THE ROLE OF HONEY BEE (*APIS MELLIFERA* L.)
QUEEN MANDIBULAR GLAND PHEROMONE
IN POLLINATION ENHANCEMENT AND COLONY MANAGEMENT

by

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THE ROLE OF HONEY BEE (APIS MELLIFERA L.) QUEEN

MANDIBULAR GLAND PHEROMONE IN POLLINATION ENHANCEMENT

AND COLONY MANAGEMENT

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Honey bee (Apis mellifera L.) queen mandibular gland pheromone (QMP) has an influence on colony state and the integration of worker activities. This study investigates the use of QMP in several contexts of honey bee management. Worker attraction to QMP has led to its successful use in pollination enhancement with crop sprays. We examined possible mechanisms for this success and determined that 1) foragers in flowering highbush blueberry and cranberry crops stayed longer in QMP plots and visited more flowers than in control plots, and 2) foragers recruited more workers to QMP-sprayed artificial feeders than to control feeders.

The effects of in-hive QMP supplements and their method of application also were examined. In colonies used for commercial cranberry pollination, pollen foraging rate and pollen load size were reduced with daily QMP supplements in a spray application, but not in long term lures that supplied less QMP. Pollen collection or brood rearing in two groups of overwintered colonies supplemented every other day with QMP on glass slides were not different between QMP and controls. Honey storage was reduced with QMP supplements, but only when pollen also was removed. We conclude that in-hive QMP supplements can affect foraging, but that results are context-specific, and depend on colony state, environmental conditions, and method and quantity of QMP supplement.
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I. INTRODUCTION: THE ROLE OF HONEY BEE QUEEN MANDIBULAR GLAND PHEROMONE IN POLLINATION ENHANCEMENT AND COLONY MANAGEMENT

Highly social insects such as the honey bee (Apis mellifera L.) are characterized by cooperative care of developing young, division of labour and reproduction, and the integration of overlapping generations to perform colony tasks (Wilson, 1971). Integration of individual behaviours within the colony is necessary to achieve the goals of colony growth and reproduction. A hierarchy of cues, both within and external to the hive, stimulate colony members to perform various functions, and inhibit workers from performing others. The colony's queen, its only fertile female reproductive, exerts considerable influence over worker activities and colony attributes, not only through her reproductive capacity, but also by her physical presence and the chemical control afforded her by pheromones.

Pheromones have a role in communication throughout many insect orders and for many purposes, including mate attraction, aggregation, oviposition, trail-marking, orientation, and alarm behaviour. In social insects, pheromones are essential for colony functioning. For example, honey bee queen mandibular gland pheromone (QMP) affects such diverse activities as the rearing of new queens, worker attraction, drone attraction during mating, suppression of swarming, and foraging (reviewed by Winston and Slessor, 1992). QMP, a complex blend of five semiochemicals, is produced in the queen's mandibular glands, from which it is secreted and distributed over her body. Workers attracted to this pheromone form a retinue around the queen, picking it up with their mouthparts, antennae and/or forelegs. They then act as messengers, distributing the pheromone to other workers and depositing it on the comb as they move throughout the hive, interacting with other colony members.
(Naumann, 1991; Seeley, 1979). Workers can detect the queen's absence from the colony within minutes of her removal, similar to the time it takes for QMP to be removed from circulation, and begin rearing replacement queens as little as 10 hours later (Seeley, 1979).

The recent elucidation and synthesis of honey bee queen mandibular gland pheromone (Kaminski et al., 1990; Slessor et al., 1988, 1990) has provided opportunities for studying its effects on honey bee colonies, and a potential tool for use in colony management. As shown in various experiments, QMP is attractive to workers, both in a retinue (Slessor et al., 1988; Kaminski et al., 1990) and in swarm clusters (Winston et al., 1989), inhibits the rearing of new "emergency" queens when a colony becomes queenless (Pettis et al., 1994; Winston et al., 1989, 1990), suppresses or delays swarming (Winston et al., 1991), and can enhance pollen foraging in newly established colonies (Higo et al., 1992).

Honey bees have become an integral part of modern agriculture because of their role in honey production and crop pollination (Winston and Slessor, 1992, 1993). Adequate pollination by honey bees affects both quantity and quality of crop yields (Free, 1993; McGregor, 1976). The attractiveness of QMP to workers has led to recent efforts to enhance pollination by applying QMP on flowering crops, including blueberry, cranberry, pear, apple, sweet cherry, onion seed, almond, kiwi fruit and canola crops (Currie et al., 1992a,b; Naumann et al., 1994). The most dramatic enhancement of pollination by spraying with QMP has occurred in crops lacking a rich nectar reward, such as cranberry and pear, to which honey bees are not readily attracted (Currie et al., 1992a,b).

Queen pheromone is not normally encountered while foraging, so its effectiveness in enhancing pollination is an interesting research subject. QMP sprays on flowering crops can result in increased forager numbers and quality and quantity of
crop yields (Currie et al., 1992a,b; Naumann, 1994), but the mechanisms responsible for this pollination enhancement are not known. One possibility is the honey bee dance language recruitment system which may generate greater forager numbers in QMP-sprayed plots. Also, honey bees may alter their behaviour or time budgets when foraging in the presence of QMP.

The effects of queen pheromones within the colony also might be used in colony management. Early results with one QMP component, 9-keto-2(E)decenoic acid (9ODA), suggested that QMP can stimulate nectar foraging (Jaycox, 1970a). Addition of the full QMP blend to newly-hived packages in the spring stimulated pollen collection, although no QMP supplement effects were detected in large established colonies in the summer (Higo et al., 1992). Under certain conditions of colony management, stimulation of foraging and brood rearing is desirable, particularly for pollination and when quick colony growth is desired. Crop pollination might be enhanced if colonies could be stimulated to increase their foraging, particularly for pollen, benefiting crops for which adequate pollination by honey bees is difficult to achieve due to low nectar rewards.

