolet light

I found that bee activity was greatest in commercial greenhouses with UV-transmitting plastic. Sensitivity to UV in honey bees and other insects is 4 to 6 times stronger than to other parts of the spectrum, suggesting that UV is an important component of insect vision (Kevan, 1970, 1979, 1983; Laughlin, 1976; Menzel and Backhaus, 1991). This may explain why bee activity was greatest under UV transmitting plastics. I found evidence in both the commercial greenhouses, and in the Y-Maze experiment that loss of bees was lowest under UV transmitting plastics. Other studies have found similar positive phototactic responses in insects to UV light, including honeybees (Berthoff, 1931; Kaiser et al., 1977; Menzel, 1979; Menzel and Greggers, 1985), and Diptera (Fischbach, 1979; Kevan, 1979). The high responsiveness to UV light is thought to be because insects use it as an indicator of an open environment (von Hess, 1913; Laughlin, 1976—dragonflies; Menzel and Greggers, 1985).

ii) Bee activity and temperature

I also found that bee activity was positively correlated with greenhouse temperature in both the commercial greenhouses and the experimental greenhouses. Heinrich (1979) calculated that foraging costs are greater for bumble bees when ambient temperatures are below 25°C because of thermoregulation costs below this temperature as well as the flight costs. At temperatures from 25°C to 35°C, foraging costs remain the same. My results were consistent with other studies that have found that bees become more active with increasing temperatures (Lundberg, 1980—bumble bees; Abrol, 1990—alfalfa-pollinating bees; Armbruster and McCormick, 1990—euglossine bees; Corbet et al. 1993—honeybees and bumble bees; Abrol, 1998—alfalfa-pollinating bees).

iii) Bee activity and tomato quality

Levels of pollination were positively correlated with bee activity levels, up to a mean of approximately 400 pollen grains per stigma per day (corresponding to between a level one and level two pollination), after which greater activity did not result in further increases in daily pollination levels. Within greenhouses, levels of pollination decreased substantially with increasing distance to the nearest colony. Fruit set and measures of tomato quality such as tomato weight, minimum and average diameters, did not increase significantly after a pollination level of one, and the number of seeds did not increase significantly after a pollination level of two.

6.1.2 Recommendations

- i) Bumble bee colonies should be spaced evenly throughout the greenhouse, to ensure adequate pollination of all flowers.
- ii) Based on the findings from the pollination level experiment (Chapter 4) and the tomato quality experiment (Chapter 5), I recommend that growers and bee suppliers should aim for an average of at least a level one pollination per flower. This should be more than adequate to ensure that flowers receive a pollination level of at least two prior to flower senescence and fruit set. I found that an average activity level of at least 2000 bee trips per hectare per day should be more than adequate to achieve this level of pollination.
- iii) Because colony activity is highly variable among greenhouses, possibly as a result of greenhouse conditions such as plastic type (Chapter 2) and internal greenhouse temperature (Chapter 2 and 3), standard recommendations for colony densities cannot be made for all greenhouses. In a greenhouse that transmits large amounts of UV light, the average activity was 4.82 ± 0.37 trips per bee per day. For an average colony size of 60, that activity level would translate to 2024 bee trips per hectare per day with a colony density of only 7 colonies per hectare. It should also be noted that greenhouses with UV-transmitting plastics will retain more bees (Chapters 2 and 3), and thus may require fewer colonies per hectare. The activity among the other plastic types did not differ, and averaged 2.37 ± 0.34 trips per bee per day, requiring approximately 15 colonies per hectare to reach an activity level of at least 2000 bee trips per hectare per day. These values of colony density are comparable to the 10 to 15 *B. terrestris*

colonies per hectare recommended by van Ravestijn and van der Sande (1991) for European tomato glasshouses. Growers with greenhouses that require greater than 15 colonies per hectare may benefit from changes that could be made to the greenhouse environment that would lower bee loss and increase bee activity. It should be noted that factors other than greenhouse plastic type and temperature, such as colony state and flower densities may contribute to differences in activity of bumble bees among greenhouses, and that our recommendations are meant only as a guide.

6.1.3 Conclusions

Maximizing bee activity and minimizing bee loss will have important economic consequences on bumble bee greenhouse pollination. Currently, bumble bee supply companies are mainly servicing tomato growers on a supply basis. Greenhouses that experience large amounts of bee loss and/or low bee activity require many more colonies than was originally estimated. Consequently, bee suppliers lose money in some situations, and have changed their billing to a fee per colony basis. For many growers this has resulted in increases in the price for crop pollination. In order to reduce costs for suppliers and growers, greenhouse environments should facilitate crop pollination as well as crop growth and health. My studies indicate that UV-transmitting plastics and high average daily temperatures promote colony activity. In addition, UV-transmitting plastics result in minimal bee loss through ventilation systems.

Although greenhouses with plastics that transmit more light within the UV range result in greater bee activity and less loss of bees, growers must balance

various factors when considering which kinds of plastics to install. Increased grey mold (*Botrytis*) spore germination has been associated with plastics that transmit ultraviolet light in the 320 to 400 nm range (Pearson et al., 1997), and this may make switching to high UV plastics less desirable. There is also evidence that UV-B light (290 to 320 nm) is damaging to growth of tomato plants (Hao et al., 1997). Also, greater transmission across the spectrum may result in temperatures that are above optimum for tomato growth during the summer months. Conversely, in the winter months, UV-transmitting plastics may help reduce heating costs. Because the peak sensitivity of bees' UV photoreceptor is 345 nm (Kevan and Backhaus, 1998; similar to Peitsch et al., 1992), it may be beneficial to use plastics that transmit down to 320 nm only.

We recommend that growers using UV-blocking plastics should use ventilation systems that prevent bee loss; such as, fan ventilation or screened gutter vents. In addition, greenhouses with UV-blocking plastics may require a greater density of colonies to ensure adequate pollination. Further studies on factors that may influence levels of bumble bee activity in commercial greenhouses are required so that more specific recommendations on required colony densities can be made.

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