FORAGING ECOLOGY AND MANAGEMENT OF *BOMBUS* SPP. (HYMENOPTERA: APIDAE) IN AGRICULTURAL LANDSCAPES

A thesis

submitted in partial fulfilment

of the requirements for the Degree of

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by

Mandy C. Barron

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FRONTISPIECE

A marked *Bombus terrestris* foraging on *Phacelia tanacetifolia* flowers

A marked *Bombus terrestris* foraging on *Lavandula* x *intermedia* cv. Grosso flowers

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Bumble bees (*Bombus* spp.) are important pollinators of agricultural crops such as red clover (*Trifolium pratense* L.) in New Zealand. However, there is a lack of knowledge about *Bombus* foraging behaviour and management at the landscape scale. This thesis evaluated habitat manipulation (i.e., provision of nest sites and floral resources) and shifting bumble bee colonies to a crop to enhance bumble bee numbers. Bumble bee flight distances from the nest and responses to different sized forage patches were also investigated.

Habitat manipulation involved placing 80, four-unit bumble bee domiciles around 16 field margins, half of which had been sown with *Phacelia tanacetifolia* Benth.. The effects of the flowers on domicile occupancy could not be determined because most plots were destroyed by plant competition and grazing animals; however, in areas with high naturally-occurring floral diversity, domicile occupancy was higher. Occupancy rates over the three years of the study were 0.31, 4.06 and 8.12 % and the main occupant was *B. hortorum* L. (67.5 %). There was a positive association between domicile occupancy in one year and nest founding in the next.

To supplement forager numbers, four commercially-obtained *B. hortorum* nests were shifted to a 4 ha red clover 'Pawera' seed crop. Foragers were marked with fluorescent powder. Reobservation rates within the crop ranged from 4-15 %. There was a virtual absence of marked foragers within 10 m of the nest. Pollen analysis showed that most (85 %) pollen collectors were visiting the red clover crop exclusively, although some were flying 200 m from the nest to forage on other species. The ratio of marked to unmarked *B. hortorum* foraging on the crop was 1:43, suggesting that adding nests contributed little.

An experiment designed to evaluate whether bees foraged close to the nest showed there was no difference in the number of *B. terrestris* L. on pots of *P. tanacetifolia* at 20 and 200 m from the nest. However, very low numbers of experimental bees were involved. More bees were observed on the *P. tanacetifolia* when the nest was downwind of the plants suggesting that upwind foraging took place; further experiments are needed to confirm this.

Patches of potted *Lavandula* x *intermedia* Lois. were created to test the effects of patch size on the numerical and functional responses of *B. terrestris.* The number of *B. terrestris* visiting the lavender increased with patch size but this was less than proportional. The number of flowers visited per foraging bout increased with patch size but the proportion of available flowers visited decreased. Combining these two responses, the net visitation per flower was independent of patch size.

Pollinator management in New Zealand is discussed. It is suggested that other bee species in addition to *Apis mellifera* L. should be used in New Zealand. Bumble bees are most promising alternative pollinators but the commercial stocking of field crops has not yet proved to be cost-effective. Habitat manipulation is therefore the recommended management option, because of the low inputs and the potential long-term benefits.

Keywords: Bumble bees, pollinator management, *Bombus terrestris, Bombus hortorum, Trifolium pratense*, habitat manipulation, *Phacelia tanacetifolia,* pollen analysis, mark-reobservation, foraging distances, *Lavandula* x *intermedia*, patch size.

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CHAPTER ONE: INTRODUCTION

1.1 Background and development of the thesis

One third of human food is derived from bee-pollinated plants (McGregor 1976). Bumble bees (*Bombus* spp.) are important pollinators of crops especially where honey bees (*Apis mellifera* L.) are ineffective pollinators or are limited by cool conditions (Corbet *et al.* 1991). Chapters two and three of this thesis investigate bumble bee management techniques for crop pollination (habitat manipulation to enhance bumble bee numbers and adding bumble bee colonies to a crop). In Chapters four and five, the focus shifts from an applied to a theoretical perspective (foraging distances from the nest and density-dependent responses to flower patch size). Although this sequence may appear to be illogical, these theoretical questions arose from the results of preceding experiments and are therefore presented in the order in which they were conducted.

The following introduction considers the value of bees, why bumble bees are important pollinators, what resources bumble bees need, the introduction of bumble bees into New Zealand and their use for field crop pollination. Bumble bee nomenclature follows Prys-Jones & Corbet (1987).

1.2 The importance of bees in agriculture

1.2.1 Pollination, a keystone process

Many agricultural crops and wild flowers are entomophilous - dependent on insects for pollination. Pollination is a key step in the sexual reproduction of plants and is defined as the transfer of pollen from the anther of a flower to the stigma of the same (self pollination) or to a different flower (cross pollination) (Free 1993). Cross pollination is essential for seed set in self-incompatible species (e.g., *Medicago sativa* L., *Trifolium* spp.), for the production of hybrid seed, to increase the oil content of seeds (*Helianthus annuus* L.), to improve the quantity of seed set (*Carum carvi* L.), to increase the earliness and uniformity of seed set (*Brassica napus* L.) and to increase fruit quality when fruit size is dependent on the number of seeds (*Fragaria* x *ananassa* Duchesne, *Cucumis melo* L., *Actinidia deliciosa*

(A. Chev.) C.F. Laing et. A.R. Ferguson) (Corbet *et al.* 1991; Free 1993). Even though well studied, the pollination requirements of most crops are still poorly understood. Those of wild flowers are even less well known (Kevan *et al.* 1990; Corbet *et al.* 1991).

Because of the crucial role of pollination in the persistence and viability of plant populations (both wild and managed) it has been called a keystone process (Kevan *et al.* 1990; Kearns & Inouye 1997; Allen-Wardell *et al.* 1998). It has been predicted that the loss of a key pollinator could lead to a decrease in plant seed set, genetic diversity and ultimately to extinction, which in turn could affect other animals dependent on the plant for food and shelter (Kearns & Inouye 1997). The severity of this ecosystem disruption will depend on whether the plant is dependent on a single pollinator, the extent of self compatibility and the dependence of the plant on seed production. A good example of these linkages is between fig trees (*Ficus* spp., Moraceae) and their obligate wasp pollinators (Agaonidae) in tropical forests. A reduction in tree or wasp numbers due to habitat fragmentation could lead to negative feed back reducing the number of fig fruits which are the staple food of many forest vertebrates (La Salle & Gauld 1993).

1.2.2 Bee-plant relationships

The relationship between a plant and its pollinator is a mutualistic one: plants are dependent on pollinators for sexual reproduction and pollinators are dependent on plants for food. Bees (Apoidea), in particular honey bees and bumble bees, are most important pollinating insects (McGregor 1976; Free 1993). They have specialised mouthparts to collect nectar, their hairy bodies are well adapted for carrying pollen, which they collect in large amounts to feed their brood. Also, their systematic foraging patterns ensure that pollen is carried and deposited on compatible flowers (Kevan & Baker 1983; Free 1993).

Bees and flowering plants have a long history of coevolution, beginning over 100 million years ago with the evolution of angiosperms (Kevan & Baker 1983). This partnership is manifested in the structure of flowers that are bee pollinated (collectively, these structures are called the pollination syndrome). Melittophilous or bee pollinated flowers are typically zygomorphic, yellow or blue in colour, have nectar guides, moderate quantities of concealed nectar, and a sweet odour (Faegri & van der Pijl 1979). Selective pressures on the plant to attract reliable pollinators and increase precision of pollination yet exclude inefficient pollinators have resulted in complex floral morphologies. Correspondingly, the bees have evolved specialised behaviours and morphologies to access the hidden rewards.

An understanding of these specialisations can aid in choosing the most efficient pollinators for a particular crop. A mismatch between the pollinator and crop species or cultivar can lead poor pollination and/or the pollinator gaining an inadequate reward from the flowers (Corbet 1991; Fairey 1993).

1.2.3 The need to manage alternative pollinators

In the past, honey bees have been used as the "default" pollinators for most agricultural crops because their management is well understood and they can be shifted in large numbers to a crop. However, recently there has been a growing awareness of the limitations of honey bees as crop pollinators and the need to diversify pollination strategies. This is because varroa mite (*Varroa jacobsoni* Oudemans), tracheal mite (*Acarapis woodi* Rennie), invasion by Africanized honey bees (*A. mellifera scutellata* Lepeletier) and falling honey prices have reduced the viability of honey bee keeping in the Northern Hemisphere (Kevan *et al.* 1990; Allen-Wardell *et al.* 1998). In North America, honey bee numbers have decreased by 25 % in just six years (1990-1996) (Allen-Wardell *et al.* 1998). Also, honey bees remain in their hives during cool weather and are inferior pollinators of some crops (see section 1.2.4 for details) (Corbet *et al.* 1991; O'Toole 1993; Cane 1997).

The reliance on just one insect species for crop pollination seems risky in light of its vulnerability to disease and its inefficiency in some crops. Therefore the management and enhancement of "alternative" (also called non-*Apis*, wild or native) bee populations is recommended (Parker *et al.* 1987; Torchio 1987; Kevan *et al.* 1990; O'Toole 1993; Allen-Wardell *et al.* 1998). These bees are often more efficient pollinators than honey bees because they have specialised behavioural and morphological adaptations to the collection of their mutualist species' nectar and pollen rewards (Parker *et al.* 1987; Torchio 1987; O'Toole 1993). But wild bee populations are also declining, as a result of habitat alteration and loss (Williams 1982; Williams *et al.* 1991; Banaszak 1992; O'Toole 1993; Buchmann & Nabhan 1996)

There are at least 30 000 bee species worldwide but fewer than 10 species are managed for agricultural pollination (Cane 1997). Wild bee populations provide an important background pollinator service, which is largely unrecognised and undervalued (Kevan *et al.* 1990; Kearns & Inouye 1997. The importance of this free pollination service was illustrated to great effect in New Brunswick, Canada in the 1970s. Large areas of conifer forest were aerially sprayed with fenitrothion to control spruce budworm (*Choristoneura fumiferana* Clemens). But the insecticide also severely depleted populations of wild bee pollinators (*Bombus, Andrena, Colletes* & *Halictus* spp.) of neighbouring lowbush blueberry (*Vaccinium angustifolium* Ait., *V. myrtilloides* Michx.) farms (Kevan & LaBerge 1979). Berry yields fell markedly due to inadequate pollination; wild plant pollination in the area was also affected (Plowright & Thaler 1979). There is therefore a need to conserve wild bee populations, not only as a potential pollinator pool for management but also for the vital pollination services they already provide for wild and cultivated plants.

1.2.4 Morphological, physiological and behavioural adaptations of bumble bees that make them important pollinators

Bumble bees have been identified as alternative pollinators with considerable management potential. They are already widely used for pollination of tomatoes (*Lycopersicon esculentum* Mill), melons (*C. melo*), aubergines (*Solanum melongena* L.) and sweet peppers (*Capsicum annuum* L.) in greenhouses (Griffiths & Robberts 1996). This is because they are capable of "buzz pollination", a behaviour honey bees do not exhibit. Some Actinidiaceae, Boraginaceae, Ericaceae and Solanaceae species have poricidally dehiscent anthers which means they release pollen from small holes at the tip of the anther rather than splitting open to release pollen (Buchmann 1983). Bumble bees collect the pollen by clinging to the stamens and rapidly contracting the indirect flight muscles producing a strong vibration that shakes the pollen out of the anthers. Also, some flowers (e.g., tomato and aubergine) produce pollen only (no nectar) so they are generally avoided by honey bees. In addition, honey bees fly out of greenhouse vents when "scout bees" recruit foragers to flowering crops outside. Bumble bees do not communicate in this manner and so most individuals remain working in the greenhouse (Griffiths & Robberts 1996).

Bumble bees are large robust insects so they can withstand the active tripping mechanism of flowers such as lucerne (*M. sativa*) and they will contact the stigmas of open flowers with widely separated styles and stamens such as kiwifruit (*A. deliciosa*) more often than do smaller bees. Bumble bees can also fly at lower ambient air temperatures than honey bees due to their superior thermoregulatory abilities (Corbet 1996). A bumble bee's thoracic temperature is maintained at 35 - 40 °C for flight (Heinrich 1979a). Heat is generated by the shivering action of flight muscles (whilst uncoupled from the wings) and a heat producing substrate cycle; their hairy coat provides insulation to retain heat (Prys-Jones & Corbet 1987). This ability to fly at low temperatures enables them to forage early in the morning and late evening and makes them more reliable pollinators than honey bees in cool climates. However, their large size also makes them vulnerable to overheating and this may explain why the numbers of bumble bees foraging often show a decline in the heat of the (mid) day (Alford 1975).

Although most species of bumble bee forage on a wide variety of plant species, i.e., are generalists, they show preferences for different flower types. Different bumble bee species prefer different types of flowers and an important characteristic is the length of the flower corolla. Tongue length (commonly measured from the base of the prementum to the tip of the glossa (Harder 1982)) varies between bumble bee species and influences the type of flowers they visit for nectar (Heinrich 1979a; Corbet 1995a) Long-tongued bumble bees (LTBB) such as *Bombus hortorum* L. and *B. pascuorum* Scopoli can reach down the long corolla tubes of flowers such as red clover (*Trifolium pratense* L.) and broad bean (*Vicia faba* L.) and they forage more rapidly on this type of flower (Ranta 1983). Also, the nectar in these long corollae is inaccessible to short-tongued bees making the flowers energetically profitable for LTBB. Bumble bees such as *B. terrestris* L. and *B. lucorum* L., with shorter tongues, have faster working speeds on shallow or open flowers such as white clover (*Trifolium repens* L.) and willow (*Salix* spp.) (Ranta 1983; Plowright & Plowright 1997). However, this correlation does not always hold true since some short-tongued bumble bees will bite holes in long-corolla flowers and rob the nectar (called "floral larceny"). These holes are then reused by other bumble bees or honey bees acting as secondary robbers (Prys-Jones & Corbet 1987).

Individual bumble bee workers often specialise on a particular flower species. Floral constancy is a type of temporal specialisation, whereby bumble bees sequentially visit flowers of a single plant species, whilst bypassing others (Waddington 1983). Pollinator constancy to a particular plant species enhances the quality and quantity of flower visits (Plowright & Laverty 1984). For the bee, learning to recognise and manipulate a particular flower species reduces flower handling times (Heinrich 1983b). A related behaviour is "majoring and minoring" (Heinrich 1976). This means that an individual bumble bee will visit mostly one species of flower but will also sample a number of different species in small amounts. An explanation for this behaviour is that the bees exploit the most profitable species available yet sample other species to track changing floral resources over time (Heinrich 1983b). They can then switch their foraging to take advantage of new or more abundant food sources. Site or patch fidelity is another behaviour pattern exhibited by bumble bees, whereby they return to a previously rewarding patch of flowers to forage, they may even visit clumps of flowers in the same sequence (Heinrich 1979a; Thomson *et al.* 1988). Floral constancy and site fidelity over time are desirable attributes in a crop pollinator, particularly if the crop has a long flowering period.

1.3 Bumble bee habitat requirements

1.3.1 Habitat resources

In order to manage and conserve populations of bumble bees, an understanding of their habitat requirements is needed. Four key habitat resources have been identified for bumble bees. These are:

nest sites
forage (especially in early spring)
courtship and mating sites
hibernation sites.

Of these resources, nest sites and forage are probably the most important and the most easily manipulated (Osborne *et al.* 1991).

1.3.2 Forage preferences

Because of their large size, bumble bees have high energy demands for flight. Energy is also needed to generate heat to warm the nest and enable them to fly at low temperatures. The flowers they visit therefore must have sufficient nectar to supply their energy requirements (Heinrich 1979a). Perennial plants generally have larger flowers with higher nectar secretion rates than do annuals (Fussell & Corbet 1992a). Some exceptions to this include tansey leaf (*Phacelia tanacetifolia* Benth.) and borage (*Borago officinalis* L.), annuals that have high nectar volumes and are very attractive to *Bombus* (Williams & Christian 1991). A survey of flowers visited by bumble bees throughout Britain showed a preference for perennials and biennials over annuals (Fussell & Corbet 1992a). A similar bumble bee preference for perennials has been shown in Norway (Dramstad & Fry 1995).

Bumble bees are eusocial species with a long foraging season. They therefore require a season-long succession of suitable forage. Some crops such as oilseed rape provide abundant nectar resources, but because of their limited flowering time they cannot support a bumble bee colony over a whole season (Williams & Carreck 1994). Undisturbed patches of perennial vegetation such as field boundaries, road sides and hedgerows are important refuges for bumble bees in agricultural landscapes (Osborne *et al.* 1991; Saville 1993; Banaszak 1996). The higher floral diversity in such refuges supports a more diverse and more abundant Apoidea community (Banaszak 1992). As well as providing a continuous succession of forage, these undisturbed areas also provide nesting, overwintering and mating sites for bumble bees (Corbet *et al.* 1994).

1.3.3 Nest sites

Natural nest sites favoured by *Bombus* spp. are characteristically open habitats with undisturbed vegetation, exposed to the sun for some part of the day and sheltered from the prevailing wind (Fussell & Corbet 1992b). There are, however, intraspecific differences. For example, *B. terrestris, B. lucorum* and *B. lapidarius* L. frequently nest underground, whilst *B. ruderatus* F. and *B. hortorum* tend to nest above ground (Donovan & Wier 1978; Prys-Jones & Corbet 1987). The main nest requirements are a dry, well insulated cavity with suitable bedding material (bumble bees generally do not import bedding material) (Prys-Jones & Corbet 1987). Abandoned small mammal and bird nests are popular nest sites (Alford 1975) and the distribution of bumble bee nests may be closely related to the distribution of rodents' nests (Harder 1986).