Honey bee colonies in temperate climates experience a dearth period throughout the winter during which little brood is reared. The population declines, and subsistence depends on utilization of adequate stores of honey and pollen. QMP stimulation of foraging and brood rearing in colonies emerging from the winter dearth might increase their resource accumulation and population growth, resulting in stronger colonies that are better able to capitalize on nectar flows as they occur throughout the season.

The experiments described here examined the use of QMP in several contexts of honey bee management. Initially, we examined potential mechanisms of enhancement of honey bee pollination with synthetic QMP sprayed on flowering crops.
We 1) observed honey bees foraging in QMP-sprayed and control plots in two different crops, and 2) examined effects of QMP encountered at artificial feeders on recruitment and associated in-hive behaviours.

We also examined the effects of in-hive supplements of QMP on foraging and brood rearing. In colonies used for commercial pollination of cranberries, we assessed foraging rate and pollen load size, and in two groups of overwintered colonies we assessed honey production and either pollen collection or brood rearing.
II. MECHANISMS BY WHICH HONEY BEE QUEEN PHEROMONE SPRAYS ENHANCE POLLINATION

Introduction

Worker behaviour in social insect colonies is mediated in part by the queen, by both her physical presence and release of pheromones. Most highly social insects use pheromones more than physical dominance to influence worker activities. In honey bees, the queen's mandibular gland pheromone (QMP) maintains social cohesion and influences many colony activities (Free, 1987; Winston, 1987; Winston and Slessor, 1992), including suppression of emergency queen rearing (Winston et al. 1989, 1990), and swarming (Winston et al. 1991), attraction of drones for mating (Butler and Fairey, 1964; Gary, 1962), and stimulation of foraging (Free, 1967; Higo et al. 1992; Jaycox, 1970a,b). Worker attraction also is one of this pheromone's most important functions, in retinue formation and queen attendance within the colony (Gary, 1961; Velthuis, 1972; reviewed by Winston and Slessor, 1992), and in swarm cluster attraction and stabilization outside the colony (Ferguson et al., 1979; Morse, 1963; Morse and Boch, 1971; Winston et al., 1989).

The attraction of honey bee workers to the queen's mandibular gland pheromone (QMP, marketed as "Fruit Boost") is currently being investigated as a management tool to enhance pollination. A major concern in pollination is the attraction of bees to the desired target crops, particularly when attractive competing flowers are present (Free, 1968). QMP sprays during pollination have been effective in increasing bee numbers, crop yields, and fruit quality in several fruit and berry crops (Currie et al., 1992a,b; Naumann et al. 1994; reviewed by Winston and Slessor, 1993), particularly those that are relatively unattractive to honey bees due to low nectar rewards, such as cranberry or pear (Hutson, 1925; Marucci, 1966).

Spraying queen mandibular gland pheromone on blooming crops to attract
foragers is a novel context for honey bee workers, as they do not naturally encounter queen pheromones while foraging (Free, 1987; Winston, 1987). Thus, its demonstrated effectiveness in pollination enhancement is intriguing. One possible mode of action of QMP encountered at flowers may involve foraging differences that increase inter-floral pollen movement. QMP may have behavioural effects, with different foraging techniques being used in its presence, or improved pollination may result from increased floral visits and resultant increases in pollen movement.

QMP sprays also might elicit increased recruitment, attracting surplus foragers from within the colony and enticing foragers away from attractive competing vegetation. Recruitment occurs in honey bees when successful foragers returning to the nest from a forage location solicit more workers to forage, and provide location and forage quality information through the dance language, particularly the well-known waggle dance (von Frisch, 1967). The waggle dance not only imparts distance and direction information to follower bees, but also releases and enhances the activity of follower and attendant bees (Bozic and Valentincic, 1991; Michelsen et al., 1989). Numerous aspects of the dance language affect recruitment, including time and number of waggle runs, as well as sound production during wagging (von Frisch, 1967; reviewed by Gould, 1976 and Gould et al., 1985; Kirchner and Sommer, 1992; Michelsen et al., 1992; Seeley and Towne, 1992; Waddington and Kirchner, 1992).

Increased recruitment induced by QMP sprays on crops could operate by heightening foragers' perceptions of forage quality, causing them to more actively promote their forage location via an increased number of dance circuits, resulting in a longer waggle dance. The forager's motivational state, which is determined by her perception of forage quality and influenced by colony stores and requirements, is an important factor reflected in the intensity of recruitment dances and foraging (von Frisch, 1967; Seeley and Towne, 1992; Stabentheiner and Hagmüller, 1991).
Increased recruitment in response to QMP odours also might occur more subtly, perhaps by making the recruitment target easier to locate by recruits, without inducing obvious behavioural differences within the hive.

Our objectives in this study were to 1) investigate potential modifications of foraging behaviour on QMP-sprayed crops, and 2) assess the impact of QMP sprays on forager recruitment. First, we observed forager behaviour and time budgets on blooming crops sprayed with QMP and compared them to those of bees in unsprayed control plots. Second, we examined the recruitment response to QMP sprays at artificial feeders in conjunction with behavioural observations of foragers returning to the colony, and explored the relationship between pheromone dose and recruitment.
Materials and Methods

1. Field observations on blueberry and cranberry

**Blueberry**  This experiment was conducted from 27 April to 6 May 1992 in Richmond, B.C., Canada, on highbush blueberry (*Vaccinium corymbosum* L., va. Bluecrop). Ten 50 m² plots within an 8 ha field were laid out daily, weather permitting, and treatments randomly assigned. Five plots were sprayed with QMP beginning at 1030 h PDT using a backpack sprayer at 500 queen equivalents (Qeq)/ha (0.05 Qeq/m²), an intermediate dosage between two that enhanced blueberry pollination (Currie *et al.*, 1992b). The other 5 plots were unsprayed controls, as no effect of spraying controls with water carrier had been detectable in previous experiments (Currie *et al.*, 1992a,b; Naumann *et al.*, 1994). One Qeq is the average amount of QMP contained in a queen's mandibular glands (Slessor *et al.*, 1988, 1990), and consists of 250 µg 9-keto-2(E)-decenoic acid, 150 µg (86% R-)-9-hydroxy-2(E)-decenoic acid, 20 µg methyl p-hydroxybenzoate and 2 µg 4-hydroxy-3-methoxyphenylethanol.