1.3.4 Threats to bumble bee abundance and diversity

Agricultural intensification in most western countries over the last 50 years has resulted in large monoculture fields of annual crops with high agrochemical inputs. This has introduced three major threats to bumble bee abundance and diversity: pesticides, habitat removal and habitat fragmentation (Kearns & Inouye 1997). Indiscriminate insecticide use kills bees; the use of fenitrothion in Canadian forests resulted in the decimation of native bee populations (Kevan & LaBerge 1979). Broad-spectrum herbicides eliminate the perennial plants bumble bees prefer and the use of fertilisers promotes the growth of rank weeds such as stinging nettle that have limited value as a bee resource (Corbet *et al.* 1994).

Reclamation of wetlands and marginal land and the removal of hedgerows to facilitate mechanisation has resulted in more land being brought into cultivation and fewer nesting and forage sites for bumble bees. Williams (1982) identified the loss of habitat providing food plants as a key cause of the decline in bumble bees in England. Similar causes have been implicated in bumble bee declines in Belgium, northern France and East Germany (Peters 1972 and Rasmont 1988, both cited in Williams 1989).

Fragmentation results in an agricultural landscape consisting of small patches of suitable, undisturbed bumble bee habitat dispersed among large tracts of unsuitable, cultivated areas. Large areas of cereal crops or of grazed or ploughed fields may act as barriers to bumble bee movement (Rathcke & Jules 1993). Patches of vegetation that are too far away from the nest or separated by barriers may not be visited because the energetic costs are too high. By altering the movement of foragers, fragmentation will affect pollinator visitation to plants in fragments. Pollen carryover and genetic diversity may thus be limited in these patches (Jennersten *et al.* 1992). The effect of different patch sizes on pollinator visitation is investigated in Chapter 5 of this thesis.

1.3.5 Habitat manipulation

Several authors (Bohart 1967; Corbet 1991; Williams *et al.* 1991; Kearns & Inouye 1997; Allen-Wardell *et al.* 1998) have recommended habitat manipulation as an effective low cost management tool for alternative pollinators. In essence, habitat manipulation involves supplementing a limiting resource to boost population numbers or alter their distribution. The biological control literature has many examples of using habitat manipulation to enhance predator numbers (see Wratten & van Emden 1995 for a review;Hickman & Wratten 1996).

Examples of successful habitat manipulations in the pollinator literature include the provision of artificial nest sites for the solitary bees *Megachile rotundata* F. (Megachilidae) and *Nomia melanderi* Cockerell (Halictidae) to enhance their populations around lucerne crops (Bohart 1972; Hickman & Wratten 1996). *M. rotundata* nests readily in drilled woodblocks or polystyrene blocks set out in the field, whilst *N. melanderi* will nest densely in specially prepared soil beds. Habitat manipulations to enhance bumble bee populations are investigated in Chapter 2 of this thesis. The provision of nest sites and forage resources to enhance bumble bee numbers is often recommended in the literature (Holm 1966; Gurr 1974; McGregor 1976; Macfarlane *et al.* 1983; Williams *et al.* 1991; Fussell & Corbet 1992a).

1.3.6 Resource distribution in time and space

It is not enough simply to provide an abundance of resources; their distribution in time and space must also be considered. As mentioned before, the temporal succession of flowers must be maintained. This is because bumble bees do not store nectar reserves as honey bees do and are thus vulnerable to disruptions in their food supply (Prys-Jones & Corbet 1987). Spring is a critical time for food resources. The newly emerged queens need a supply of pollen to mature their ovaries, and nectar to fuel their nest-searching activities (Alford 1975). The spatial availability of resources is also important. Habitat fragmentation may lead to partial habitats supplying only one or two of the resources required (Westrich 1996). Bumble bees are central place foragers (Plowright & Laverty 1984) (nectar and pollen must be transported back to the nest) so foraging sites must be within flight range of the nest. Therefore, an understanding of the spatial aspects of bumble bee foraging behaviour is also required for their management.

1.3.7 Bumble bee movement

An understanding of bee movement is important for both agricultural and conservation goals. For example, knowledge of a bee's foraging range can be used to calculate the minimum area of habitat to be conserved. In agroecosystems, flight range information can be used to answer management questions such as how far the bees move into the crop, where to place bee colonies, overlap of colony foraging areas, isolation distances and optimal location for refuge areas. The movement of bees from their nests is studied in Chapters 3 and 4 of this thesis.

Most previous movement studies on bumble bees have focussed on within-flower, within-patch or between-patch movements (but see Saville 1993; Dramstad 1996b; Osborne *et al.* 1997; Schaffer 1997). These small-scale studies have described many important forager behaviour patterns such as traplining, majoring and minoring, area-restricted searching, patch fidelity and departure decision rules (see Heinrich 1976 for a review). But for a complete picture of bumble bee foraging behaviour, analysis at a larger scale needs to be included. Several authors (Bronstein 1995; Dramstad 1996b; Corbet 1997; Schaffer 1997) have advocated the study of bee movement at the landscape scale or community level. This is because pollination is a landscape-scale process, performed by different but overlapping and interacting communities of plants and their pollinators.

1.4 Bumble bees in New Zealand

1.4.1 The importation of bumble bees

In New Zealand, the majority of food and forage crops have been imported from the Northern Hemisphere. New Zealand native bees (Hymenoptera: Colletidae, Halictidae) are too small and few in number to pollinate introduced crops effectively (Gurr 1961). So the pollinators of introduced crops also had to be imported. Honey bees were introduced in 1839 but did not raise seed yields of red clover to economic levels (Gurr 1961). The importance of bumble bees as pollinators of red clover was recognised by Darwin (1951), so attempts to introduce bumble bees to New Zealand were begun in 1870 (Gurr 1961).

The first successful introduction of bumble bees in 1885 saw the establishment of *B. terrestris* and *B. ruderatus.* Within 5 years of their introduction, populations had built up rapidly and yields of red clover seed had increased dramatically (Thomson 1922). But after a peak of 10 years, seed yields declined despite the introduction of another two bumble bee species (*B. hortorum* and *B. subterraneus* L. subsp. *latreillellus* Kirby) in 1906. This decrease in seed yields was attributed to a lack of pollination caused by a decline in bumble bee populations (Palmer-Jones *et al.* 1966). However, Gurr (1974) contended that no continuing long-term decline in seed yields had been demonstrated and that initial high yields did not persist because cropping was intensified, creating a 'dilution' effect on local bumble bee populations. For both sets of introductions no accurate records were kept of what species were liberated and there was confusion as to which species had established up until 1957 when Gurr (1957a) published a thorough review. Ironically, one of the first species to establish and subsequently become the most abundant and widespread species in New Zealand was the short-tongued bumble bee *B. terrestris,* which is a poor pollinator of red clover because of its nectar-robbing habit.

1.4.2 Species and their distributions

There are four species of bumble bees in New Zealand: *B. hortorum, B. ruderatus, B. subterraneus* and *B. terrestris.* The first three are referred to as long-tongued bumble bees (LTBB) with tongue lengths of 13.5, 9.3 and 7.9 mm respectively (Prys-Jones & Corbet 1987; Clifford & Scott 1989). *B. terrestris* has a relatively short tongue, 5.4 mm long (Clifford & Scott 1989).

B. terrestris is a widespread species found throughout the North, South, Stewart and 27 other offshore islands (Macfarlane & Gurr 1995). *B. ruderatus* is also widespread throughout the North and South islands, but is less common in the North Island than *B. terrestris* and appears to be absent from the south-west of the South Island (Macfarlane & Gurr 1995). *B. hortorum* is present in Southland, Otago, Canterbury, Marlborough, Nelson (South Island), and Manawatu (North Island). In the last 35 years, it has increased its range from Palmerston North to Lower Hutt but has not expanded into North Canterbury (Macfarlane & Gurr 1995). This failure to spread into North Canterbury is puzzling because it is described as a "widespread ubiquitous species" in Britain (Williams 1989); *B. hortorum* may be somehow limited by droughts that are common in early summer in North Canterbury (Macfarlane & Gurr 1995). The fourth species, *B. subterraneus,* is confined to inland Canterbury and Otago (Macfarlane & Gurr 1995).

1.4.3 Life History

Bumble bees are primitively eusocial and their colonies follow an annual life cycle. Fertilised queens hibernate in underground cavities throughout winter and emerge in spring when soil temperatures rise (Alford 1975). Upon emerging, queens search for a suitable nest site, lay their eggs, then incubate and tend the brood. The first batch of workers emerges after three to four weeks and the queen can then relinquish her foraging duties (Prys-Jones & Corbet 1987). There follows a period of colony growth, the 'ergonomic phase' (Plowright & Laverty 1984), as progressive batches of workers are produced and the size of the colony increases. Later, the colony switches from an ergonomic to termination phase (Plowright & Laverty 1984) with the rearing of reproductive bees (drones and queens). The new queens and drones leave the nest to mate and the colony eventually dies out. After mating, the new queens forage extensively to build up their fat reserves to sustain them through the winter hibernation.

In New Zealand, *B. terrestris* queens are the first to emerge in September to November, followed by *B. hortorum* in October and November (Donovan & Macfarlane 1984). Colonies of *B. ruderatus* and *B. subterraneus* are founded later in late November and early December (Donovan & Macfarlane 1984). *B. hortorum* colonies mature four to five weeks after the emergence of the first workers and there is evidence that *B. hortorum* can have two, possibly three generations per year in New Zealand (Donovan & Macfarlane 1984). Because of New Zealand's mild temperate climate, nest founding can be prolonged in *B. terrestris, B. ruderatus* and *B. hortorum* and the presence of foraging queens in winter suggests some queens do not hibernate or may break hibernation (Donovan & Wier 1978). Some *B. terrestris* colonies also overwinter. In contrast, the marked seasonality in the high country and competition from other bumble bee species ensures that *B. subterraneus* has a strictly annual cycle and complete hibernation (Donovan & Wier 1978).

1.5 Management of bumble bees for red clover pollination

1.5.1 Why use bumble bees?

Red clover is a high-value forage plant in the family Leguminosae. It is a low growing herbaceous perennial identified by its fine leafy stems, hairy trifoliate leaves and crimson inflorescences (McGregor 1976). The inflorescence is composed of 50-200 florets which open from top to bottom over six to ten days (Free 1993). The flowers must be cross pollinated to set seed. Honey bees and bumble bees are the most important pollinators of red clover (McGregor 1976; Free 1993). The pollinating mechanism is a pistol type: when a bee lands on and exerts pressure against the standard and wing petals the stigma and anthers pop out (are "tripped") and contact the head of the visiting bee (Fig. 1.1).

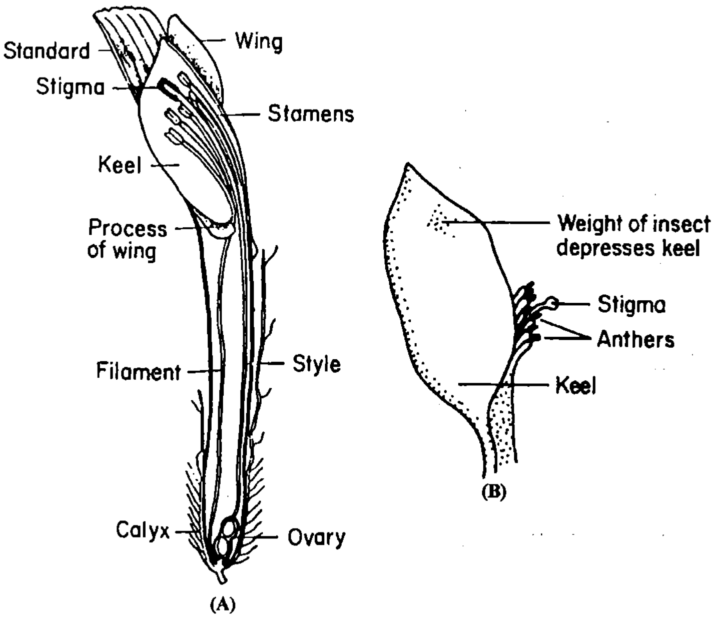

Figure 1.1. Flower of red clover (*Trifolium pratense*): a, longitudinal section; b, side view with keel depressed (from Free 1993).

Long-tongued bumble bees are the most efficient pollinators of red clover, particularly of the tetraploid varieties that have longer corollae than diploid varieties (Free 1993). This is because their long tongue enables them to access readily the nectar from the long corolla so they visit 2-3 times more flowers per minute than honey bees (Holm 1966). In addition, LTBB are more reliable pollinators because they work under adverse weather conditions, they collect both nectar and pollen from the flowers and they remain faithful to red clover. In contrast, honey bees tend to visit the crop for pollen only and often desert the crop for more attractive or accessible flowers. However, not all bumble bees are efficient red clover pollinators. Short-tongued bumble bees (STBB) such as *B. terrestris* and *B. lucorum* bite holes in the corolla to access the nectar, bypassing the pollinating mechanism. These holes are often re-used by honey bees acting as secondary nectar robbers.

1.5.2 Previous studies and management recommendations

There has been a long history of research into red clover pollination; Holm (1966), McGregor (1976) and Free (1993) provide thorough reviews. Their main conclusion was that, although bumble bees were more efficient pollinators than honey bees on an individual basis, their populations were too low and unpredictable to be relied upon, so they advocated the use of honey bee colonies to achieve maximum pollination. This advocacy was made in spite of the honey bees' limitations associated with their short tongue length and relatively high temperature activity threshold. Management recommendations also included: to ensure synchrony between bee and flower peaks, to keep crop sizes small, to provide and/or conserve forage and nesting sites for bumble bees, to plant crops in areas suitable for bumble bees, to keep competing crops to a minimum and to ensure plant nutrients and irrigation were adequate.

In New Zealand, low red clover seed yields have been attributed to inadequate pollination (Clifford & Scott 1989). Stocking the crop with honey bees has met with variable results because they are inefficient pollinators of tetraploid varieties such as Pawera and desert the crop if alternative flowers are available nearby (Clifford & Anderson 1980). Hence a considerable amount of research has looked at ways of enhancing bumble bee numbers and their management for pollination of tetraploid red clover crops. Macfarlane *et al.* (1983) listed three management options:

1. Collection of queens in spring from other localities for release near the crop.
2. Placement of domiciles close to the crop for queens to found nests in.
3. Colony introduction from outside the crop area. (To date, commercial rearing of LTBB is not possible, so these would have to be field collected.)

Option 1 was studied by Clifford (1973), who found that introducing queens to an area in spring increased the summer bumble bee population densities threefold. These results have never been repeated and seem inconsistent with the theory that it is nest site and forage resources that limit bumble bee populations. Option two is more consistent with this theory and has had some success in New Zealand (Donovan & Wier 1978; Pomeroy 1981), and is considered in further detail in Chapter 2 of this thesis. Supplementing red clover crops with LTBB colonies (option 3) has been a successful strategy, in that increased LTBB colony densities have resulted in increased seed yields (Macfarlane & Griffin 1985; Macfarlane *et al.* 1991). However, the economic benefits of this practice have yet to be proven (see next section).

1.5.3 Use of bumble bee colonies for field crop pollination

Because of the small size and high cost of bumble bee colonies, the use of bumble bees for field crop pollination is limited to high value crops that are inefficiently pollinated by honey bees. Examples include: high and lowbush blueberries and cranberries (*Vaccinium* spp.), kiwifruit (*A. deliciosa*), almonds (*Prunus dulcis* (Miller) D.A. Webb), apples (*Malus domestica* Borkh), pears (*Pyrus communis* L.), melons (*C. melo*) (van Doorn 1993), red clover, lucerne and cicer milkvetch (*Astragalus cicer* L., Richards & Myers 1997). Their use is cost effective if the benefits (i.e., seed yields) outweigh the costs of colony purchase (Corbet 1991). But cost effectiveness analyses are often incalculable because there is uncertainty about the relationship between bee density and seed set and therefore the correct stocking rate to use. This lack of knowledge is reflected in the wide variety of honey bee stocking rate recommendations for a particular crop (Torchio 1987; Corbet *et al.* 1991).

Some field evaluations of crops supplemented with bumble bees may be incorrect because they have been based upon unproven assumptions of bumble bee foraging behaviour. For instance, stocking rates are usually calculated from bee density and flower density assessments and assume that that x number of bees from a colony will result in y extra flowers being pollinated. R.C. Plowright (pers. comm.) suggested that the assumption that one bee visit results in one seed set is an overestimate because bumble bee foraging paths will overlap, so some flowers will be visited many times, others not at all. This estimate will be lowered further if a flower requires more than one visit to effect pollination or it is not receptive at the time of visitation or if the non-random foraging behaviour of bumble bees is taken into account. In fact the relationship between seed set and pollinator density is likely to be described as in fig. 2 (Plowright & Hartling 1981). The effect of supplementation on seed set will be modified by the background pollinator abundance . So at low pollinator densities, supplementation may enhance pollination but at higher densities, extra bees will add little to overall seed set. In New Zealand, low crop seed yields have been associated with low LTBB densities (Donovan & Wier 1978; Clifford & Anderson 1980; Macfarlane *et al.* 1983; Read *et al.* 1989), so in theory supplementation would be beneficial.

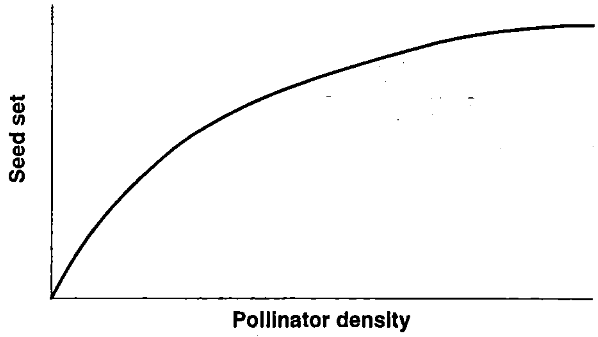

Figure 1.2. Relationship between seed production and pollinator density for an obligate outcrossing entomophilous plant species (from Plowright & Hartling 1981).

When evaluating the efficacy of bees shifted to a crop, researchers have often assumed that bumble bees observed on a patch of flowers are from nests placed or located nearby (Fussell 1992); or that a decline in bee densities in the centre of a crop (Braun *et al.* 1956; Bond & Pope 1974), or with increasing distance from the nest (Macfarlane *et al.* 1994), means that most bumble bees are foraging within a few hundred metres of their nest. However, Dramstad found high densities of *B. terrestris* foraging within 250 m of a known *B. terrestris* nest, yet when she marked 77 of these bees, not one was seen returning to the nest. Conversely, reobservation rates of nest-marked bees at the forage patch were low. This suggests that most of the bees observed foraging did not originate from the nearby nest and highlights the importance of marking bees to identify them conclusively as coming from a certain nest. Often observations of individuals are assumed to be representative of the colony as a whole. Schaffer (1997) found that most marked bees observed within a lucerne crop were foraging within 50 m of their nests. However, by calculating reobservation rates, it was found that this was not representative of where most (99 %) of the marked bees were foraging. These examples highlight the need to include spatial data in any field trials of bumble bees for crop pollination. A spatial evaluation of *B. hortorum* shifted to a red clover clop is included in Chapter 3 of this thesis.

1.6 Thesis objectives

This thesis aimed to evaluate two bumble bee management techniques for field crop pollination: habitat manipulation to enhance local bumble bee populations and, stocking crops with commercially obtained bumble bee colonies. Because management practices are reliant on an understanding of bumble bee biology and behaviour, aspects of their foraging behaviour were also investigated. The main objectives of this thesis were:

- To monitor the occupation of artificial nest sites by *Bombus* spp. and to determine if the provision of a floral resource enhances occupancy and nest productivity (Chapter 2).
- To evaluate the usefulness of shifting *B. hortorum* colonies to a red clover crop by investigating their contribution to the total number of bumble bees foraging on the crop, their crop specificity, foraging behaviour on the crop, distribution within the crop and diurnal activity patterns (Chapter 3).
- To test the hypothesis that bumble bees prefer to forage close to their nest (Chapter 4).
- To test the effects of forage patch size on the numerical and functional responses of *B. terrestris* (Chapter 5).

CHAPTER 2: HABITAT MANIPULATION TO ENHANCE BUMBLE BEE NUMBERS BY PROVIDING NEST SITE AND FORAGE RESOURCES

2.1 Introduction

2.1.1 Habitat resources limit bumble bee numbers

In New Zealand, there is a desire to increase bumble bee populations within agroecosystems to enhance pollination of field and orchard crops such as lucerne, red clover and kiwifruit. In order to manipulate bumble bee numbers it is necessary to identify the factors limiting their populations. In New Zealand, it is thought that two key habitat resources, nest sites and forage sites, are limiting bumble bee populations (Gurr 1957b; Gurr 1974). This hypothesis is based on indirect evidence from both New Zealand and Northern Hemisphere studies. First, high bumble bee abundance and diversity has been associated with areas such as undisturbed, uncultivated perennial vegetation that provide abundant nest and forage resources (Gurr 1957b; Clifford 1973; Williams 1982; Osborne *et al.* 1991; Banaszak 1992; Saville 1993; Macfarlane & Patten 1997). Therefore it is thought that the lack of critical habitat (nest sites and floral resources) in intensively managed agricultural landscapes limits bumble bee numbers. But queen overwintering and male patrolling sites (Saville 1993; Corbet *et al.* 1994), competition from other bee species (Gurr 1957b; Clifford & Anderson 1980; Woodward 1990; Paton 1993), predators and parasites (Hobbs *et al.* 1962; Alford 1975; Donovan & Macfarlane 1984; Schmid-Hempel & Durrer 1991), insecticide based mortality (Kevan & LaBerge 1979; Osborne *et al.* 1991; Kearns & Inouye 1997) and climatic factors (Williams 1986; Macfarlane & Gurr 1995) may also be important.

Other indirect evidence of a lack of nesting habitat includes the low occupancy rates of artificial nest sites in a UK study (Fussell & Corbet 1992b) compared with the higher occupancy in a New Zealand study (Donovan & Wier 1978). Fussell & Corbet (1992b) considered this difference to indicate a lower nest site availability in New Zealand, probably due to a restricted fauna of small burrowing mammals. A high incidence of bumble bee nest invasion in domiciles by queens of the same and other species (Donovan & Wier 1978; Richards 1978) also suggests that there is a limited supply of nest sites. Because bumble bee colonies store only small amounts of pollen and nectar (Shelly *et al.* 1991) their survival, growth and reproduction may be reduced if even short-term food shortages occur, so a continuous succession of flowers is required (Patten *et al.* 1993; Carreck & Williams 1997). Bowers (1985) found that the persistence, ontogeny and reproduction of *Bombus* in meadow habitats was governed mainly by mid-late summer meadow floristics, suggesting that floral resources are limiting to *Bombus*. Because bumble bees are central-place foragers (Plowright & Laverty 1984), forage patches have to be near the nest; Macfarlane & Patten (1997) observed that small growing colonies readily fail if there is little food within 100 metres.

To remedy this lack of bumble bee resources in agricultural landscapes, habitat manipulation techniques have been advocated (Bohart 1967; Gurr 1974; Macfarlane *et al.* 1983; Banaszak 1992; Corbet *et al.* 1994; Allen-Wardell *et al.* 1998). Habitat manipulation is a potentially effective, low-cost management technique (Corbet 1991) which, if maintained, can provide long-term positive effects on local bee populations because it directly rectifies the lack of resources. In contrast, moving bumble bee colonies to a crop is only a short-term option, and new inputs would be required annually. This is because factors contributing to the low numbers of bumble bees in the locality in the first place would also limit the success of any new queens produced from supplemented nests. Studies on other bee species (e.g., *N. melanderi, M. rotundata, Osmia cornifrons* Radoszkowski, *O. lignaria propinqua* Cresson) have shown that modified spray programmes and the provision of resources such as nest sites and supplementary forage can enhance local populations (Parker *et al.* 1987; O'Toole 1993). For bumble bees, nest sites can be provided in the form of domiciles (nest boxes) (Holm 1960), and floral resources ("bee forage") that provide a continuous supply of nectar and pollen can be purposely sown (Engels *et al.* 1994; Carreck & Williams 1997).

2.1.2 Artificial domiciles

Bumble bees will readily nest in domiciles placed in the field, both in the Northern Hemisphere (UK, Canada, USA, Europe) (Sladen 1912; Frison 1926; Fye & Medler 1954;

Holm 1966; Richards 1978) and in New Zealand (Donovan & Wier 1978; Pomeroy 1981; Macfarlane *et al.* 1983). Domicile acceptance rates in New Zealand range from 8-93 %, depending on immediate habitat characteristics, domicile design, domicile placement, local bumble bee populations and local availability of nest sites (Donovan & Wier 1978; Pomeroy 1981; Macfarlane *et al.* 1983). The optimal domicile design satisfies the bees' requirement for a dry, well insulated nest cavity with fibrous nesting material to cover the growing colony (Donovan & Wier 1978). Abandoned small-mammal sites are frequently used by bumble bees for nest sites in their native northern-temperate habitat because they provide a ready made nest cavity complete with bedding material (Fye & Medler 1954; Fussell & Corbet 1992b). The cues used by a queen bumble bee when searching for a suitable nest site are poorly understood. With abandoned mouse nests, it may be the cavity entrance and the presence of nesting material or mouse odour that prompts an investigatory response from the queen. The use of mouse nest material may be a simple technique to increase bumble bee nest-founding rates in domiciles.

2.1.3 Supplementing forage resources

The provision of spring forage has been recommended, in conjunction with the provision of domiciles (Macfarlane *et al.* 1983; Donovan & Macfarlane 1984). Gurr (1957b) regarded food availability in early spring as a critical factor in nesting success. Queens that emerge from hibernation need large quantities of protein from pollen to mature their ovaries, and nectar to fuel their nest-searching activities (Prys-Jones & Corbet 1987). The provision of floral resources at this critical time could increase nest-founding rates or increase nest productivity, but this has never been experimentally tested. For crops such as red clover that are generally late flowering, it is important to have a continuous source of forage to sustain bee populations over summer until the crop starts to flower. Field margins, in particular floristically diverse margins, can enhance beneficial insect population diversity and densities, for example, those of predatory arthropods (Dennis & Fry 1992; Cowgill *et al.* 1993; Hickman & Wratten 1996) and pollinating insects (Lagerlöf *et al.* 1992; Engels *et al.* 1994; Banaszak 1996). Field margins in Canterbury are characteristically low in floral abundance and diversity, because of the high use of herbicides and fertilisers, and cultivation up to the field edge (M.Barron pers. obs.). In this study, field margins were sown with *P. tanacetifolia,* a North American annual. *P.*

tanacetifolia was chosen because it is very attractive to bumble bees (Williams & Christian 1991). It is also resistant to low temperatures and frost and may flower as early as October in Canterbury (Bowie *et al.* 1995).

The purpose of this study was to monitor the acceptance of domiciles along field margins and to evaluate the ability of *P. tanacetifolia* to attract *Bombus* spp. to field margins to increase occupancy rates in the domiciles. The effect of mouse odour or the presence of a mouse nest on domicile occupancy by bumble bees was also investigated.

2.1.4 Objectives

- To monitor the occupancy rates of domiciles placed in field margins.
- To determine if the presence of *P. tanacetifolia* increases occupancy rates and productivity in domiciles.
- To determine if there is an association between previous use of a domicile by mice or bumble bees and subsequent nest founding by bumble bee queens.

2.2 Methods

2.2.1 Definition of a nest

This study follows Donovan & Wier (1978) and Pomeroy (1981) in defining a nest as occupied if any attempt at nest founding is made, even if no workers are produced. A breakdown of nest productivity and state at termination is presented in the results section 2.3.2.

2.2.2 1995/96 season

Eighty bumble bee nest boxes were constructed from 4 mm plywood; the lids were covered in silver-coated rubber (Fig. 2.1). The boxes consisted of four compartments, each supplied with a piece of folded felted-fibre carpet underlay.

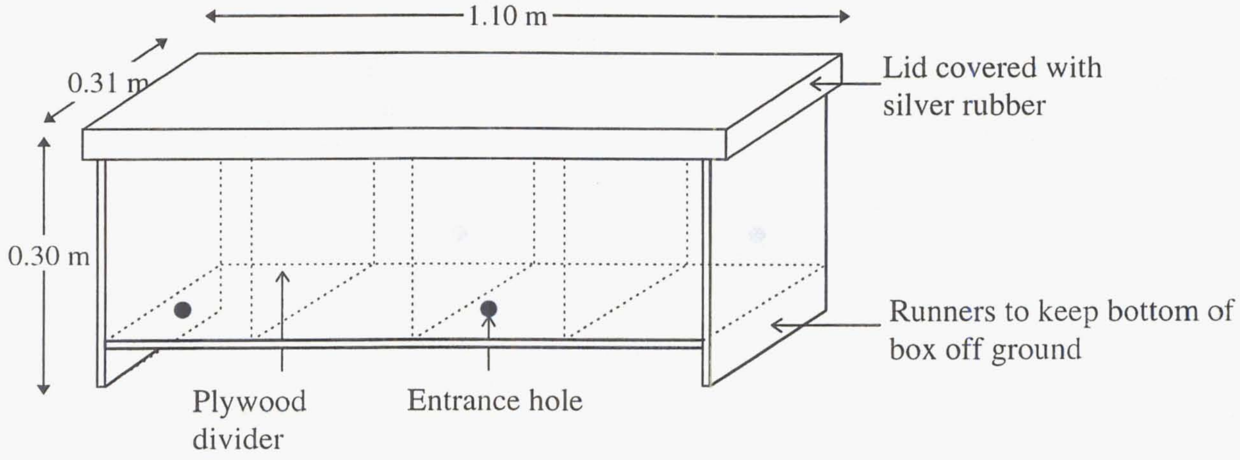

Figure 2.1. Diagram of a bumble bee domicile

The units were placed along 16 field margins in Lincoln University farms (Fig. 2.2). Five domiciles were placed along each field margin, 15-25 m apart, depending on the availability of a 'suitable' site (e.g., the base of a tree). Placing the domicile against a landscape feature such as a tree or fence post aids bee orientation (Macfarlane *et al.* 1983). Eight of the 16 field margins were drilled in late October 1995 with a 100 m x 0.15 m strip of *P. tanacetifolia* at a rate of approximately 10 kg/ha (Plate 2.1, Fig. 2.2).

Plate 2.1. A strip of *Phacelia tanacetifolia* with domicile in the background.

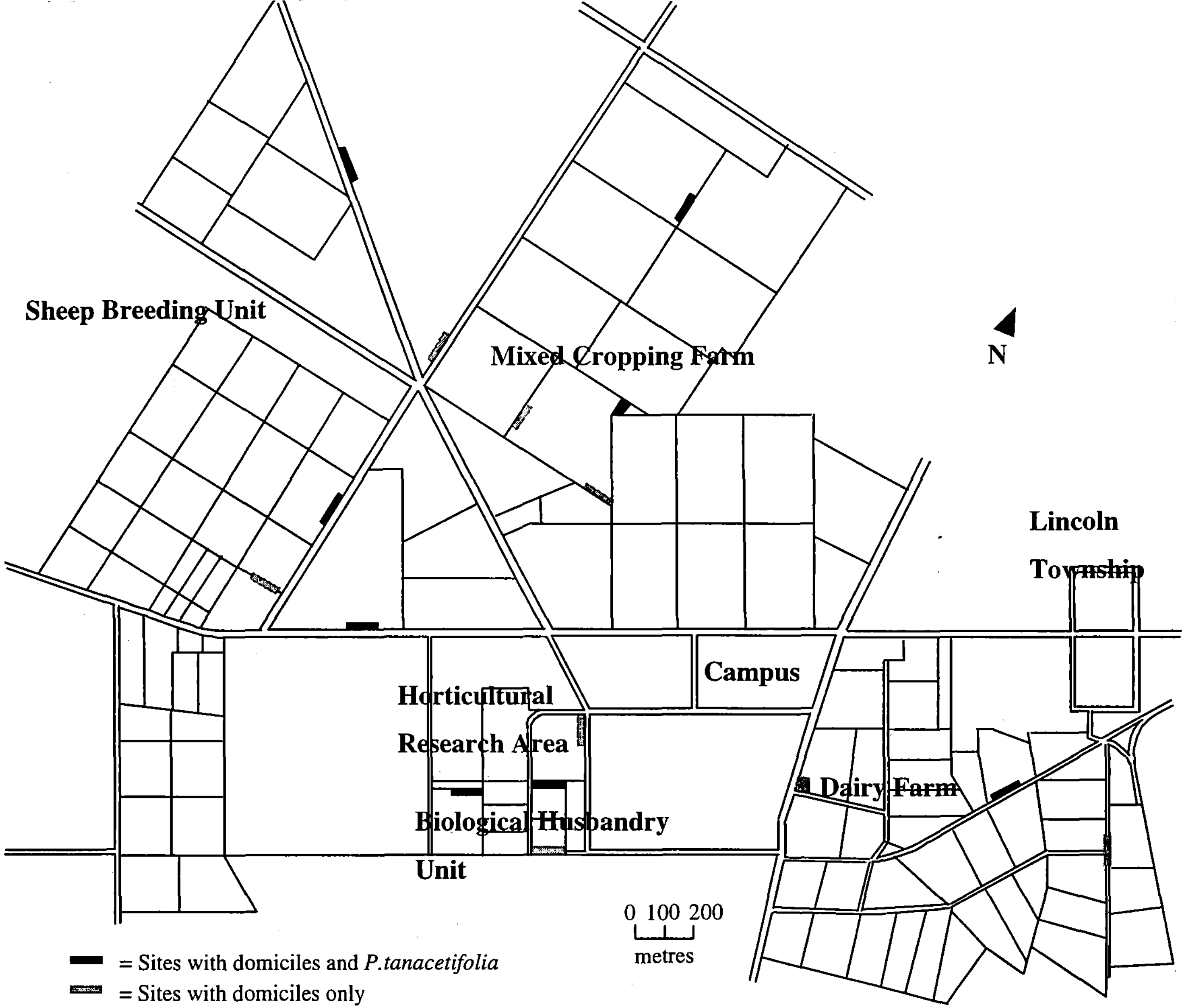

Figure 2.2. Map of domicile placement and *Phacelia tanacetifolia* strips around Lincoln University farms.

The domiciles were placed in the field on 25 & 26 November 1995. Domiciles were checked weekly for occupancy from 28 November 1995 to 18 March 1996. Mice and rats were controlled during the summer by placing Talon® (brodifacoum) baits in domiciles that showed signs of rodent occupancy. The number of bees visiting each site was determined by "bee walks" (Banaszak 1980). A 100 m transect was marked out at each field margin and the observer walked along the margin at a steady pace, recording the number, species and caste of bumble bees, what flowers they were visiting and whether they were foraging for nectar and/or pollen. The bee walks were conducted at each site 1-2 times a week when *P. tanacetifolia* was in flower (4 December 1995 - 22 January 1996). The number of *P. tanacetifolia* flower heads in three randomly selected 1 m strips was counted at each observation period. The number of bees visiting sites with or without *Phacelia* was compared using a Mann-Whitney rank sum test.

2.2.3 1996/97 season

In August 1996, domiciles with mouse nests in them were recorded. The carpet underfelt and mouse nests were removed from all domiciles and replaced with new underfelt. The number of bumble bee nests founded in domiciles with or without previous occupation by mice was compared with a Chi-squared test.

In 1995, *P. tanacetifolia* started flowering too late (December) to influence the main nest-founding period, which is October-November for *B. hortorum* and September-November for *B. terrestris* (Donovan & Macfarlane 1984). Therefore, in 1996, *P. tanacetifolia* was sown at eight sites between 21-28 August, so it would be in flower by October. Unfortunately, mowing, cows, herbicide drift or competition from grass destroyed five of the eight P. tanacetifolia sites. For this reason, the *Phacelia* versus no *Phacelia* comparison was abandoned and the domiciles were simply checked for occupancy on 16 October 1996, 7 November 1996 and 3 March 1997. Mice and rats were controlled over the summer by the use of Talon® baits placed in the domiciles.