We began recording forager behaviour one hour after spraying using a portable laptop computer and EventlogR software. A maximum of five individual foragers were observed consecutively in each plot for a total of 20 minutes. Forager time budgets were monitored by measuring total time in plot, duration and number of flower contacts, and inter-floral flight time for each forager. This experiment was replicated on four dates. Data were checked for normality and homogeneity of variance, and log-transformed and analyzed by anova (SAS 1988). All data are presented as untransformed means.

**Cranberry**  Forager behaviour was observed on QMP-sprayed cranberry (*Vaccinium macrocarpon* Ait.) crops on two dates and locations: on 11 June 1992 in Pitt Meadows, B.C. (va. Stevens), and on 15 June 1992 in Richmond, B.C. (va. Bergman). QMP was aerially applied between 0600 and 0900 hours PDT at 100 Qeq/ha (the most
effective dose tested on cranberry by Currie et al., 1992b) and observations began at 1130 hours. In Pitt Meadows, foragers were observed at random locations in 5 control and 5 QMP-sprayed plots (0.4 ha) for a maximum of 20 minutes per plot. In Richmond, foragers were observed at 5 random locations within one 7.8 ha sprayed and one neighbouring 3.2 ha control plot for a maximum of 20 minutes per location. Observations for each forager included total time in plot, inter-floral flight time, and floral contact time, and the number of flowers visited. Data were checked for normality and homogeneity of variance, and log-transformed and analyzed by ANOVA (SAS 1988). All data are presented as untransformed means.

2. Recruitment Experiment

This experiment was conducted from 16-31 July, 1992 and 10 June-28 July, 1993. Foragers from a 2-frame glass-walled observation colony with approximately 4000 workers (1992) or 4-frame observation colony with approximately 8500 workers (1993) were trained to two separate artificial feeders using standard training techniques (von Frisch, 1967; Gary and Witherell, 1971). Entrance and exit of bees was restricted to one side of the hive so that the dances of returning foragers could be observed (Seeley and Towne, 1992). Each artificial feeder consisted of a 45 cm square polyethylene- and paper-covered table, with a 100 ml, 5 cm diameter glass jar containing sugar syrup. This was inverted over filter paper on the top cover of a disposable Plexiglass petri dish vented with 5 mm holes (the scent reservoir). Feeders were located 300 m from the colony, at a 30° angle from each other to permit directional interpretation by recruits and minimize directional effects of wind. Sugar concentration at both feeders was left at 0.25 M (moles/liter) between experimental sessions and raised simultaneously to a concentration of either 2.0 or 2.5 M during experiments. Solutions were laced with anise extract (5 µl per 100 ml) which also was used in the scent reservoirs at both locations. Foragers were paint-marked on the
abdomen at each feeder and number-tagged on their return to the colony. Once a base group of 20 numbered foragers was returning to each feeder, treatments were sprayed on the upper surface of the feeder platform prior to beginning the experiment. Doses were lower than in field sprays due to the two-dimensional quality of the feeder surface, and consisted of a low ($10^{-4}$ Qeq/m$^2$, 1992 and 1993), or a high ($5 \times 10^{-3}$ Qeq/m$^2$, 1993) dose QMP in water application and a water control.

Following spray application, new (unmarked) recruits to each feeder were counted and captured for a total of 90 minutes. Sugar concentration at both feeders was then reduced to eliminate recruitment for a minimum of one hour. Weather and time permitting, treatments were then reversed and the experiment repeated. The effect of location on number of recruits was tested with analysis of variance, and no location differences were found ($p>0.05$). Total recruitment in each observation period varied widely, from 15 to 288 recruits, due to changing colony and environmental conditions throughout the experiments. For this reason, the number of recruits at each feeder was converted to percent of total recruits for that observation period and analyzed using the Wilcoxon rank sum test (SAS, 1988).

**In-hive observations** Concurrent with the recruitment experiment, numbered foragers returning to the colony were observed and their in-hive behaviour recorded using EventlogR software on a portable laptop computer. Each bee was observed only once per observation period, and parameters associated with dance language recruitment or forage quality were measured, including: total in-hive time, search time (time until unloading began), time spent unloading, walking over the comb, and dancing, total time and number of waggle runs and number of separate dance bouts performed by each bee. Control vs. low QMP treatment data from 1992 and 1993 were pooled, and analyzed by anova (SAS 1988). Linear regression was used to characterize the effects of in-hive behaviours and dance language parameters on the number of recruits.
and analyses of regression line slopes to detect differences between the low QMP and control treatments on parameters affecting recruitment (Student's t-tests, Zar, 1984).

Two-tailed tests were used throughout the analyses as honey bee responses to pheromones may be positive or negative, depending on context and concentration.
Results

1. Field observations on blueberry and cranberry

Blueberry  Flower visitation rates were similar in QMP-sprayed and control plots (2.81±0.23 and 2.95±0.45 flowers per minute, respectively, \( p>0.05 \)). Foragers spent significantly longer time periods in QMP-sprayed plots than in control plots (Fig. 1, \( p=0.005 \)). In QMP plots, bees spent more time in inter-floral flight (\( p=0.005 \)), brief floral contacts (\( p=0.03 \)), and nectar removal (time in flowers, \( p=0.41 \)), and visited significantly more flowers (\( p<0.04 \)).

Cranberry  Flower visitation rates were similar in QMP-sprayed and control plots (5.32±0.42 and 6.79±0.58 flowers per minute, respectively, \( p>0.05 \)). In the QMP treatment, mean plot and floral contact time were significantly longer than in control plots (Fig. 2, \( p=0.02 \) and \( p=0.04 \), respectively). Mean inter-floral flight time and number of flower visits also were greater, but these results are equivocal, as the differences were not statistically significant (\( p=0.06 \) and \( p=0.13 \), respectively).