2.2.4 1997/98 season

On 5 September 1997, only the wet and fungus-colonised underfelt in the domiciles was replaced. Mice nests were left in the domiciles. A Chi-square analysis was used to test for association between the presence of a mouse nest and occupancy by bumble bees. The domiciles were checked for occupancy on three occasions: 27 October 1997, 15 January 1998 and 23 March 1998. Mice and rats were not controlled. To test if there was any association between box occupancy by bumble bees in the previous season and occupancy in the current season a Chi-square test was used.

2.3 Results

2.3.1 Overall occupancy trends

Occupancy was very low (0.31%) in the first summer of this study but increased annually over the three seasons of monitoring (Table 2.1). Between November 1995 and March 1998, 40 (12.5 %) of the domiciles had bumble bee nests founded in them. Over all seasons, *B. hortorum* was the main occupant; founding 65 % of all recorded nests. There was a highly significant association between the presence of a bumble bee nest in a unit in the previous year and occupancy in the following year (Yates' corrected Chi-square; $\chi^2=31.83$, df=1, $P<0.001$).

Table 2.1. Occupancy of field domiciles by bumble bees at Lincoln 1995-1998 (number of domiciles set out = 320 in units of four).

Season	Number of nests founded	% occupancy	% *B. hortorum* nests	% *B. terrestris* nests	% unidentified nests
1995/96	1	0.31	100	-	-
1996/97	13	4.06	76.9	7.7	15.4
1997/98	26	8.13	57.7	30.8	11.5

2.3.2 Colony cycle

Only four out of 40 nests established produced new queens. Most (67.5 %) produced workers (Table 2.2). Nests observed in late October 1996 and 1997 already had workers present. As it takes approximately 1 month from nest foundation to worker emergence, and no nests were observed during August maintenance checks, these early nests must have been founded in mid September. For all seasons combined, 23 of 40 nests (57.5 %) were founded before the end of October. Five new *B. hortorum* nests were founded between 15 January and 23 March 1998.

Table 2.2. Number and state at termination of bumble bee (*Bombus* spp.) nests founded in field domiciles 1995-1998 (n=40).

Species	Queen cavity & egg clump	First cocoons	First workers	Fewer than 50 worker/male cocoons	More than 50 worker/male cocoons	Number of nests producing queens
B. hortorum	1	3	4	10	7	3
B. terrestris	1	3	1	2	1	1
Unknown	2	2	1	-	-	-

2.3.3 Effect of *P. tanacetifolia* on domicile occupancy by bumble bees

Because the *Phacelia* flowered too late in 1995 and only one nest was founded that season, its effect on occupancy rates could not be determined. Most of the *Phacelia* strips sown in 1996 were destroyed, so again the effects on nest occupancy could not be assessed.

2.3.4 Effect of *P. tanacetifolia* on bumble bee numbers, 1995/96 season

There were significantly more bumble bees observed at field margins planted with *Phacelia* than at margins without (Fig. 2.3: Mann-Whitney; U=2398, df=53, $P<0.001$). There was a strong positive relationship between the number of *Phacelia* flower heads and the number of bumble bees observed per transect (Spearman rank; Rs=0.708, df=123, $P<0.001$)

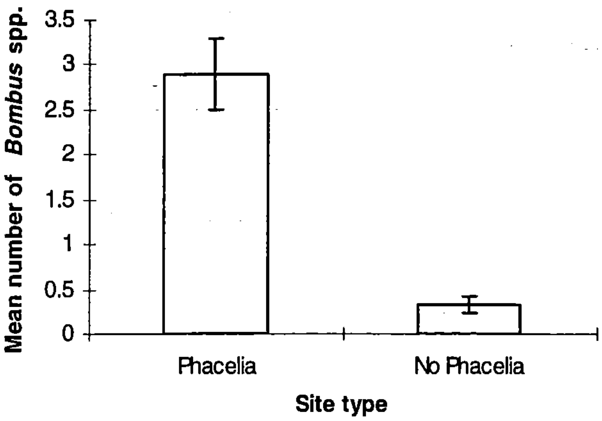

Figure 2.3. The number of bumble bees observed along 100 m transects at sites with and without *Phacelia tanacetifolia* around Lincoln University farms, summer 1995/96 (means±SE).

The first site at which flowering began was site 3, on 4 December 1995; all sites were in flower by 13 December 1995. Flowering lasted for approximately 4 weeks but some sites had shorter flowering periods; for example, flowering at sites 1 and 7 lasted only c. 2 weeks, because of competition with grasses. Figure 2.4 shows the flowering phenology at site 8, with the numbers of bumble bees observed on transect walks.

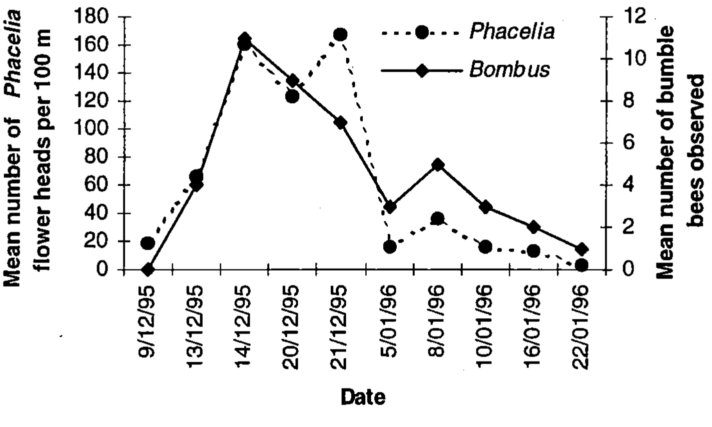

Figure 2.4. Mean number of *Phacelia tanacetifolia* flower heads (left axis) and the number of *Bombus* (right axis) observed per 100 m transect, site 8.

2.3.5 Effect of mouse nests on nest founding by bumble bees

No association was found between the presence of mouse nests in the domiciles and subsequent nest founding in the domiciles in 1996/97 (Yates' corrected Chi-square; χ^2=0.49, df=1, P=0.4840). There was also no association in 1997/98 (Yates' corrected Chi-square; χ^2=2.07, df=1, P=0.1500).

2.4 Discussion

2.4.1 Domicile occupancy trends

There was an increase in domicile occupancy over the three nest-founding seasons. Frison (1926) and Donovan & Wier (1978) found a similar increase, which they attributed to both improved domicile design and placement to reduce dampness in the domiciles. In the present study, however, the boxes were left in the same location from year to year. Another explanation suggested by Donovan & Wier (1978) for annually increasing occupancy rates, was queen bumble bees returning to the vicinity of their maternal nest to found their own nests. This seems an unlikely explanation for the 10-fold increase in nest founding from the first to second season in this study, because no queens were produced from the one nest that was founded in the first year. Queens reared in domiciles in 1996/97 may have returned to found nests in 1997/98, since a significant association between unit occupancy in the previous year and occupancy in the current year was found. Pomeroy (1981) found that overwintered queens visited their maternal nest site and D.Woodward (pers. comm.) found enhanced attraction and nest founding in laboratory and domicile trials when bumble bee nest odour was present. Alternatively, the weathering and increased camouflaging of domiciles with time possibly increased their acceptability to bumble bee queens. Richards (1978) speculated that the camouflaging of tunnel entrances to his domiciles by founding queens was a defence mechanism against inclement weather, social parasites and predators.

Compared with other New Zealand studies (Donovan & Wier 1978; Pomeroy 1981; Macfarlane *et al.* 1983), the occupancy rates in this study were low. This could be due to a number of factors: nest sites may not have been limiting, the domicile design or placement may not have been satisfactory, or local bumble bee populations may not have been as large in previous studies. It is difficult to elucidate which factors were the most important. The low occupancy rates in the first season of the study were probably due to the fact that domiciles were not set out until late November, missing the main nesting period of *B. terrestris* and *B. hortorum* (Donovan & Macfarlane 1984). As most of the nests were founded before the end of October in subsequent years, this seems a likely explanation. The domiciles were of a similar design to those of Donovan & Macfarlane (1984) except that the present ones had four compartments. Most boxes remained dry throughout the three seasons and any wet bedding material was replaced each spring. Although some of Donovan & Wier 's (1978) domiciles were set out in the same area as this study (Lincoln), they may have been placed in more favourable (undisturbed, floristically diverse) sites than in the present study, in which a range of, often floristically poor, field margins were utilised. The high incidence of nest invasion by other *Bombus* queens suggests that nest sites were limiting in some locations (see also Richards 1978).

2.4.2 Species occupying the domiciles

The main occupant of the domiciles was *B. hortorum* (65 %); this is similar to the 52.4 % of Donovan & Wier (1978). It also agrees with descriptions of nest sites in the European habitat of *B. hortorum* where it is predominantly a surface nest-builder (Prys-Jones & Corbet 1987). In contrast, *B. terrestris* is usually a subterranean nester (Prys-Jones & Corbet 1987) and preferentially occupies underground domiciles (Donovan & Macfarlane 1984). The relatively high acceptance rate of domiciles by a long-tongued species of bumble bee is a promising result, because it is these species that are most useful for pollinating red clover (Free 1993). The founding of *B. hortorum* nests in late summer supports the finding of Donovan & Macfarlane (1984) that *B. hortorum* has a second generation in New Zealand. Although *B. terrestris* was the dominant species in the locality, it comprised only 20 % of the domicile occupants, indicating that the domiciles did not satisfy its nest site requirements. Richards (1978) found a similar situation in his field trials; the most locally abundant species was not the most numerous in the domiciles. In the present study, only two bumble bee species inhabited the domiciles. *B. subterraneus* was not expected as it is absent from the Lincoln area (Donovan & Wier 1978), but the absence of *B. ruderatus* is difficult to explain. Donovan & Wier (1978) found that 20.2% of their domiciles were occupied by *B. ruderatus*. Some *B. ruderatus* individuals are completely black but the morphs with yellow markings have a similar banding pattern to those of *B. hortorum* (Donovan & Macfarlane 1984). As it is difficult to distinguish banding patterns when a bee is flying, it is possible that some of the *B. hortorum* nests were misidentified and were in fact light-coloured morphs of *B. ruderatus*.

2.4.3 Effect of mouse nests on bumble bee nest founding

Although mouse nests are frequently used by bumble bees in the wild, they appear not to influence nest founding in domiciles (but see Fye & Medler 1954). This is probably because domiciles, such as the ones used in this study, already provide the necessary insulated cavity and nesting material, therefore mouse nests are not necessary to induce nesting (Hobbs *et al.* 1960; Fussell & Corbet 1992b). D. Woodward (pers. comm.) also found that the presence of mouse odour had no effect on nest founding by queen bumble bees. In addition, mice are predators of nests and in this study several nests were destroyed by them.

2.4.4 Effect of a floral resource on bumble bee populations

Because of problems in maintaining the *Phacelia* strips, the effect of a spring forage resource on occupancy rates and nest production could not be tested. Circumstantial evidence that the provision of floral resources increases domicile occupancy is provided by comparing the occupancy of domiciles in the Biological Husbandry Unit (BHU) at Lincoln University (33.3 %) with occupancy rates of the remaining sites (7.7 %). The BHU manages its orchards and crops in accordance with organic principles and has a policy of floral diversification to augment beneficial insect populations (R.Crowder pers. comm.). In spring, tree lucerne (*Chamaecytisus palmensis* (Christ), *Phacelia (P. tanacetifolia),* cow parsley *(Anthriscus sylvestris* L.*),* mustard *(Sinapis alba* L.*),* fruit trees *(Prunus* spp., *M. domestica*) are in flower and provide nectar and pollen for emerging bumble bee queens.

Phacelia attracted bumble bees to the field margins and there was a close correlation between flower abundance and bee numbers. The most abundant bumble bee visitor was *B. terrestris,* which constituted 99 % of all *Bombus* observed. Williams & Christian (1991) also found a close dependency of bumble bee numbers on flower density and a dominance of *B. terrestris* on *Phacelia* patches. The dominance of *B. terrestris* is likely to be due to two factors: *B. terrestris* is the most abundant species in rural Lincoln (Donovan & Wier 1978), and short-tongued bumble bees preferentially visit flowers with shorter corollae (such as *Phacelia*), whereas for long-tongued species the opposite is true (Plowright & Plowright 1997).

2.4.5 Choice of floral resource

The *Phacelia* in this study performed poorly compared with other studies; it flowered for only c. 4 weeks compared with the 8 weeks recorded by Williams & Christian (1991) and Bowie *et al.* (1995). This is because it was smothered by vigorously growing grasses and was probably water-stressed (irrigation was impractical because of access problems). A longer-flowering, more competitive and drought resistant species is required to provide a continuous floral resource. Engels *et al.* (1994) developed a mix of annual species ("The Tűbingen mix") to provide a continuous succession of floral resources for bees in Germany. But when tested under UK conditions, *Phacelia* dominated the mixture, so was of little advantage over sowing *Phacelia* alone (Carreck & Williams 1997). Development of a similar mix for Canterbury conditions would require the assessment of the agronomy and flowering phenology of the component species under the prevailing climate and soil conditions (see Bowie *et al.* 1995) The constituent species in an annual mix would require resowing every 1-2 years, which would mean continuing financial and labour inputs. Also, regularly ploughing the field margins could facilitate the invasion of arable annual weeds (Corbet *et al.* 1994). A longer-term option would be to plant nectar- and pollen-rich perennial plants or trees and shrubs such as clovers (*Trifolium* spp.), bergamot (*Monarda fistulosa* L.), lavender (*Lavandula* spp.), rosemary (*Rosmarius officinalis* L.), and tree lucerne (*C. palmensis*). Perennial and biennial plants are 'preferred' by bumble bees over annual species (Fussell & Corbet 1992a), and can suppress annual weeds and would require less maintenance once established (Corbet *et al.* 1994). In the UK, set-aside schemes, where arable land is taken out of food production, have great potential to be managed as bumble bee habitat (Williams & Carreck 1994). Although annually ploughed rotational set-aside and conservation headlands can provide abundant nectar and pollen sources, they tend to redistribute local populations rather than enhance them (Corbet 1995b). Therefore non-rotational set-side offers greater scope for the enhancement of bee numbers and diversity because the lack of cultivation means nest sites are not disturbed and a diverse perennial vegetation develops with time (Corbet 1995b).

2.4.6 Practical Implementation

As a means of enhancing pollination via increased bumble bee populations, the setting out of domiciles has variable success. In this study, occupancy rates were low compared with other studies. This was probably because in this study sub-optimal habitats, e.g., floristically poor paddock edges, were utilised. For the amount of money and time spent building the 320 units, the results (low occupancy) did not justify the effort. However, for a red clover grower with just one or two paddocks the number of domiciles required would be much lower. If she/he could adapt containers already at hand (e.g., nail boxes), then outlay could be minimised. The number of bumble bee colonies occupying the domiciles should build up over the years. The seeding of domiciles with pieces of old bumble bee nest is recommended as means to attract bumble bee to the domiciles. The prospects for enhancing bumble bee populations in New Zealand are promising, because many of their natural enemies, for example, cuckoo bumble bees (*Psithyrus* spp.), conopid flies (Diptera: Conopidae), badgers (*Meles meles* L.), and shrews (*Sorex* spp.), are absent from New Zealand (Holm 1966; Donovan & Wier 1978; Fussell & Corbet 1992b).

The choice of an appropriate pollen and nectar source for the field margin is difficult; the flowering phenology, agronomy, weed status, ease and cost of establishment and management, nectar and pollen availability and bumble bee preferences all must be considered (Patten *et al.* 1993; Carreck & Williams 1997). Also, the companion plant could draw pollinators away from the crop if both are flowering at the same time. Mowing the margin when the crop starts to flower could prevent this. Another option would be to place domiciles and to plant bee forage in a area of undisturbed, uncultivated land, then shift occupied domiciles to the crop when required.

These habitat manipulation techniques have been recommended for many years (see Gurr 1957b) but it is not known whether farmers actually practise them. Some growers put out domiciles and approximately 30-80 *B. hortorum* nests are sold to red clover growers in the South Island each year, but exact details of grower practices are not known (B. Donovan pers. comm.). In this study, the relationship between the number of bumble bee nests in field margins and seed yields was not investigated but other studies have shown that bees from domiciles adjacent to a crop, move into the crop (Chapter 3, this thesis) and the number of domiciles supplied to a crop has been positively correlated with seed yields (Macfarlane *et al.* 1983; Macfarlane *et al.* 1991).

CHAPTER 3: FORAGING BEHAVIOUR OF *BOMBUS HORTORUM* WITHIN A RED CLOVER SEED CROP

3.1 Introduction

3.1.1 Red clover seed production in New Zealand

Red clover (*T. pratense*) is self incompatible and therefore reliant on insects (primarily honey bees and bumble bees) to cross pollinate its flowers. Bumble bees were introduced to New Zealand from England in 1885, specifically for red clover pollination (see section 1.4.1). Initial high seed yields following the first introduction of bumble bees did not last, probably because local bumble bee populations were spread more thinly when more and more acreage was put into seed production (Gurr 1974).

Average seed yields of red clover in New Zealand are low compared with those of the Northern Hemisphere, and this is thought to be due to inadequate pollination (Gurr 1974). The seed yields of tetraploid clovers are even lower than those of diploid cultivars; 150 kg/ha compared with 200 kg/ha (P. Clifford pers. comm.). A significant reason for lower seed yields in tetraploids is that they have longer corolla tubes than diploids, making it difficult for bees with short tongues to reach the nectar and hence pollinate the flowers (Holm 1966; Free 1993). Red clover is a valuable seed crop, worth $5-7/kg for diploid cultivars and $11-12/kg for tetraploids (P. Clifford pers. comm.), so the incentive for increasing yields is great.