2. Recruitment Experiment

  The percent recruits in the \( 10^{-4} \) Qeq treatment was greater than in the control in both 1992 (\( p<0.04 \)) and 1993 (\( p<0.07 \)) and was highly significant (\( p<0.004 \)) when data from both years were pooled (Fig. 3). In 1993 there was no statistically significant difference in percent recruits in the high QMP dose (\( 5\times10^{-3} \) Qeq/m²) when compared to either the control (\( p=0.999 \)) or the low QMP dose (\( p=0.14 \)), although the percent of recruits in the low QMP dose was greater, and the differences may have been significant with greater replication (Fig. 3).
Fig. 1. Mean total time per bee (±S.E.) spent in control or QMP-sprayed plots, composed of time in inter-floral flight, in flowers, and in brief floral contacts (i.e., bee lands on flower but does not probe corolla), and the mean number of floral visits/bee while foraging on highbush blueberry (*Vaccinium corymbosum* L.). Pairs of bars topped by (*) were significantly different (p<0.05).
Fig. 1
Fig. 2. Mean total time per bee (±S.E.) spent in control or QMP-sprayed plots, composed of time in inter-floral flight and floral contacts, and the mean number of floral visits/bee while foraging on cranberry (*Vaccinium macrocarpon* Ait.). Pairs of bars topped by (*) were significantly different (p<0.05).
Fig. 3. Mean percent (±S.E.) of total new recruits to each feeder per 90 min. period in paired tests of control vs. low dosage (1992/93, n=15) and control and low vs high dosage (1993, n=5). Pairs of bars topped by (*) were significantly different (p<0.05). Actual mean number (±s.e.) of recruits was 23.4±6.4 vs 34.3±7.6 for control vs low doses (1992/93); 101.2±23.4 vs 89.4±9.2 for control vs high (1993); and 67.8±25.1 vs 33.0±8.8 for low vs high (1993).
Fig. 3
**In-hive behaviours.** Low dose QMP bees began unloading more quickly than control bees (p=0.04) and spent less time unloading (p=0.02, Fig. 4c). We also found a shorter in-hive time (p=0.42), with more time spent dancing (p=0.39), including waggle run time (p=0.36) and less time spent walking on the comb surface (p=0.13) in the low QMP treatment, but these differences were not significant. The mean number of separate dance bouts in the low QMP treatment also was greater than in controls, but not significantly so (p=0.40). In-hive behaviours in response to the high dose QMP spray were not statistically different from either the controls or the low QMP treatment (Figs. 4a & 4b, p>0.05).

Regressions of the number of recruits on mean dance time, waggle time, and number of waggle runs were positive and significant in the low QMP treatment and control groups (Fig. 5). There was a significant regression in the QMP, but not in the control treatments for time spent walking in the hive and number of separate dance bouts. Slopes of regression lines were significantly different (p<0.05) between QMP and control treatments for both number of separate dance bouts, and mean time spent in waggle runs.
Fig. 4. Mean (±S.E.) of total in-hive time, total time in observed behaviours, and number of separate dance bouts per forager returning from low ($10^{-4}$ Qeq) vs. high QMP ($5 \times 10^{-3}$ Qeq, fig. 4a), control vs. high QMP (fig. 4b), and control vs. low QMP (fig. 4c), treatments sprayed at artificial feeders. Pairs of bars topped by (*) were significantly different ($p<0.05$).
Fig. 4

Mean dance bouts per bee (±S.E.)

Mean Seconds (±S.E.)

- a. Low vs. High QMP
  1993 n=4

- b. High QMP vs. Control
  1993 n=4

- c. Low QMP vs. Control
  1992/93 n=13

- Control
- 5x10^-3 Queq
- 10^-4 Queq
Fig. 5. Regressions of in-hive behaviour on the number of recruits for the low QMP and control treatments. Significant differences in slopes (B, p<0.05) of the lines are indicated by (*).
Fig. 5

- ▲ Low QMP treatment
- ▼ Control

### Mean time (s) walking in hive per bee

- $r^2 = 0.39$
- $p = 0.01$
- $B = -0.72$

### Mean no. of waggle runs per bee

- $r^2 = 0.10$
- $p = 0.16$
- $B = -0.41$

### Mean dance time (s) per bee

- $r^2 = 0.71$
- $p = 0.0002$
- $B = 5.863$

### Mean dance bouts per bee

- $r^2 = 0.31$
- $p = 0.035$
- $B = 5.183$

### Mean time (s) in waggle runs per bee

- $r^2 = 0.71$
- $p = 0.0002$
- $B = 6.668$

### Mean dance time (s) per bee

- $r^2 = 0.68$
- $p = 0.0003$
- $B = 2.46$

### Mean dance bouts per bee

- $r^2 = 0.31$
- $p = 0.03$
- $B = 2.07$

### Mean dance time (s) per bee

- $r^2 = 0.43$
- $p = 0.009$
- $B = 38.11$

### Mean dance bouts per bee

- $r^2 = 0.04$
- $p = 0.48$
- $B = 9.67$
Discussion

Our results indicate that two different factors contribute to increases in forager numbers and yields in QMP-treated crops. First, foragers stayed longer in QMP-treated plots, and visited more flowers, which would increase both forager numbers and inter-floral pollen movement. Second, foragers recruited more workers to feeders sprayed with the low dose of QMP, and some effects were seen on their in-hive recruiting behaviours. The high QMP concentration was not effective in increasing recruitment.

Blueberry and cranberry field observations

Field observations demonstrated that foragers spent more time in QMP-sprayed plots. This result was consistent in both blueberry and cranberry, suggesting that increased plot time, time in flowers, and floral visits are mechanisms by which QMP sprays may enhance pollination. The increased time spent on the crop included increased time in floral contacts and inter-floral movement. Increased numbers of flowers visited before a forager returns to the colony to deposit nectar and/or remove her pollen loads would result in increased pollen transfer between flowers. Pollination is enhanced by multiple pollinator visits, particularly in fruits that are multi-seeded such as cranberry or blueberry. These berries require good pollination and fertilization for large, regularly-shaped fruits, with berry size correlated to the number of seeds per fruit (McGregor, 1976; Westwood, 1978; Free, 1993). In addition, more time spent by foragers in sprayed plots would increase bee numbers over time as additional foragers accumulate, and flowers in those plots would benefit from increased opportunities for multiple visits by foragers.

Recruitment

The percentage of recruits arriving at the low QMP treatment feeders compared to the control feeders was consistently higher in both years of the
experiment, despite fluctuations in recruitment due to changing colony and environmental conditions. Thus, increased recruitment to sprayed plots is a second mechanism that explains increased bee numbers and enhanced pollination and yields following QMP sprays on blooming crops. The process of recruiting new foragers to a location can have a compounding effect, with new recruits motivating and recruiting more foragers to the same location (Bozic and Valentincic, 1991; Kirchner, 1993).

Our results are even more striking because compounded recruitment was prevented by our experimental design in which we continuously removed new recruits. In a normal field situation it would take only a small initial increase in recruitment to produce a large increase in the foraging force at a sprayed site, assuming no negative feedback on recruitment from the rising number of foragers.

**Dose Response**

Our finding that a low QMP dose was more effective than a higher dose is consistent with other QMP dose-response experiments in which higher pheromone dosages did not result in the best response. In QMP crop sprays on cranberry (Currie et al., 1992b), forager numbers and yield as measured by both number of berries and total weight were higher with the application of 100 Qeq than with 1,000 or 10,000 Qeq/ha. In blueberry, the highest dosage of 10,000 Qeq/ha was not different from the controls in number of bees/bush, and produced the lowest yield in g/100 flowers of three dosages tested (100, 1000, and 10,000 Qeq/ha, Currie et al., 1992b). Within colonies, Winston et al. (1990) added various dosages of QMP to de-queued colonies and obtained equal suppression of queen rearing with 1 and 10 Qeq/day. In retinue bioassays, attraction to and contacts with lures at dosages higher than $10^{-2}$ Qeq is reduced (Pankiw et al., 1994; Kaminski et al., 1990). Colonies newly started from packages displayed greater foraging and larger nectar and pollen loads with 1 Qeq daily supplements than with 10 Qeq, which was not different from the control
treatment (Higo et al., 1992). From these experiments, conducted in many contexts, it appears there is an upper threshold beyond which excess QMP becomes ineffective.

In-hive behaviours

The significantly shorter search and unloading times for foragers returning from the low QMP treatment compared to controls were similar to the shorter search and unloading times found for foragers returning with a higher value nectar reward (Seeley, 1986). Honey bee foragers choose more concentrated (35-60%) sugar solutions, all else being equal (Waller, 1972). Further, foragers returning from preferred nectar sources search more vigorously for unloaders and thus are unloaded more quickly by in-hive bees (Seeley et al. 1991), and also display more intense recruitment dances with a higher rate of recruitment (von Frisch, 1967; Seeley, 1986; Waddington, 1982).

The mechanism responsible for the more rapid unloading we observed is not clear, but there are several possibilities. First, workers may be returning to the hive with a small amount of queen pheromone, which might cause nectar receivers to treat them preferentially. However, this seems unlikely due to the small amount present in the area used for feeding by a single bee (approx. $10^{-8}$ Qeq/cm²), of which a forager could pick up only a very small portion. Preliminary experiments in which we topically applied aliquots of QMP ($10^{-4}$ Qeq) to foragers at artificial feeders gave no indication of any effect on recruitment (H. Higo, unpublished observations), but a full range of dosages would need to be tested to properly evaluate this hypothesis.

A second possibility is that foragers exposed to QMP in sprayed crops may experience an overall increase in activity level. Seeley (1979) noted a general increase in activity in colonies by messenger bees that move throughout the colony at an increased pace following queen contact, thereby distributing queen pheromone (Naumann et al., 1991, 1992). Increased forager activity levels may result in greater
recruitment through an increased number of waggle runs, or a greater likelihood of dancing, and may be reflected in dances of greater intensity, the increased "vivacity" or "liveliness" of the dance referred to by von Frisch (1967) and Gould (1976). Queenless bees show an increased metabolic rate in response to some queen mandibular gland components, queen head extracts, and live queens (Moritz and Crewe, 1988). Increased metabolic rates may result in increased body temperatures, similar to that found in foragers at artificial feeders (Schmaranzer and Stabentheiner, 1988; Waddington, 1990), and by dancers back in the colony in response to more concentrated nectar sources (Stabentheiner and Hagmüller, 1991). Potential recruits may be more readily recruited by a high temperature (QMP) dancer, possibly switching from other in-hive activities, or from cursory attending of dances to more intense dance-following prior to foraging (Bozic and Valentincic, 1991; Seeley and Towne, 1992). However, this speculation remains to be demonstrated experimentally. Stabentheiner and Hagmüller (1991) suggested this hypothesis might be examined using a heated model of a dancing bee (Michelsen et al., 1989), but tests with a heated mechanical model were unsuccessful due to bees in the colony attacking the model as the "dancer's" temperature rose above 34°C (Michelsen et al., 1992).

An increased metabolic rate and/or activity level in QMP-exposed foragers would also explain the trends we found towards a shorter in-hive time, with more time spent dancing, less time spent walking on the comb surface, and more separate dance bouts. Although these particular in-hive behaviours were not statistically different between treatments, the regression analyses of their effects on the number of recruits support their importance as potential enhancers of recruitment in this experiment. These variables all produced significant regressions as expected from previous research in which dance duration and the number of waggle circuits were related to recruitment (von Frisch, 1967; Kirchner, 1993; Seeley and Towne, 1992). Higher
levels of significance and $r^2$ values in the QMP treatment relative to controls in each case indicate less variability and a better fit of the QMP data to the regression line. It is interesting that QMP enhanced some aspects of recruitment that were not significant in controls. Significantly higher slopes of the low dose QMP treatment regression lines in the number of dance bouts and waggle run time indicate that QMP at the forage site affected in-hive behaviour to make these variables more important in predicting recruitment, at least in this foraging situation.