3.1.2 Management of long-tongued bumble bees

It is generally agreed that long-tongued bumble bees (LTBB) such as *B. hortorum* and *B. ruderatus* are the best pollinators for tetraploid red clover crops (Clifford & Scott 1989; Free 1993) because of their fast working speeds, their preference for flowers with long corollae and their ability to work under adverse weather conditions (Donovan & Macfarlane 1984). The problem with using LTBB as pollinators is that their natural populations in New Zealand are low and fluctuate widely (Donovan & Macfarlane 1984).

It is not yet possible to rear *B. hortorum* colonies commercially, so natural populations have to be relied upon for pollination. One option is to transfer nests to the crop from other localities. There are few stocking rates of bumble bees for field crops in the literature: Macfarlane *et al.* (1994) used 10 colonies per ha of *B. occidentalis* Greene for cranberry bogs and Richards & Myers (1997) recommended more than five colonies per ha of *B. occidentalis* for cicer milkvetch fields. Recommendations for managing bumble bee colonies for New Zealand red clover crops include placing the nests as close to the crop as possible (Macfarlane *et al.* 1983) and supplying them at a stocking rate of six colonies per hectare (Macfarlane *et al.* 1991). Macfarlane *et al.* (1991) calculated this stocking rate from estimates of the number of flowers/ha needing pollination each day and the number of flowers/ha pollinated each day by the average number of bees/ha observed on different crops.

To evaluate the effectiveness of bumble bee nests shifted to field crops, an understanding of where individual bees, and the colony as a whole, forage in relation to their nest site is also necessary. This introduces the spatial component of bumble bee foraging behaviour that has hitherto been ignored in studies of bumble bee field-crop pollination (but see Schaffer 1997). This spatial information is needed to answer fundamental management questions such as: where and how far apart should nests be placed; how far into the crop do the bees forage; do the bees visit the crop exclusively or do they forage on competing plants?

3.1.3 Bumble bee movement from the nest

It has generally been accepted that bumble bees prefer to forage as close to their nests as possible, because of energy and time constraints on foraging (Alford 1975; Heinrich 1976; Bowers 1985). These observations have been only anecdotal, not explicitly tested for in the experimental design and the shorter foraging distances observed may have been an artefact of small sampling areas (see Dramstad 1996a for review). Butler (1951) concluded that most foraging flights from a *B. agrorum* F. nest adjacent to a red clover paddock were within 18.3 m of the nest. However, no methodology for calculating this result was presented. Often researchers assume that high bumble bee densities observed on flowers near a known nest site are a result of bumble bees foraging close to their nest

(Braun *et al.* 1956; Bond & Pope 1974; Macfarlane *et al.* 1994). This assumption may be flawed since Dramstad (1996a) found that none of the bees she marked foraging near (< 250 m) a known nest were seen entering the nest and very few of the bees she marked leaving the nest were seen foraging in the area. In the present study, *B. hortorum* foragers were marked with a fluorescent powder to identify them positively in the red clover crop. Marking the bees also distinguished them from wild bees, so the contribution of the introduced bees to the total pollinator community can be assessed. To see if observations of marked individuals represent the whole colony, reobservation rates must be calculated (Schaffer 1997). In the current study, video recordings were also used to estimate the number of bees from a particular nest that were foraging, and hence calculate reobservation rates. Records of nest activity can also be used to determine peak activity periods and duration of foraging throughout the day.

3.1.4 Pollen analysis

Inspection of pollen loads can be used as an indirect method of determining where pollen-collecting bees have been foraging (Waddington 1983). Whidden (1996) successfully used pollen load analysis to show that *Bombus impatiens* (Cresson) foragers were faithful to lowbush blueberry (*V. angustifolium*) crops, but he looked at pollen collection over only a two-day period. By analysing pollen loads, the specificity of the bumble bees to the crop over time can be assessed. This may change with flowering intensity of the crop or when other plants in the locality come into flower.

3.1.5 Study design

'Grasslands Pawera' is a New Zealand tetraploid red clover cultivar that has good herbage characteristics and is a valuable seed crop. A small number of bomibculturalists in the South Island supply *B. hortorum* and *B. ruderatus* nests specifically for red clover pollination. Four such nests were purchased for this study from Donovan Scientific Insect Research. Four nests at approximately $65 each was considered to be the outlay a grower would initially be prepared to pay for each paddock (R. MacCarthy, pers. comm). This study was carried out on a 4 ha crop of red clover, which has the advantage of being a realistic area for this seed crop ($\leq$ 5 ha is the recommended paddock size for red clover seed crops) (Clifford & Anderson 1980). Many other studies have been carried out on very small experimental crops (e.g., Brown 1989, 0.36 ha; Fussell 1992, 0.17 ha; Schaffer 1997, 0.24 ha), the results of which may not extrapolate to a larger crop area, due to 'dilution' of pollinator numbers. Because the crop was an abundant source of nectar and pollen and because of the well-documented preference of *B. hortorum* for red clover, the null hypothesis was that most bees from the supplied nests would visit the crop.

3.1.6 Objectives

- To quantify the population density, species composition and foraging behaviour of bumble bees present on the red clover crop.
- To compare the number of nest-marked bees reobserved at different distances (10, 50, 100 & 200 m) into the crop.
- To use time-lapse video recordings of bumble bee nest traffic to determine diurnal activity patterns and to estimate the number of marked bees foraging (this estimate will be used to calculate reobservation rates of nest-marked bees within the crop).
- To use pollen analysis to determine bumble bee specificity to the crop and to see if this changes over time.
- To evaluate the usefulness of bumble bee nests placed on the edge of field crops by investigating their contribution to the total number of bumble bees foraging on the crop, their crop specificity, foraging behaviour on the crop and nest productivity.

3.2 Methods

3.2.1 Study area

The study area was a 4 ha tetraploid red clover crop (*T. pratense* cv. Pawera) at Tai Tapu, Canterbury, New Zealand. The crop was grazed until 13 December 1996 when it was closed for seed production; it began flowering around 10 January 1997. It was bounded by a hawthorn hedge (*Crataegus monogyna* Jacq.) and a ditch to the west, a *Macrocarpa* spp. hedge to the north and a river along its south-east boundary (Fig. 3.1).

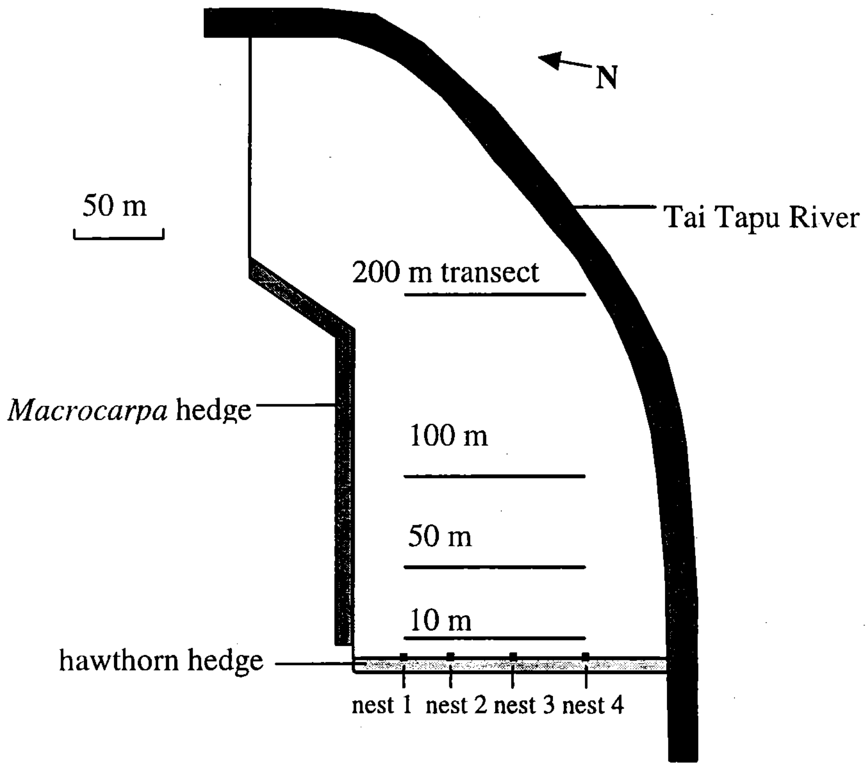

Figure 3.1. Diagram of Tai Tapu study site and location of transects.

Four *B. hortorum* nests were moved to the crop edge on 15 January 1997. The nests were sourced from the Lincoln area where they had been founded in artificial domiciles placed in the field. These domiciles were a standard wooden type with a metal-covered roof (see Donovan & Macfarlane 1984 for design details). The nests were placed along the western edge of the field against the hawthorn hedge, affording them some shelter from the sun and the prevailing north-west wind. The boxes were placed 30-40 m apart, exploiting where possible a distinct landscape feature such as a tree trunk or fence post, to aid bee orientation to the box (Macfarlane *et al.* 1983; Plowright *et al.* 1995).

3.2.2 Marking

The bees were marked at the nest entrance using a fluorescent powder marking system designed by M. Schaffer (Schaffer 1997). Marking tunnels were used, which consisted of a plastic PET soft drink bottle painted black, with corrugated cardboard lining the inside and a cosmetics brush projecting into the narrow end of the bottle. The wide end of the bottle was screwed on the nest entrance and the narrow end rested on a foam "landing pad" (Plate 3.1). As a bee left the nest, its legs and ventral surface became coated with fluorescent powder in the grooves of the cardboard. When the bee walked out of the entrance hole its dorsal surface was doused with powder from the cosmetics brush. Four colours of "Day Glo ™" fluorescent powder were used: nest 1 - saturn yellow (yellow); nest 2 - signal green (green); nest 3 - strong magenta (red); and nest 4 - strong orange (orange). The marking tunnels were filled with c. 5 cc of fluorescent powder, shaken vigorously and attached to the nest one hour before each observation session, to allow time for all active foragers to be marked.

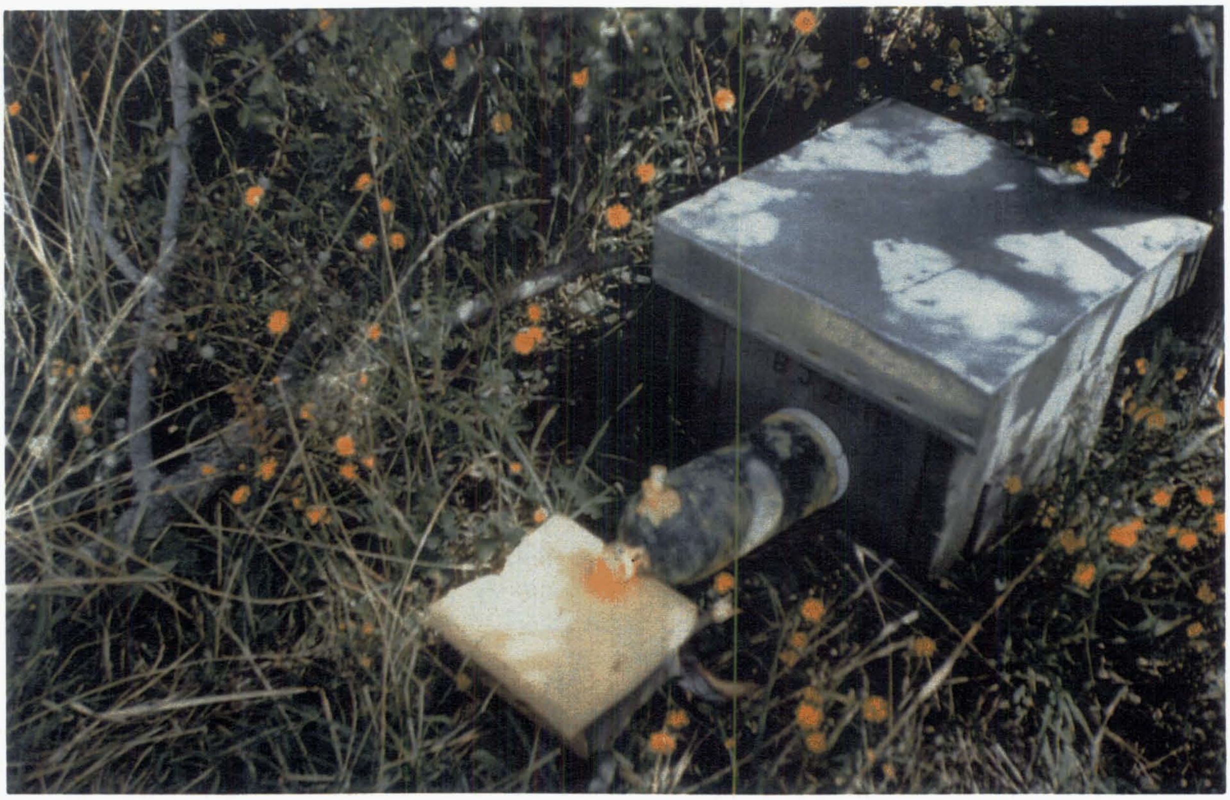

Plate 3.1. Fluorescent-powder marking tunnel attached to bumble bee domicile.

The efficiency of the marking tunnels was tested by an observer sitting outside the nest for 15, 30 or 60 mins and recording the number of marked and unmarked bees leaving and entering the nest.

3.2.3 Transect walks

Transect walks, 100 m long, were used to estimate bee numbers (Banaszak 1980). Transect walks were carried out at distances of 10, 50, 100 and 200 m from the bumble bee nests (Fig. 3.1). The observer walked down the transect at a steady pace, looking 1.5 m to either side, recording the bumble bee species, caste, markings, foraging behaviour and presence and colour of pollen loads. The number of honey bees was also recorded on the transect walks until 18 February 1997, when their numbers became large and counting them slowed the observer too much.

Bee activity can be affected directly by microclimate fluctuations and indirectly through the production of nectar and pollen (Free 1993). At the beginning of each transect walk, temperature (standard dry bulb thermometer), humidity (sling psychrometer), radiation intensity (INS DX-100 digital lux meter), wind direction and wind speed (anemometer) were recorded at crop height (approx. 0.5 m).

Transect walks were carried out between 15:00 and 18:00 h (NZ Summer Time), a time of high bumble bee activity (Fussell 1992; Schaffer 1997; section 3.3 this Chapter). It took approximately two hours to complete the set of four transects. Thirteen sets of transect walks were carried out between 20 January and 13 March 1997. The transect order was reversed each time a new set was started.

3.2.4 Flowering intensity

The number of red clover inflorescences in 10 randomly placed 0.5 m^2 quadrats was counted each time a set of transect walks was completed. At peak flowering, a random sample of 30 inflorescences was taken and the number of florets per inflorescence was counted.

3.2.5 Nest traffic

To calculate reobservation rates, an estimate of the number of bees foraging is required. This was done using time-lapse video recording. A black and white JVC camera (model SR-L900E) with a 50 mm macro lens was connected to a JVC time-lapse recorder (model TK-S240) and powered by a 12 v Portalac battery. Because of technical difficulties, including such things as battery failure and loose connections, the timing of recordings was sporadic; for a list of recorded dates and times see Appendix 3.1. Five of the recordings were of the green nest and one was of the orange nest. When replayed, the video recordings were viewed in contiguous 15-minute segments, tallying the number of bees entering and leaving the nest in each 15-minute period. The number entering was subtracted from the number that had left, to give an estimate of the number of bees out of the nest at the end of that 15-minute recording period. This total was carried over to the next 15-minute period, from which the number of entries was subtracted and the number of exits added, to give a cumulative total of the number of bees foraging throughout the day.

To emulate the temperature a bee experiences, a thermocouple was inserted into a "black-globe" (Corbet 1990), made of 20 mm diameter ball of Blu-tack ball painted black. "Black-globe" temperature was measured in conjunction with video recordings using a Tiny-talk® data logger, but all but one day was lost whilst down-loading the data.

3.2.6 Intensive searches

Because of the low number of marked bumble bees found in the crop, two intensive searches of the crop were carried out with a team of workers. On 27 February 1997, six observers systematically searched the whole crop for marked bumble bees. Standing 4 m apart, they made their way back and forth across the crop on a north-south axis looking 2 m to either side of them. The numbers of marked and unmarked *B. hortorum* were recorded. A similar search was carried out on 12 March 1997 but only four observers were present and only one quarter of the crop was covered.

3.2.7 Pollen analysis

Pollen samples were taken from worker bees returning to their nests. Pollen was collected at least once a week from the yellow nest (starting 20 January 1997) and at least twice a week from the green nest (starting 21 January). Pollen was initially collected from the yellow and green nests only, but the bees in the yellow nest stopped collecting pollen around 17 February 1997, so some additional pollen samples were taken from the red and orange nests (see Appendix 3.2 for a list of collection dates and number of samples taken).

Pollen presentation schedules (the time of day anthers dehisce and/or flowers open) and hence insect activity can vary throughout the day (Free 1993). Therefore, pollen was collected within the same time period on each collection date: between 10:00 and 12:00 h (yellow and orange nests) (NZ Summer Time) and between 14:00 and 16:30 h (green and red nests). Pollen was collected over a one-hour period with a maximum of six samples taken per hour. Pollen collection was avoided on days that transect walks were made, in case pollen removal affected foraging behaviour. However, sometimes both types of sampling were done on the same day because of poor weather. One hundred and five pollen samples were taken between 20 January and 12 March 1997 when bees in the last nest still collecting pollen (orange) ceased collecting.