The number of separate dance bouts elicited a significant regression on the number of recruits in the low QMP treatment, but not in the controls. Although separate dance bouts are mentioned in previous research (Fergusson-Kolmes et al, 1992; Seeley, 1994; Waddington and Kirchner, 1992), this factor has not been evaluated for an effect on recruitment. If a forager alternates dancing and unloading via trophallaxis, and then resumes dancing in a different location or to a different group of followers and attendants, the potential for recruitment should be enhanced. This aspect of recruitment behaviour deserves further study.

The use of QMP sprays on blooming crops can enhance pollination (reviewed by Winston and Slessor, 1993), but the mechanisms responsible for the success of this unnatural context in which worker bees encounter queen pheromone have previously been unknown. This study has revealed two mechanisms that could result in enhanced pollination by foraging honey bees in the presence of QMP: 1) increased recruitment, and 2) increased time spent and flowers visited by foragers in QMP-sprayed plots. These results at least partly explain why queen mandibular pheromone, an essential component within the honey bee colony, can be used successfully in the field as a management tool to manipulate foraging behaviour.
III. SUPPLEMENTAL QUEEN MANDIBULAR PHEROMONE IN HONEY BEE
(APIS MELLIFERA L.) COLONIES: EFFECTS ON
FORAGING AND BROOD REARING

Introduction

Individual honey bee behaviours are mediated by a hierarchy of environmental and within-colony cues. Some of these cues originate from the queen, largely via her chemical influence provided by pheromones. For example, queen mandibular pheromone (QMP), a blend of five compounds produced in the queen's mandibular glands, affects queen rearing, swarming, foraging, worker attraction for retinue formation and swarm clustering, and drone attraction for mating (Butler and Fairley, 1964; Ferguson et al., 1979; Free, 1967, 1987; Gary, 1961, 1962; Higo et al., 1992; Jaycox, 1970a,b; Morse, 1963; Morse and Boch, 1971; Pettis et al, 1994; Velthuis, 1972; Winston, 1987; Winston et al., 1989, 1990, 1991; Winston and Slessor, 1992).

Supplementing the queen's natural pheromone production with synthetic QMP can induce an increase in pollen collection under certain conditions (Higo et al., 1992). Colony and environmental conditions are important in determining the response to QMP supplements, as this stimulation of pollen collection only occurred in package bees established early in spring, but was not found in larger, well-established colonies during the summer honey flow.

In-hive QMP supplements also might improve crop pollination by inducing higher levels of pollen foraging in those colonies. The attractiveness of queen pheromone to workers and the recent elucidation of the complete pheromonal blend from the queen's mandibular glands (Slessor et al., 1988, 1990; Kaminski et al., 1990) have led to recent advances in spraying QMP on blooming crops and increasing bee visitation, thus enhancing pollination and subsequent crop yields (Currie et al., 1992a,b; Naumann et al., 1994; Winston and Slessor, 1992, 1993). The mechanisms
involved in QMP-enhanced pollination include increased recruitment of workers to QMP-sprayed plots by foragers returning to the nest, and increased time spent and number of flowers visited by foragers (Chapter II). Similarly, in-hive applications of QMP might be beneficial in crops that produce pollen but do not have high nectar rewards for honey bees, thus making adequate pollination difficult to achieve (Hutson, 1925; Marucci, 1966). Increased pollen foraging in colonies may be reflected in either more pollen collected and stored or higher levels of brood-rearing in colonies that consume larger amounts of collected pollen (Allen and Jeffree, 1956; Al-Tikrity et al., 1972; Free, 1967; Hellmich and Rothenbuhler, 1986; Todd and Reed, 1970). Changes in pollen collection can occur both at the colony level, in numbers of returning pollen foragers, and at the individual level, in pollen load size (Eckert, 1990; Fewell & Winston, 1992; Higo et al., 1992).

Nectar collection also may be influenced by the presence of the queen and QMP. The addition of one QMP component, 9-keto-2(E)-decenoic acid (9-ODA), to groups of caged queenless workers resulted in nectar foraging similar to that of caged queenright workers, and greater than in queenless controls (Jaycox, 1970a). However, the context of pheromone supplements must be stressed. Additions of 9-ODA to colonies in a field situation did not affect nectar collection (Jaycox, 1970a), and the addition of the full QMP blend to large queenright colonies during the summer honey flow or to colonies newly established from packages did not significantly affect nectar foraging and honey storage (Higo et al., 1992).

In the current experiments, we examined the effects of in-hive QMP supplements on foraging and brood rearing in two different contexts: 1) colonies used for cranberry pollination, in which an increase in foraging, particularly for pollen, may have enhanced pollination of the cranberry crop, and 2) colonies moved outdoors in the spring from an indoor overwintering building, which may have benefitted from an
early growth in colony population resulting from stimulation of foraging and/or brood-rearing.
Materials and Methods

1. Cranberry pollination colonies

This experiment was conducted in cranberry (*Vaccinium macrocarpon* Ait., Bergman cultivar) fields in Richmond, B.C., from 19 to 27 June, 1991. Two days after commercial honey bee colonies were moved in for crop pollination, 30 queenright colonies, each housed in 2 Langstroth hive bodies, were selected based on a minimum colony strength of 8 frames of bees and 3-4 frames of brood. Colonies were not manipulated during the experiment, except for pheromone applications.

Treatments were assigned randomly to colonies, with 10 replicates per treatment, and consisted of (1) an untreated control, (2) a one queen equivalent (1 Qeq) pheromone spray, diluted in isopropanol and applied each morning, and (3) two stationary lures (500 \( \mu \)m mesh bubble caps loaded with 10 Qeq of pheromone, supplied by Phero Tech, Delta, B.C.) mounted in the center of the brood area at the beginning of the experiment. One Qeq is the amount of pheromone contained in an average pair of queen mandibular glands, and consists of 250 \( \mu \)g 9-keto-2(\( E \))-decenoic acid, 150 \( \mu \)g (86\% R-) 9-hydroxy-2(\( E \))-decenoic acid, 20 \( \mu \)g methyl-p-hydroxybenzoate, and 2 \( \mu \)g 4-hydroxy-3-methoxyphenyl ethanol. At the end of the experiment, the stationary lures were removed and analyzed for remaining QMP. Correlation analyses were conducted between the amount of QMP removed from the lures and forager counts and pollen loads (SAS, 1988).

Returning foragers were counted twice daily, weather permitting, for 2.5 minute periods, commencing at 0945 and 1330, PDT. Foragers with and without pollen loads were counted separately. Forager count data were checked for normality and homogeneity of variance, log-transformed and analyzed by anova and Tukey's means separation test to detect differences between treatments (SAS, 1988).

Ten pollen foragers per colony were collected daily, between 1130 and 1230
and immediately frozen on dry ice. Pollen in their corbiculae was later removed and weighed.

2. Overwintered colonies:

The effects of QMP supplements on brood rearing and pollen collection were examined separately in two groups of colonies located near Fairview, Alberta. Colonies had been prepared as nuclei with queen cells the previous June, wintered indoors as single-chambered colonies and moved outdoors in early April to a spring apiary site. Colonies for the two studies were moved to different apiary sites on 11 May 1992 and equalized for brood and bees. The brood experiment utilized 20 colonies (10 per treatment) that consisted of single brood chambers (all standard deep Langstroth equipment) and commenced on 23 May 1992, with the addition of either 3 Qeq of QMP or isopropanol (solvent) controls on glass slides. Slides were replaced on alternate days until 6 June (8 treatment applications) and sealed brood area was measured on 5 and 23 June. Four colonies became queenless during the experiment and were therefore removed from the statistical analysis.

The pollen collection experiment also used 10 colonies per treatment and was initiated on 27 May 1992 with the addition of 3 Qeq of QMP or solvent controls on glass slides. Slides were replaced on alternate days, ending on 10 June (8 treatment applications). Colonies had been fitted with an Ontario Agricultural College type pollen trap before the experiment began, and pollen was removed from traps and weighed on alternate days from 27 May to 10 June, and on the 15th, 21st, and 23rd of June. Three colonies became queenless and were removed from the data analysis.

Colonies in both the pollen and brood rearing experiments were managed for honey production throughout the experiment by adding brood chambers and honey supers as required. Honey supers were weighed before and after placement on
colonies to obtain honey storage data. Data from the brood rearing and pollen collection experiments were analyzed by anova (SAS, 1988).
Results

1. Cranberry pollination colonies

Forager numbers  The mean number and percent of pollen foragers were significantly lower for the spray treatment than either the control or stationary lure treatments (Fig. 6, p<0.05). There were no differences in means of either non-pollen or total foragers between treatments (p>0.05).

Pollen loads  Foragers in the spray treatment carried significantly smaller pollen loads than in the control (p<0.05), but the lure treatment was not different from either (Fig. 7).

Pheromone lure uptake  Lure analysis at the end of the experiment determined that a mean of 2.21±0.24 Qeq of QMP per colony was removed during the experiment, resulting in an average daily QMP uptake of .28 Qeq/day. A significant negative correlation was found between QMP uptake and pollen load size (r = -0.72, p = 0.02); the more QMP removed from the lures, the smaller were the pollen loads (Fig. 8).

2. Overwintered colonies

Brood Rearing Experiment:  There was no difference between treatments in sealed brood area on either assessment date or when the two brood measurements were combined, or in mean weight of stored honey at the end of the summer(p>0.05, Fig. 9).

Pollen Collection Experiment:  The quantities of incoming pollen taken from pollen traps were not significantly different between QMP and control colonies when analyzed by date or combined (p>0.05, Fig. 10). Colonies in the control treatment stored significantly more honey (p=0.034, Fig. 10) when compared to those in the QMP treatment.
Fig. 6. Mean number of foragers with and without pollen, total foragers, and percent pollen foragers returning to colonies in 2.5 minute observation periods. Treatments consisted of 1) 2 bubble cap lures each loaded with 10 Qeq of QMP, 2) a 1 Qeq spray applied to colonies each morning, and 3) untreated controls. Within each parameter, bars with the same letter are not significantly different (p>0.05).
Fig. 6
Fig. 7. Mean pollen load mass removed from the corbiculae of 10 pollen foragers per colony, taken on five days of the experiment. Bars with the same letter are not significantly different (p<0.05).
Fig. 7
Fig. 8. Correlation between mean pollen load mass removed from the corbiculae of foragers from each colony in the stationary lure treatment, and QMP uptake for each colony over the course of the experiment.
QMP uptake (Qeq)

Fig. 8

![Graph showing the relationship between QMP uptake (Qeq) and mean pollen mass (mg). The correlation coefficient (r) is -0.72 with a significance level (p) of less than 0.02.

Fig. 8
Fig. 9. Mean brood area on two assessment dates and their total, and stored honey in QMP-treated and control colonies.
Fig. 9
Fig. 10. Mean weight of trapped pollen and stored honey in QMP-treated and control colonies. (* significantly different, p<0.05).
Fig. 10
Discussion

Colony QMP supplements in this and other experiments produced effects on foraging and brood rearing that differed depending on 1) QMP application method and dose, and 2) the context of the colonies. It should be emphasized that in these QMP in-hive experiments the queen's own production of QMP was supplemented. Differences between controls and QMP treatments should therefore be viewed as effects of the level of QMP available in the colony, not as effects of its presence or absence. In the cranberry pollination experiment, colonies supplemented with a 1 Qeq spray displayed suppression of pollen foraging, both in forager number and pollen load size. Although this method of QMP application provided immediate distribution as sprayed workers moved throughout the colony, the presence of QMP may have been short-lived due to degradation and removal from circulation (Naumann, 1991; Naumann et al., 1991, 1992; Seeley 1979). Pollen and total forager numbers and pollen load size were similar in colonies supplemented with QMP in long-term bubble-cap lures and controls. However, the significant negative correlation between mean pollen load size and QMP uptake from these lures suggests that QMP supplements, ranging from 1.25 to 3.5 Qeq over the course of the experiment, diminished pollen load size in bees from treated colonies. The actual QMP uptake per day is unknown, as stationary lures were analyzed only at the end of the experiment.