Pollen was collected from workers by catching the bees in a net, transferring them to a holding tube and removing a single corbiculum pollen load with a needle. The pollen was then transferred to a 5 ml centrifuge tube containing approximately 3 ml of 70% ethanol. The needle was cleaned with ethanol between collections to avoid cross contamination of pollen. Pollen samples in the centrifuge tubes were placed in a Vortis™ mixer to blend the layers of pollen. The samples were then centrifuged for 5 mins at 6000 rpm and the ethanol was evaporated over a hot-plate. A square (approx. 5 mm^2) of glycerine jelly stained with saffranin (Erdtman 1943) impaled on a needle was used to pick up the pollen grains in the tube. The jelly and attached pollen grains were then transferred to a slide. This procedure was repeated to pick up any remaining pollen grains in the centrifuge tube. The slides were then warmed on a hot plate to melt the glycerine jelly, the pollen was stirred in and coverslips applied.

Pollen grains were identified and counted in six different, non-overlapping fields of view at 400x magnification. Preliminary observations showed that this number of fields of view was sufficient to detect all pollen types present on a slide. Pollen grains were identified with the aid of reference slides, reference books (Erdtman 1943, Sawyer 1981) and the help of a pollen taxonomist (Neville Moar, Landcare Research). Although red clover pollen is quite distinctive, some other pollen types could be identified only to the family level (e.g., Rosaceae).

3.2.8 Data analysis

Because of the non-normality and heteroscedasticity of the data gathered in this study, it was analysed by non-parametric methods. Spearman rank correlations were used to test for associations between forager numbers and climate variables and a Kruskal-Wallis ANOVA was used to test for differences in the proportions of red clover pollen collected over time. There were too many zeros in the data to perform even a non-parametric test on the numbers of marked *B. hortorum* found at different distances from the nest.

3.3 Results

3.3.1 Red clover flowering phenology

The crop began flowering on approximately 10 January 1997 and the number of flowering inflorescences reached a maximum on 18 February 1997 (Fig. 3.2). At peak flowering, there were 2220000 (± 190555 SE) inflorescences per hectare and the mean number of florets per inflorescence was 115 (± 3.7 SE). The crop was harvested in late April 1997 and yielded 245 kg/ha of dressed seed (980 kg total) (R. McCarthy, pers. comm.). The numbers of *B. terrestris* observed on the crop were strongly correlated with the density of red clover inflorescences (Spearman rank; Rs=0.817, df=12, *P*=0.0007); there was no such correlation with *B. hortorum* numbers (Spearman rank; Rs=0.296, df=12, *P*=0.326).

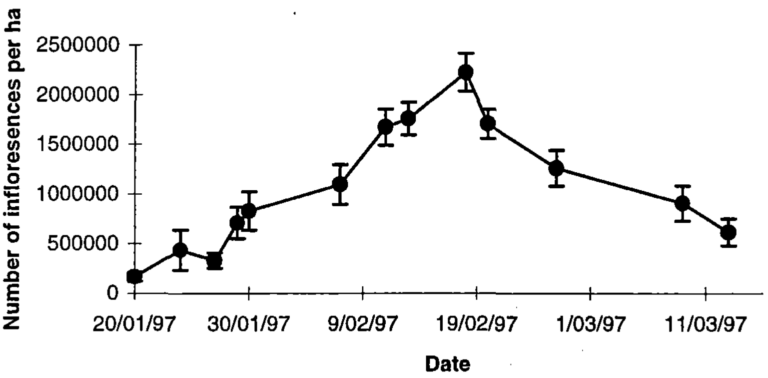

Figure 3.2. Estimated number of *Trifolium pratense* 'Pawera' inflorescences per hectare on a 4 ha crop at Tai Tapu, 1997 (means ± SE).

3.3.2 Bees foraging on the crop

Three bumble bee species were observed foraging on the crop: *B. hortorum*, *B. terrestris* and *B. ruderatus*. Only one *B. ruderatus* was observed, during an intensive search on 27 February 1997. *B. terrestris* was the main bumble bee species on the crop, comprising 76 % of all *Bombus* observed on the transect walks. *B. terrestris* numbers peaked on 26 February 1997 (Fig. 3.3), and the number of *B. hortorum* foraging on the crop peaked later, around 9 March 1997 (Fig 3.3). Mean density of *B. hortorum* (excluding intensive search data) was 80 bees/ha (range: 17-258 bees/ha) and for *B. terrestris* was 258 bees/ha (range: 75-483 bees/ha). The average ratio of marked to unmarked *B. hortorum* foraging on the crop was 1:43 (range: 2:1-1:152). All castes of bumble bees were observed foraging on the crop, although *B. hortorum* males were not observed until 9 March 1997.

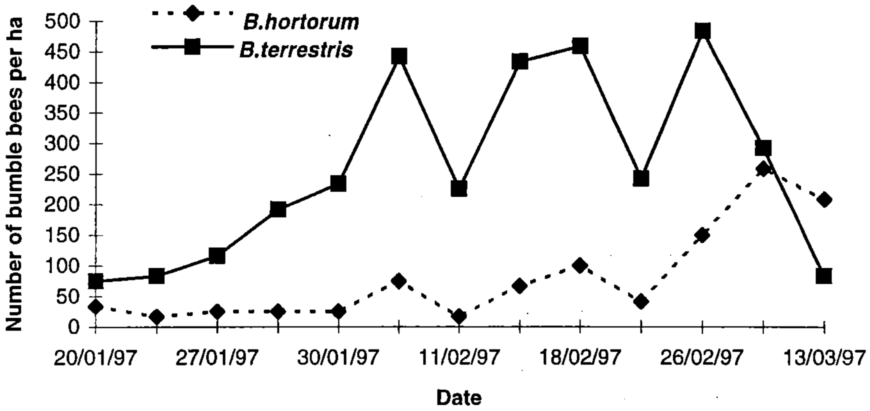

Figure 3.3. Number of bumble bees observed on transect walks within a 4 ha *Trifolium pratense* 'Pawera' crop, Tai Tapu, 1997.

All the *B. hortorum* individuals observed on the crop were visiting flowers through the front of the floret (64 % for pollen, 36 % for nectar only), contacting the anthers and stigmas. However, only 24 % of *B. terrestris* visits to the red clover were of this type. Most *B. terrestris* (76 %) were nectar-robbing, biting holes in the base of the corolla tube to obtain nectar. Most of the honey bees observed (90%) were secondarily nectar robbing, i.e., taking nectar through holes made by *B. terrestris*.

3.3.3 Climatic factors

Microclimate variables did not differ much between transect observation periods, although 26 February 1997 and 9 March 1997 had low relative humidity and light intensity (Fig. 3.4). The wind was mostly north-easterly. The number of *B. terrestris* observed on the crop was positively correlated with temperature (Spearman rank; Rs=0.634, df=11, *P*=0.027). The number of *B. hortorum* foraging on the crop was negatively correlated with light intensity (Spearman rank; Rs=-0.681, df=11, *P*=0.015), and with relative humidity (Spearman rank; Rs=-0.713, df=11, *P*=0.009).

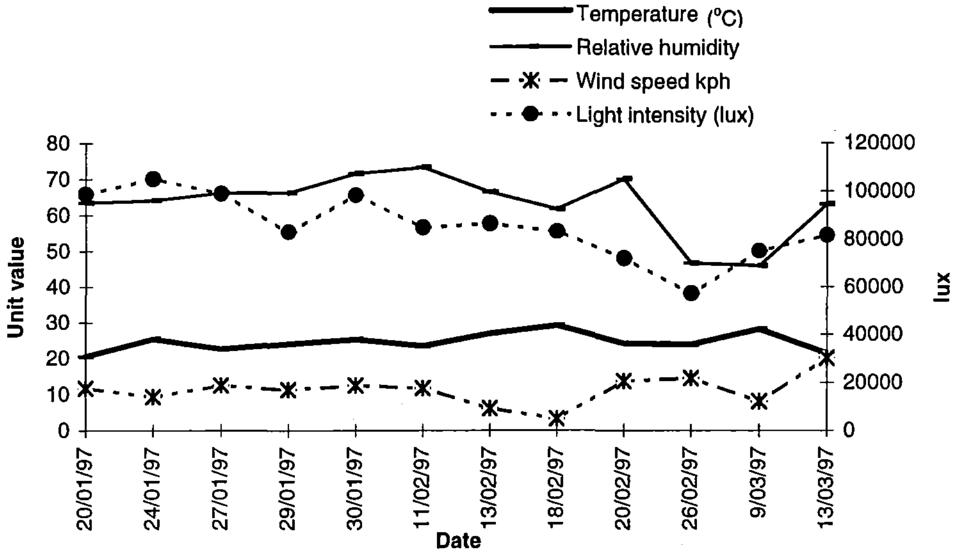

Figure 3.4. Microclimate data recorded during each transect walk (means ± SE presented). Left axis: temperature (°C), relative humidity, and wind speed (mph). Right axis: light intensity (lux).

3.3.4 Efficiency of marker tunnels

Records of nest activity, 1 h after recharging the marker tunnels, showed that on average 82% (range 11-100 %) of the bees leaving the nest were clearly marked with fluorescent powder. The powder was clearly visible in the field and 5 cc of powder was sufficient to mark bees leaving the nest for 3 h.

3.3.5 Transect walk reobservation data

In total, 125 *B. hortorum* were recorded on transect walks; 10 (8 %) were marked bees. Two yellow-, three green- and five orange-marked bees were reobserved; but no red-marked individuals were seen. Using an estimate of the number of bees foraging (from video records) on 28 January 1997 and 27 February 1997, reobservation rates of 3.82 % and 5.41 % respectively were calculated. No marked bees were reobserved at the 10 m transect (Fig. 3.5) and reobservations were too few to compare statistically the mean number of marked bees observed at each transect. No marked bees were observed during casual searches on the weeds (*Malva sylvestris* L., *Symphytum officinale* L., *Taraxum* spp., *Carduus* spp.) surrounding the crop.

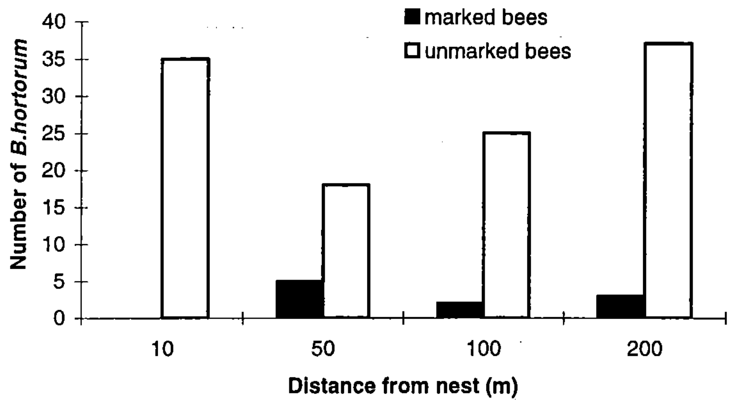

Figure 3.5. Number of *Bombus hortorum* observed on transect walks in a *Trifolium pratense* 'Pawera' crop over 13 observation sessions 24 January - 13 March 1997.

3.3.6 Intensive search reobservation data

On 27 February 1997, the whole crop was searched and 453 *B. hortorum* bees were observed, of which 14 (3.1 %) were marked. Five yellow-, two green-, two red- and five orange-marked bees were reobserved. The reobservation rate of marked bees calculated from video recording data was 6.67 %. There was a peak of marked *B. hortorum* at 150-199 m and unmarked at 200-249 m from the nests (Fig 3.6).

On 12 March 1997 the western end of the crop up to 75 m in from the hawthorn hedge was intensively searched. *B. hortorum* numbers were very high that day and 613 were observed. Four of these (0.7 %) were marked and all were from the orange nest. The reobservation rate was 14.8 %. The marked bees were found at distances of approximately 5, 15, 50 and 60 m from their nest.

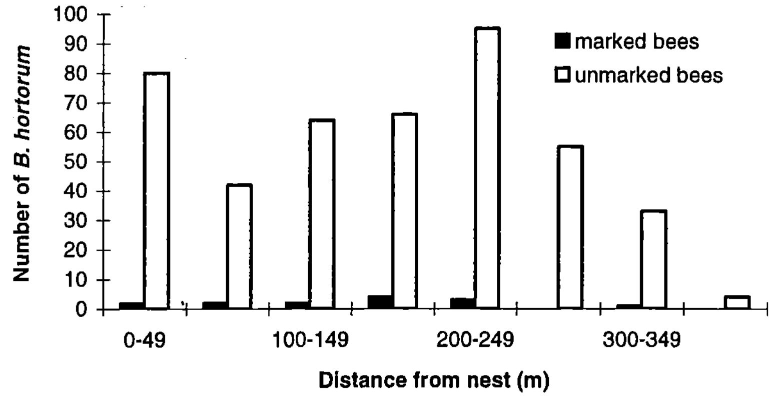

Figure 3.6. Number of marked and unmarked *Bombus hortorum* foraging on a 4 ha *Trifolium pratense* cv. Pawera crop with increasing distance from four *B. hortorum* nests.

3.3.7 Diurnal patterns of foraging activity

Video records showed that the number of *B. hortorum* foraging increased steadily throughout the day and peaked between 15:00 and 16:00 h (NZ Summer Time) (Fig 3.7). No mid-day decline in bee activity was evident. Black globe temperature was positively correlated with the number of bees foraging (Spearman rank; Rs=0.583, df=27, *P*=0.001). Bees from the study nests were active for at least 14 h per day.

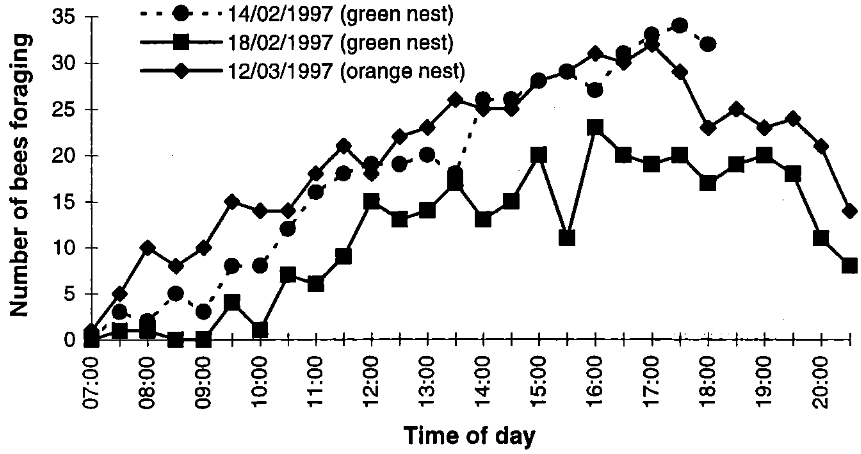

Figure 3.7. Foraging activity of a *Bombus hortorum* nest throughout the day.

3.3.8 Pollen analysis

Most *B. hortorum* from the four nests were collecting red clover pollen (Table 3.1); this was assumed to be collected from the adjacent crop because there were no other red clover crops within a 2 km radius. Most red clover pollen loads (84 %), were 'pure' [sensu Free (1970) who classified 'pure' as >98 % of one type of pollen]. Nest four (orange) was an exception; over half the pollen loads sampled from this nest were collected from other than red clover (Table 3.1). Over all nests, twelve pollen types were identified (see Appendix 3.3). The percentage of red clover pollen collected by nest two (green) changed over time (Kruskal Wallis: H=24.011, P=0.02, df=12) but this was not biologically important since the percentage of red clover in pollen loads ranged only from 94.12 - 100%.

Table 3.1. Pollen loads collected by workers from four *Bombus hortorum* nests adjacent to a red clover crop.

	N	No. of loads with > 98% red clover pollen (%)	No of pollen types	Mean % of red clover pollen per load
All Nests	105	89 (84.8 %)	12	90.4
Nest 1	14	14 (100 %)	2	99.6
Nest 2	67	63 (94.0 %)	6	99.5
Nest 3	3	3 (100 %)	2	99.7
Nest 4	21	9 (42.9 %)	9	53.7

3.3.9 Nest productivity

Nests were variable in their size and productivity. Nest 4 (orange) was the largest and survived the longest (Table 3.2).

Table 3.2. Dimensions (mm) and productivity of four *Bombus hortorum* nests shifted to a red clover crop on 15 January 1997.

Nest	Marking colour	Nest size at 17/01/97 ht	Nest size at 17/01/97 diam	Nest size at 7/04/98 ht	Nest size at 7/04/98 diam	Approx. date bees stopped collecting pollen	Approx. date all bees dead	Queens produced?
1	yellow	80	150	100	190	17/02/97	9/03/97	Yes
2	green	100	200	140	220	3/03/97	25/03/97	Yes
3	red	70	100	80	130	10/03/97	15/03/97	No
4	orange	80	180	150	240	12/03/97	6/04/97	Yes

3.4 Discussion

3.4.1 Bee densities

Mean densities of *B. hortorum* (80 bees/ha) were similar to those found in early New Zealand studies on diploid clover (Forster & Hadfield 1958; Palmer-Jones *et al.* 1966), but were low compared with more recent New Zealand studies on tetraploid red clover, which reported densities of 1250-7300 LTBB/ha (Clifford 1973; Macfarlane & Griffin 1985; Brown 1989; Clifford & Scott 1989; Macfarlane *et al.* 1991). Most of these higher densities can be explained by a concentration effect on bumble bee populations by the much smaller field sizes (Clifford 1973, 0.8 ha; Brown 1989, 0.36 ha; Clifford & Scott 1989, 0.8 ha; Macfarlane *et al.* 1991, 0.36 ha) compared with those in the current study (4 ha). However, in commercial-sized crops supplemented with similar stocking rates of LTBB (c. 1 colony/ha), average LTBB densities were still higher at 365 and 465 LTBB/ha (Macfarlane & Griffin 1985) than in this study. This difference could be due to many factors such as differences in local bumble bee populations, inflorescence density (Clifford & Scott 1989), yearly bumble bee population fluctuations (Forster & Hadfield 1958) or the time of day that observations were made (Clifford & Scott 1989). *B. terrestris* densities were three times as high as those of *B. hortorum* in this study; Clifford & Scott (1989) suggested that when local populations of *B. terrestris* are high, they compete through exploitation (i.e., nectar removal) with LTBB on red clover.

Peak densities of bumble bees occurred at a later date than the peak of red clover inflorescences per hectare. Most red clover seed is produced from the early and main flowering heads (Clifford & Anderson 1980) so, for optimal pollination, it is recommended that the peaks of crop flowering and bumble bee populations are synchronised (Holm 1966; Free 1993). That was not so in the current study thus seed yields may have been limited.

3.4.2 Foraging behaviour

The foraging behaviour of the different bee species on the crop was markedly different. All observed visits by *B. hortorum* were through the front of the flower and hence would have tripped the flower's pollinating mechanism. Marked *B. hortorum* did not display any difference in their foraging behaviour on the crop compared with unmarked bees. Most *B. terrestris* (76 %) were not effecting pollination as they were robbing nectar through holes in the side of the corollae. This is slightly lower than the 93-100% reported by other workers studying tetraploid red clover crops in New Zealand (Forster & Hadfield 1958; Palmer-Jones *et al.* 1966; Clifford 1973; Clifford & Scott 1989; Fussell 1992). This difference may be due to the high numbers of *B. terrestris* queens observed; these have longer tongues than workers, enabling them to extract nectar legitimately through the front of the flower. The holes bitten in the corolla of red clover flowers by robber bumble bees do not affect the ability of the flower to set seed if they are subsequently visited by a legitimate pollinator (Free 1993). The impact of nectar robbers on crop pollination has yet to be quantified. Free (1993) described two possible scenarios: 1. The holes bitten by STBB may attract large numbers of honey bees (acting as secondary robbers), some of which will enter the flowers and pollinate them; or 2. the activities of robber bees may reduce the number of legitimate pollinators visiting the crop by depleting the amount of nectar available to them. Despite this 'dishonest' behaviour, *B. terrestris* may be a significant pollinator of red clover crops because of its sheer numbers (Hawkins 1956; Forster & Hadfield 1958; Gurr 1961). In this study, an estimated average of 61 *B. terrestris* per hectare were legitimately visiting the red clover (24% of 258 bees/ha); this is similar to the average density of *B. hortorum* on the crop. Also, *B. terrestris* numbers were at their peak earlier and were therefore available to pollinate the high seed-setting early and main flowering heads.

Honey bees were present in large numbers on the crop (mean density of 1157 bees/ha) but most (90%) were robbing the nectar through holes bitten by *B. terrestris.* Benedek (1976, cited in Free 1993) and Macfarlane & Griffin (1985) found that the percentage of pollinating honey bees declined as red clover flowering proceeded because of an influx of robbing nectar-collecting honey bees. A similar trend is suggested in this study but honey bee numbers were not recorded throughout the whole flowering period. Again, the high numbers of honey bees may make them important pollinators of red clover in spite of their robbing behaviour (Forster & Hadfield 1958; Palmer-Jones *et al.* 1966; Free 1993). However, they are generally regarded as inefficient pollinators of tetraploid red clover because of their tendency to switch to alternative forage sources, their slow working speeds, their limited ability to work at low temperatures, and their highly fluctuating numbers on the crop, both throughout the day and over the crop flowering period (Macfarlane & Griffin 1985; Clifford & Scott 1989; Free 1993).

3.4.3 Climatic effects on bumble bee foraging

Climatic variables were not very useful in explaining differences in bumble bee densities among observation periods. This is probably because climatic variables did not differ markedly between observation periods as transect walks were deliberately carried out on fine sunny days. The negative correlation of *B. hortorum* and *B. terrestris* numbers with light intensity was probably coincidental because two high lux measurements were recorded at the start of crop flowering when bumble bee densities were low. However, high radiation can lower *B. subterraneus* numbers (Clifford & Scott 1989). Bumble bees tolerate strong winds; gusts of up to 20 kph were recorded in this study, yet bumble bee densities were not correlated with wind speed. On two occasions (11 and 20 February 1997) densities of both bumble bee species dropped significantly. Relative humidity recordings were high on those days. The number of *B. hortorum* observed was negatively correlated with relative humidity, yet *B. terrestris* numbers were not. Whether a real effect of microclimate on bumble bee densities was demonstrated or if it was simply a spurious result cannot be determined. If relative humidity did have an effect it would probably be indirectly through nectar concentration and pollen presentation rather than via a direct effect on insect activity itself (Kearns & Inouye 1993). Temperature affects bumble bee activity directly (Clifford & Scott 1989; Corbet *et al.* 1993) and in this study the numbers of *B. terrestris* were positively correlated with temperature.

3.4.4 Diurnal foraging patterns

Video records showed that nests were active for long periods each day (at least 14 hours on fine sunny days). The ability of bumble bees to regulate their body temperature enables them to forage for these extended periods and gives them an advantage over honey bees, which are more limited by temperature and have shorter foraging days (Macfarlane *et al.* 1991; Fussell 1992; Corbet *et al.* 1993). Video records did not reveal the midday lull in bumble bee activity reported by other authors (Alford 1975; Clifford & Scott 1989; Fussell 1992). This may be because midday peaks of radiation intensity and temperature or densities of honey bees (Clifford & Scott 1989; Fussell 1992; Schaffer 1997) were not sufficiently high to depress bumble bee numbers. Black globe temperatures, recorded in conjunction with video recordings, were positively correlated with the cumulative number of bees foraging, again illustrating the strong effects of temperature on foraging activity (Corbet *et al.* 1993). Although not measured in this study, diurnal patterns of floret opening and nectar production could affect nest activity. The peak in the rate of floret opening between 1200 h and 1500 h (Clifford & Scott 1989; Fussell 1992) and peak nectar rewards in early evening (Fussell 1992) could account for the peak in *B. hortorum* activity in late afternoon. Video recordings showed that transect walks were done at an optimal time for reobservation of nest-marked bees.

3.4.5 Was *B. hortorum* visiting the crop?

Reobservation rates in the current study (4.6 %) were higher than those reported by Saville *et al.* (1996: 0.148 %) and Schaffer (1997: 0.9 %). However, it was not known where over 95 % of the bees were foraging. Reobservation rates may have been higher than in previous studies because more bees were actually visiting the crop due to the strong preference of *B. hortorum* for red clover and because the 4 ha crop was a considerably larger resource compared with the small patches of flowers studied by Saville *et al.* (1996) and Schaffer (1997). Reobservation rates may have been low for a variety of reasons: the bees were visiting the crop but were not detected, the bees were in the crop but did not have markings on them, or most bees were not visiting the crop. Transect walks are not the ideal method for detecting nest-marked bees because, when two intensive searches were carried out, the reobservation rates rose from 4.6 % to 6.67 and 14.8 %. The fluorescent marking tunnels were a reliable method of marking most (82 %) of the foragers, so it can be assumed that this was not the reason for low reobservation rates. No marked bumble bees were observed foraging on weed species surrounding the crop.

Using mark-reobservation methods alone did not give a good indication of where most of the bumble bees were foraging. Pollen analysis was a much more direct method for detecting where the bumble bees had been foraging. This showed that most of the pollen-collectors were 'majoring' (Heinrich 1979a) on the red clover pollen. They were unlikely to be foraging elsewhere because there were no other red clover crops within a 2 km radius. Only two of the marked bees observed on the crop were collecting pollen, yet most pollen-collectors from the nest were collecting red clover pollen and, at the height of pollen collection, 80-91 % of the foragers were returning with pollen loads. These results suggest that the nectar-collecting bees were also targeting red clover but were not detected by the observation methods. A better method of detecting where nectar-collectors are foraging is required. Spencer-Booth (1965) showed that pollen from flowers from which bumble bees are collecting nectar accumulates in the proboscidial fossa. This method could be used to study the foraging of nectar-collecting bees in future studies, but this method also has limitations. For example, pollen would not accumulate in the mouthparts of nectar robbers.

The transect observation method used in this study was not optimal for reobservation of nest-marked bees. This was probably because the area covered by the transects was too small to detect the few marked bees foraging in a large crop area. Rates of reobservation may have been affected by the tendency of bees to follow traplines and/or show forage patch fidelity (Saville *et al.* 1996) or by the decreased probability of detecting a bee the further away it is from the nest due to the distance/area relationship (Schaffer 1997). Strong winds may have also interfered with observations on some days, because the moving plants made it difficult to detect bees. Intensive searches gave good results but much labour was needed.

3.4.6 Bumble bee movement from the nest

Because the transect walks and intensive searches used different search methods and effort, the results cannot be pooled, so they will be discussed separately. The low numbers of marked bees reobserved during transect walks meant that no statistical analyses could be performed on bee numbers at different distances from the nest. Over all transect walk observation periods, no marked bees were observed at the 10 m transect, yet the number of unmarked *B. hortorum* at this transect was high. Casual searches within 10 m of the nest on three occasions failed to reveal any marked bumble bees. This suggests that *B. hortorum* from the supplied nests was generally not foraging within 10 m of the nest. It has been suggested that because bees collect a pollen load more quickly than a nectar load, there is a tendency for pollen collectors to forage closer to the colony (Free 1993); reobservation rates in the current study were too low to detect this.

The intensive search of the whole crop on 27 February revealed that most marked bumble bees on the crop were foraging within 200 m of their nest. Only one marked *B. hortorum* was observed further than 300 m from the nest. However, most unmarked bees were also found in this area of the crop and because of the irregular shape of the field, the areas of each transect were different, so no valid comparisons can be made. The intensive search on 12 March was the only time a marked bumble bee was found within 10 m of its nest.

This bee came from the nest (orange) that was not collecting the majority of its pollen from the red clover crop; the orange nest was also the largest nest of the four. The bees from the orange nest would have to fly at least 200 m to collect the Rosaceae and Ericaceae pollen types present in their pollen loads. Dramstad (, 1996 #213]) and Schaffer (1997) also found that bumble bees did not necessarily visit the neighbouring forage resource and instead foraged some distance away. Dramstad (1996a) suggested that this was because bumble bees evolved not to forage close to their nest. The low reobservation rates in current and other studies (Dramstad 1996a; Saville *et al.* 1996; Schaffer 1997) suggest that earlier findings on bumble bee foraging distances (Butler 1951; Free & Butler 1959; Alford 1975; Macfarlane *et al.* 1994) may be suspect because the bees under observation were not marked and/or reobservation rates were not calculated. Because no definite conclusions could be reached with this experiment, the question of how far bumble bees fly from the nest to forage is further explored in Chapter 4 of this thesis.

3.4.7 Evaluation of B. hortorum nests as pollinators of tetraploid red clover crops

From the pollen load study it may be concluded that the pollen-collecting bumble bees from nests adjacent to the red clover were faithful to the target crop. Pollen-collecting bees are reputed to have faster working speeds (Skovgaard 1952) and a greater pollinating efficiency than nectar collectors (Free & Williams 1972). Of the *B. hortorum* that visited the crop, all were potential pollinators (both nectar- and pollen-collectors) because they were foraging through the front of the flower and therefore would have tripped the flower's pollinating mechanism. It was expected that all four nests would collect similar types and proportions of pollen because of their proximity to each other and their similar access to forage resources, but this was not the case. This phenomenon of different exploitation of available resources is commonly reported in the literature for both bumble bees (Free 1970) and honey bees (Free 1993) when nests are placed along one crop edge. Therefore it cannot be assumed that all nests shifted to a crop or all foragers from a nest will visit the target crop and this must be taken into account when calculating stocking rates. The bees will also be less constant to red clover than indicated by pollen analysis alone, because pollen-collectors may forage on many different species throughout the day and the flower types visited by nectar-collectors cannot be deduced.

The mark-reobservation data showed that the marked bees were moving at least 200 m into the crop to forage, so there should have been no 'cold spots' of inadequate pollination in the centre of the crop.

The ratio of marked to unmarked *B. hortorum* foraging on the crop was 1:43, suggesting that the extra four colonies supplemented the natural *B. hortorum* population by only 2 %. If other pollinating bees were included, this percentage would be even lower. The nests were within the normal size range of naturally occurring nests (Donovan & Wier 1978) so the number of foragers from each placed nest would have been typical of a natural *B. hortorum* nest. This suggests that very high numbers of colonies would have to be moved to a crop to significantly enhance bumble bee numbers foraging on the crop. At approximately $65 per nest, this could be expensive. Donovan (unpub. data) estimated that a single *B. hortorum* nest can produce 126.65 kilograms of seed and at $10 per kg is worth $1266.54. It is unlikely in this study that the nests contributed this much to the yields, given their very low proportion of the pollinator population.

The relationship between seed set and pollinator density can be described by a hyperbola and is therefore governed by the law of diminishing returns (Fig. 1.2, Chapter 1) (Plowright & Hartling 1981). Palmer-Jones *et al.* (1966) found that caging very high concentrations of honey bees and LTBBs caused no further increases in pollination compared with that in the surrounding crop. The supplementation of natural populations with four *B. hortorum* nests may have added little to the resulting seed yields because the existing pollinator population was sufficiently high to adequately pollinate the crop. The resulting seed yield in this study (245 kg/ha) was higher than the national average (150 kg/ha, P.Clifford pers. comm.) suggesting that pollination was adequate. However, much higher tetraploid seed yields have been reported in New Zealand (Macfarlane *et al.* 1991, 528 kg/ha; Clifford & Anderson 1980, 600 kg/ha), and given the lower densities of LTBB compared with those in other New Zealand studies, it can be inferred that the crop in this situation was not near the asymptote of the seed set/pollinator abundance curve described by Plowright & Hartling (1981).

An important point in comparing yields between different crops is that pollination is not the sole determinant of seed set; other factors such as soil moisture, soil nutrients, plant row spacing, seed contamination, closing date and timing of harvest can affect yields (Clifford & Anderson 1980). Harvest losses of seed may also be significant; they were over 50 percent for the crop in this study (R.McCarthy pers. comm.) which is similar to the mean of 49 % reported by Macfarlane *et al.* (1991). Therefore it may be meaningless to compare yields and try to relate them to pollinator densities when agronomic conditions differ widely.

3.4.8 Management of colonies

This study has shown that when placed near a red clover crop, most pollen-collecting bees from a *B. hortorum* nest will visit the crop and remain faithful to it as flowering progresses. Weed species such as mallow (*Malva* spp.) and thistle (*Carduus* spp.) were not major competitors for foragers' attention, but flowering shrubs and trees attracted some bees from one nest away from the crop.

The colonies should be shifted to the crop when it has just begun to flower so that the bees are available to work it when the early and main inflorescences are in flower, to maximise yields. This is especially important when local LTBB populations do not peak until late in the flowering period, as occurred in this study. Free (1959) and Free *et al.* (1960) found that shifting honey bees to a crop when it had just started flowering resulted in the bees' collecting more of the target pollen than when they were shifted before flowering or at its peak.

Colony placement is not critical in small fields because the bees will move at least 200 m into the crop. Nests should be placed on the edge of the crop that is downwind of the prevailing wind (Chapter 4). Placement should be modified to some extent with respect to the location of shelter for the nests as overheating can be a problem in summer (Chapter 4). Data from this study suggest that the bumble bees did not forage close to their nests (within 10 m) but the concentration of naturally occurring bumble bee populations at the edges more than compensated for this.

A stocking rate of *B. hortorum* colonies is difficult to calculate because it depends on the existing natural populations of both *B. hortorum* and other bee species plus their interactions with each other; ideally, these should be monitored before deciding on a stocking rate. Supplementation may not significantly increase pollinator numbers or seed yields and therefore would not warrant the $65+ outlay per nest. Macfarlane *et al.* (1991) recommended a stocking rate of six LTBB colonies per hectare for maximum seed set based on flower and bee density counts. For the crop in this study that would cost $1560, and would need an extra 141 kg in seed to pay for the colonies alone. The relationship between *B. hortorum* and red clover seed set has been estimated (Donovan unpub. data), but this considered *B. hortorum* in isolation; its interactions with other pollinators and other floral resources were not considered. Existing knowledge on the quantitative relationship between seed set and pollinator abundance is meagre (but see Plowright & Hartling 1981). In the future, more complex models incorporating the temporal and spatial dynamics of *B. hortorum* foraging behaviour could be constructed to better estimate stocking rates and to aid pollinator management.

B. hortorum is a very effective pollinator of tetraploid red clover but, because of the high cost per bee, purchasing bumble bee colonies for pollinating field crops has not yet proven to be cost effective. Purchase may be warranted when natural populations are low or asynchronous with crop flowering, or when increasing the crop area. If local pollinator populations are adequate, a low-cost management technique such as provision of forage and shelter resources (see Chapter 2) would be the best option.

CHAPTER 4: FORAGING DISTANCES OF *BOMBUS TERRESTRIS* FROM THE NEST

4.1 Introduction

4.1.1 Background

Optimal foraging theory predicts that bumble bees will minimise flight distances between the nest and flowers to maximise their net rate of energy intake (Heinrich 1975). Consequently, the prevailing view in the literature has been that "As long as food is locally abundant it is probable that the bumble bees forage close to the hive, specialising on the local flora." (Heinrich 1976, pg 126). However, there are many examples of bumble bees forsaking presumably rewarding patches of flowers close to their nest and flying further afield to forage (Hobbs *et al.* 1961; Dramstad 1996a; Saville *et al.* 1996; Schaffer 1997, J. Osborne pers. comm., Chapter 3) . These results suggest that the bees prefer to forage at greater distances from the nest than those predicted by energetic models.