The application of QMP on glass slides in the overwintered colony experiments, was similar to previous experiments (Higo et al., 1992; Winston et al., 1989, 1990, 1991), but with a dose of 3 Qeq on alternate days. This QMP supplement did not affect brood rearing or pollen collection. However, QMP-treated colonies stored less honey than controls, but only in the experiment when pollen was trapped. Previous analyses of QMP-laden glass slides after 6, 8, or 24 hours in colonies indicated virtually all of the QMP was removed (Higo et al., 1992; Winston et al.,
1989, 1990, 1991), suggesting that in the present experiments there may have been little QMP left on slides after the first day. It is not clear if QMP supplements were ineffective in influencing brood rearing and pollen collection due to the "pulsed" application, the level of QMP supplements used, or the context of overwintered colonies. Since there was a significant difference between treatments in stored honey in the pollen collection experiment, I suggest that sufficient QMP was provided and that brood rearing and pollen collection were unaffected by QMP supplements in this context. However, it is puzzling that QMP would suppress honey storage in one experiment but not the other.

Colony context, in terms of both internal and external conditions, must be considered in evaluating the effects of QMP supplements. A QMP component (9-ODA) stimulated nectar foraging with queenless caged bees when compared to controls (Jaycox 1970a), and the complete QMP blend added to newly established colonies stimulated pollen foraging. However, these effects were not observed in larger, well-established colonies (Higo et al., 1992; Jaycox, 1970a). Further, QMP supplements either were ineffective or interacted with one or more colony attributes to suppress either pollen foraging or honey storage in both the cranberry and overwintered colony experiments. The pheromonal mechanisms mediating foraging are complex, and difficult to assess in isolation. For example, pollen collection is strongly influenced by the amount and stages of brood and stored pollen in colonies (Barker, 1971; Eckert, 1990; Fewell and Winston, 1992; Fewell et al., 1991; Free, 1967; Moeller, 1972; Schmid-Hempel et al., 1993), so that QMP effects could vary considerably depending on these factors. Stimulation of pollen collection was not detected when pollen forage was abundant, but was evident later in the year when pollen had become scarce (Free, 1967), indicating that environmental factors such as resource availability also could influence QMP effects.
Pollen foraging was only found to be stimulated by QMP supplements in newly hived packages of bees early in the spring (Higo et al., 1992), similar to the establishment of a new swarm. Packages or swarms do not have many attributes of established colonies that might influence foraging, such as stored honey, pollen, and brood, at least when initially founded. Perhaps QMP stimulates pollen foraging only in the absence of these or other stimuli. It is interesting that the stimulatory effect of QMP on pollen foraging in the package experiment was strongest in the first ten days following package establishment, with the effect diminishing as brood rearing and honey and pollen storage increased.

QMP supplements within the colony appear to have both stimulatory and suppressive effects, depending on the application method and colony context. Context-specific effects of pheromones are well-recognized. Weaver (1983) states that "the information transmitted by a pheromone depends on the context in which it is transmitted" and provides examples of honey bee queen pheromone being attractive at a distance to workers in the context of swarming (Butler and Simpson, 1967; Winston et al., 1989) and to drones in mating flights (Gary, 1963), but not at a distance to workers or drones within the colony (Butler et al., 1973).

The effects of QMP supplements applied in the colony on foraging have been variable and difficult to predict due to many parameters, including method of pheromone presentation, dosage supplied, and variations in colony state and external environment. For these reasons, in-hive QMP supplements remain interesting biologically, but do not yet have practical value in manipulating foraging and brood rearing for colony management or crop pollination.
IV. SUMMARY

This study has examined the effects of synthetic queen mandibular gland pheromone (QMP) on colony regulation and worker behaviour. It provided insights useful for the development of QMP applications for pollination enhancement and colony management.

In Chapter II, the mechanisms responsible for the success of QMP in pollination enhancement were examined. Behavioural observations of foragers in sprayed and unsprayed plots in flowering blueberry and cranberry crops revealed that foragers stayed longer and visited more flowers in QMP plots than in controls. Another mechanism of QMP pollination enhancement is the honey bee dance language recruitment system, in which foragers returning to the nest solicit more workers to join them in foraging at their forage site. We found that recruitment to QMP-sprayed feeders was greater than to controls, and foragers returning from QMP feeders were unloaded more quickly. In addition, several in-hive behaviours that are part of the waggle dance recruitment system produced stronger, more highly significant regressions on recruitment in the QMP treatment than in the controls. We conclude that at least two mechanisms may be responsible for the success of QMP sprays in enhancing pollination: 1) longer time spent and more flowers visited by foragers when QMP is present, and 2) increased recruitment to QMP-sprayed crops.

In Chapter III, we investigated the effects of QMP supplements in the hive in several contexts that could be advantageous in colony management. Colonies used for cranberry pollination showed reduced pollen foraging and smaller pollen loads when supplemented with a daily 1 Qeq QMP spray, but pollen foraging in colonies with long term lures that dispensed less pheromone was not different from either the QMP spray or control colonies.

QMP in-hive supplements also were examined in colonies recently moved
outdoors from an indoor overwintering building. No differences were found between QMP and control colonies in brood area or in pollen collected. However, less honey was stored in the QMP treatment, but only in colonies in which pollen was removed in traps.

These experiments underlined the importance of context when using QMP supplements in the hive. QMP is one of many interacting stimuli that determine worker behaviour and overall colony attributes. Effects of QMP supplements in the hive are dependent both on colony and environmental conditions, and may vary depending on dosage and application method.

These experiments have provided insights into the mechanisms responsible for the successful use of QMP in pollination enhancement, and furthered our knowledge of its effects within the colony in different contexts. Its future potential in colony management depends on establishing these parameters and improving our understanding of the effects of this essential queen signal.
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