4.1.2 Bumble bee foraging distances from the nest

Dramstad (1996a) reviewed the literature and presented results of three mark-reobservation studies that suggested bumble bees tended not to forage close (within 50 m) to their nest. She concluded that they 'prefer' to forage some distance from their nest and she suggested reduced predation risk and intraspecific competition/depletion of resources as explanations for this behaviour. A study designed to test this hypothesis involved placing three *B. lucorum* nests directly adjacent to a 2 m x 210 m strip of *P. tanacetifolia,* carrying out mark-reobservations, then relocating the nests to over 200 m away and repeating observations (Dramstad 1996b). The mean number of bumble bees foraging on *Phacelia* before the nests were moved was 11.5, but this rose to 18.3 when the nests were over 200 m away. This result was regarded as supporting the hypothesis that bumble bees 'prefer' to forage at some distance from their nest. However, this result could have been confounded by time; over time the *Phacelia* may have become more attractive or more bees may have encountered it. Similarly, other studies have been confounded by the differential distribution of resources in space (Dramstad 1996a; Saville *et al.* 1996) or were merely anecdotal observations within other studies (Hobbs *et al.* 1961). To test whether this avoidance of foraging close to the nest is a real phenomenon, it is necessary to remove the confounding effects of floral quality, floral density and spatial arrangement, and the presence of competing floral resources. In this study, competing floral resources were minimised by conducting the experiment in grazed paddocks and floral rewards were standardised in time and space by using pots of *P. tanacetifolia*.

4.1.3 Bumble bee flight distances

Bumble bees are capable of flying long distances from the nest; distances of at least 2.4 km have been reported (Rau 1924). However, it is probable that like honey bees, most bumble bee foragers will range an average distance from the hive (Visscher & Seeley 1982; Roubik 1989; Buchmann 1991) and for the purposes of crop pollination, it is important to know where most bees forage most of the time. To find this out, observations must be repeated over time to see if the bees are consistently flying a certain distance. Also, an estimate of reobservation rate must be calculated to see if the observed behaviour is representative of the whole nest. Some previous studies (Butler 1951; Free & Butler 1959; Macfarlane *et al.* 1994) failed to fulfil these requirements and thus the validity of their findings is questionable. Also, Schaffer (1997) suggested that the leptokurtic distribution (many bees close to the nest, tailing off with increasing distance) found by some workers may be an artefact of their sampling method. This is because as linear distance from the nest increases, the area available for a bee to forage in increases disproportionately (by the square factor), hence there is a reduced probability of encountering a bee with increasing distance from the nest. The full 200 m radius foraging area was not covered in this study, but an attempt was made to correct for this problem. Ten times as many pots were placed at the furthest distance from the nest, giving a bee an equal probability of encountering a flower pot and also an equal probability of being observed if equal search effort is allocated per pot.

4.1.4 Objective

- To compare the number of nest-marked bees foraging at 20 and 200 m from their nest.

4.2 Methods

4.2.1 Experimental design

The study site was two 4 ha pastures on the Lincoln University sheep breeding unit. These paddocks were heavily grazed before the experiment. A commercially-reared *B. terrestris* nest (Zonda Resources Ltd.) was placed at one end of the paddock and pots of *P. tanacetifolia* were placed at distances of 20 and 200 m from it. As a bee moves further from its nest, the probability of it encountering a flower pot decreases because of the radius/circumference relationship (2πr). Therefore, to correct for this, more pots of *Phacelia* were placed at the 200 m distance (20 pots) than at 20 m from the nest (2 pots) (Fig. 4.1). The nests were left to acclimatise for two days before any observations were made. After three-four days of observations the orientation of the experiment was reversed i.e., the nest and pots were shifted to opposite ends. The experiment was repeated twice in different fields with a different nest and pots of *Phacelia* but the last replicate (number three) was abandoned because both the *Phacelia* and the bees were killed by excessive heat.

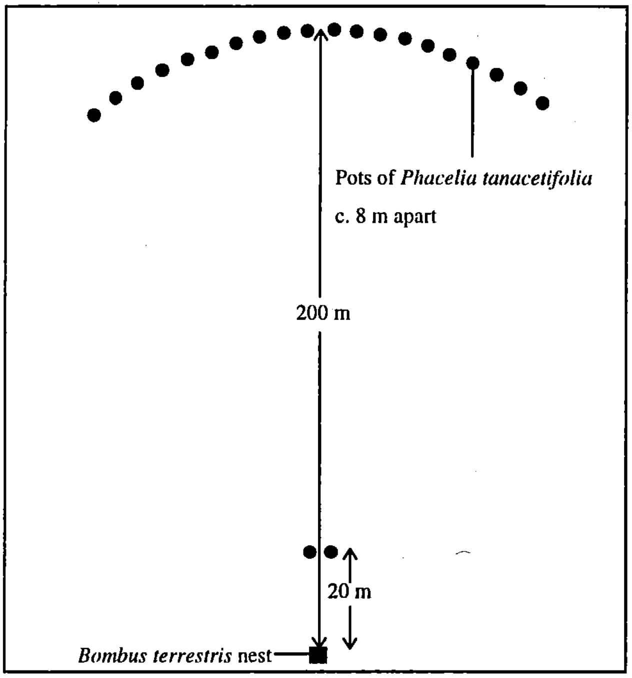

Figure 4.1. Diagram of the experimental design within a 4 ha paddock.

4.2.2 Study plant: *P. tanacetifolia*

P. tanacetifolia is a very attractive floral resource for bumble bees, especially *B. terrestris* (Williams & Christian 1991) and is often used in 'bee pasture' floral mixtures (Engels *et al.* 1994). *P. tanacetifolia* was sown in potting mix in 20 litre plastic plant tubs and thinned at the seedling stage to eight plants per pot. The pots were shifted to the field one day after flowering began. The pots of *Phacelia* were watered every day. The number of flowers per pot was estimated every second day of observations by counting the number of flowering stalks per pot, then counting the number of open flowers per stalk on five randomly selected stalks.

4.2.3 Mark-reobservations

The bees were marked at the nest using a marking tube (Kwak 1987) and a water-based paint. Some workers from unknown nests were marked with a numbered tag while foraging on the *Phacelia*. Marking was done in the morning and observations were generally carried out in the afternoon between 1400 h and 1800 h (NZ Summer Time), a time of high bumble bee activity (Fussell 1992; Schaffer 1997). Observations consisted of making 10 second counts of the number of bumble bees and honey bees foraging on each pot. The presence of markings, the species and caste of bumble bee and the type of foraging behaviour were also recorded. When a field assistant was available, counts at the 20 m and 200 m distance were made simultaneously, but most of the time one observer covered all the pots. The order of observation of the pots was reversed for each recording session. Counts were made over three-four days for each site/orientation combination and at six-ten times per day. Observations for replicate one were made between 27 November - 11 December 1997, and for replicate two, between 17 December - 31 December 1997. Microclimate measurements (temperature, humidity, wind speed and light intensity) were recorded before the start of each observation session.

Video recordings were to be used to estimate the number of bees foraging from the nest but only one recording was made before the recorder broke down. Instead, exits and entries from the nest were monitored for 30 min each day between observation sessions.

4.2.4 Pollen analysis

Because of the low reobservation rate of marked bees, pollen samples were taken from worker bees returning to the nest at the second replicate to determine what flowers they had been visiting as well as *Phacelia.* Methods followed those given in Chapter 3. Forty pollen load samples were taken over nine days between 17 December and 31 December, 1997.

4.2.5 Data analysis

The mean number of marked bees per pot was calculated for each walk by dividing the number of marked bees observed at 20 m by two, and at 200 m by 20. Because the data were highly skewed they were log-transformed (log $(x+1)$) before being analysed by ANOVA. A two-sample t-test was used to test for differences in the mean number of flowers per pot between pots at 20 and 200 m from the nest.

4.3 Results

4.3.1 Foraging distance from the nest

There was no significant difference between the mean number of marked bees reobserved at 20 and 200 m from their nest (Fig. 4.2: ANOVA; F=0.285, df=1, P=0.631). The mean number of marked bees observed on *Phacelia* was low, 0.028 (95 % CL=0.004-0.053) and 0.040 (95 % CL=0.029-0.050) bees per pot for 20 and 200 m, respectively.

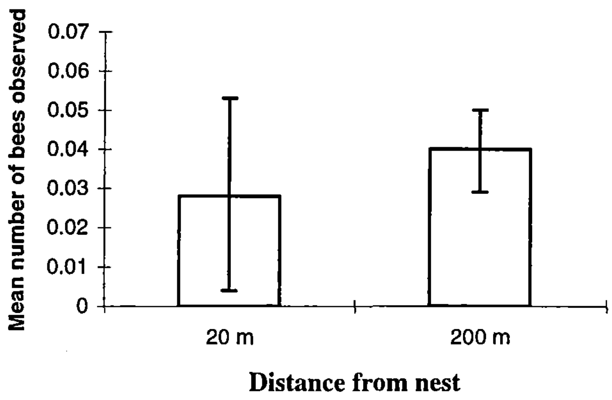

Figure 4.2. Mean number of marked *Bombus terrestris* per pot foraging on *Phacelia tanacetifolia* at 20 and 200 m from the nest (means ± 95 % CI).

4.3.2 Mark-reobservation

The mean percentage of bees entering or leaving the nest that had markings was 76.8 and 82.2 % for replicate one and two, respectively. Over the whole study, the ratio of marked to unmarked *B. terrestris* foraging on the *Phacelia* was 1:7. Reobservation rates of 0.049 and 0.032 were calculated for replicate one and two, respectively. The number of nest-marked bees observed on *Phacelia* was greater when the nest was at the south end of the paddock compared with when it was at the north end (Mann-Whitney rank sum; U=0.5, df=1, P=0.005).

4.3.3 Phacelia flowering

The *Phacelia* flowered for approximately 4 weeks, 3 weeks, and 6 days for the first, second and third replicates respectively. There was no significant difference (*t*-test; *t*=1.984, df=19.8, *P*=0.061) between the mean number of flowers per pot at 20 m (mean=263.9, SE=20.17) and 200 m (mean=222.3, SE=5.65) from the nest, for replicates one and two combined.

4.3.4 Bees foraging on the Phacelia

Most (453; 98.7 %) bumble bees foraging on *Phacelia* were *B. terrestris*; only six (1.3 %) *B. hortorum* were observed. *B. terrestris* foraged for both pollen (53 %) and nectar (47 %) from *Phacelia*. On average, 5.6 (±0.4) *B. terrestris* were observed on each walk.

The number of honey bees foraging on *Phacelia* was variable (range 0-37 per walk) and most (97 %) were foraging only for nectar. There was no correlation between the number of bumble bees and honey bees foraging on *Phacelia* (Spearman rank; Rs=0.23, df=80, *P*=0.04).

4.3.5 Pollen analysis

Only two pollen loads from the 40 samples contained *Phacelia* pollen (5 %). Most pollen loads contained a mixture of predominantly pasture legumes such as white clover (*T. repens*), lucerne (*M. sativa*) and lotus (*Lotus corniculatus* L.), with a small amount of arable weed pollen such as *Taraxacum* and *Carduus* spp. White clover was the predominant pollen in 55 % of the samples collected. One pollen load contained mostly cornflower (*Centaurea cyanus* L.) pollen; this was presumed to have come from a domestic garden approximately 500 m away.

4.4 Discussion

4.4.1 Preferred foraging distance from the nest

There was no significant difference between the number of marked bees foraging at 20 or 200 metres from the nest. However, the number of marked bees reobserved overall was very low. Because it was not known how far from the nest over 95 % of the bees were foraging, no conclusions on the 'preferred' foraging distance can be drawn. Instead most bees appeared to be foraging on the surrounding pasture, as shown by pollen load analysis. It was hoped that by conducting the experiment in a recently grazed paddock, competing floral resources could be kept to a minimum. However, there were still white clover flowers scattered throughout the surrounding paddocks. Casual surveys of the number of white clover flower heads in the experimental and surrounding paddocks revealed densities of 0-25 flower heads/m^2. The dispersed white clover flowers appeared to be more attractive than the clumps of *Phacelia* flowers.

B. terrestris foragers from other nests in the area were consistently foraging on *Phacelia*, so it is not known why *B. terrestris* from the supplied nest were not visiting it. The blue *Phacelia* flowers would have been highly visible in the field, making a stark contrast to the surrounding largely brown paddocks. The bumble bees from the commercially-reared nest were naïve foragers so they would not have had any learned floral preferences that influenced their subsequent foraging. Bumble bee visitation is sensitive to the nectar volume and nectar secretion rates of *P. tanacetifolia* (Williams 1997). Perhaps the local populations of bumble bees and honey bees reduced the floral rewards to a level where they were not profitable for nest bees to visit, i.e., some kind of exploitative competition may have been operating. Some *B. terrestris* individuals foraging for nectar on the *Phacelia* had brown pollen loads typical of white clover, suggesting that they 'preferred' white clover pollen over *Phacelia* pollen.

4.4.2 Forager behaviour

Repeated reobservation of individually marked bumble bees as the observer walked down the line of *Phacelia* suggested that they were regularly visiting the same sequence of pots, a type of trap-lining behaviour described by Heinrich (1979a). Another interesting behaviour evident in the current study was the increase in the number of marked bumble bees observed when the orientation of the experimental design was reversed. In both replicates, there were more nest-marked bees observed when the nest was at the southern end and the *Phacelia* at the northern end of the paddock. It was first thought that these were workers that had been left behind when the nest was shifted but closer examination revealed that some were collecting pollen from *Phacelia*. Pollen collection would be unlikely if they had lost the nest and were foraging for their own needs so they probably were returning to the nest.

The prevailing wind throughout this experiment was from the north-west; this can be a very strong Föhn wind in Canterbury with gusts up to 37 km/h recorded during this study. It would be energetically efficient for a bee to fly into the wind when leaving the nest to forage, thereby having a tail wind behind it when returning laden with pollen and/or nectar. Also, the scent of the flowers would travel downwind attracting the bees to them. Wenner *et al.* (1991) found an upwind colony foraging bias in honey bees that they attributed to the bees using odour to locate their food sources. Brian (1952) found that most bees from a *B. agrorum* nest foraged in five distinct trajectories away from the nest but their direction with respect to the prevailing wind was not known. Direction of foraging paths from the nest warrants closer inspection in future studies. An alternative explanation for the increase in the number of nest-marked bees observed could be that over time the bumble bees increased their foraging area, as has been found with honey bees (Free 1993)

4.4.3 Limitations of the study

Although this experiment did not show any 'preference' by bumble bees for foraging at the greater distance from the nest, it does not mean the phenomenon does not exist. The number of marked individuals observed was too low to detect any trends, but it is evident that at least some individuals were foraging 500 m away from the nest. Perhaps the distinction between the 'near' and 'far' distance was not great enough to detect any difference in behaviour. It was planned also to have pots of *Phacelia* at 400 m from the nest but there was difficulty in finding a large enough paddock or two adjacent empty paddocks in which to place them. Future studies should look at a wider range of flight distances from the nest. The relatively new technique of using harmonic radar (Riley *et al.*

1996) to track bumble bee movement should shed further light on how far most bumble bee fly from the nest to forage. Early reports suggest flights in the 200-300 m range are common in agricultural landscapes (J. Osborne unpub. data). It is thought that the opportunity cost (time not spent foraging), rather than the energy spent flying, limits the distance flown from the nest to forage (Heinrich 1979a). If it is assumed that a bumble bee flies at 5 m/s (Brian 1954), then it would take 4 s to fly to the pots of *Phacelia* at 20 m and 40 s to fly 200 m. Would this 36 s loss in foraging time have any great effect on the bees' energy budget? A new quantitative model predicts that when nectar rewards are meagre, an outward flight of 2-4 km is not a significant energetic cost to the bee because the greater distance covered increases the bee's probability of encountering a more rewarding nectar patch (J. Cresswell pers. comm.). Heinrich (1983b) warned against viewing bumble bee behaviour solely in terms of optimising nectar rewards because these insects are not governed by energetics alone; other conflicting constraints such as pollen collection (Rasheed & Harder 1997) and predation aversion (Dramstad 1996a) could be operating.

CHAPTER 5: EFFECTS OF PATCH SIZE ON *BOMBUS TERRESTRIS* ABUNDANCE AND BEHAVIOUR

5.1 Introduction

5.1.1 Background and significance of the work

Floral density has profound effects on the abundance, composition and behaviour of pollinators visiting a patch of flowering plants (Heinrich 1979b; Roubik 1982; Thomson 1982; Rathcke 1983; Campbell & Motten 1985; Sih & Baltus 1987; Jennersten *et al.* 1992). For entomophilous plants, dependent on insect visitation for pollen movement, these density effects on pollinator behaviour can alter the pollen carry-over and hence the genetic structure of plant populations (Real 1983). Habitat fragmentation is a common by-product of agricultural intensification worldwide (Fry 1989; Krebs 1994), resulting in disparate patches of vegetation of differing sizes. By quantifying the effects of patch size on pollinator abundance and behaviour, the consequences of fragmentation on plant-pollinator systems can be predicted. For example, in small isolated populations of *Phyteuma nigrum* FW Schmidt (Campanulaceae) in the Netherlands, seed set may be reduced due to low pollinator visitation and/or heterospecific pollen transfer (Kwak *et al.* 1991). There is some evidence that the viability of plant populations is reduced super-proportionately with a decline in their size due to fragmentation effects upon their pollinators' behaviour and abundance (the "Allee effect": Lamont *et al.* 1993).

5.1.2 Competition and facilitation

Interactions between plants (both inter- and intraspecific) may affect their reproductive success. A patch composed of two or more plant species may compete for pollination via pollinator preference (one plant attracts pollinators away from another) and interspecific pollen transfer (the pollinator switches between plant species as it forages) (Waser 1983). Interspecific pollen transfer (IPT) can reduce a plant's reproductive success through a loss of donor pollen, a loss of receptive stigmatic surface to the recipient and possibly the production of inviable or sterile hybrids (Waser 1983). Interspecific pollen transfer is believed to be the more common form of the two mechanisms of competition and is thought to select for divergence in floral traits such as floral morphology, reward systems and flowering times (see Waser 1983 for a review).

Facilitation (sometimes called mutualism) may also occur, whereby the presence of another species increases pollinator visitation by the attraction of, and energetic support (provision of nectar) for shared pollinators (see Rathcke 1983 for a review). The adaptive significance of facilitation is not well understood and may be an incidental rather than an adaptive feature of plant-pollinator systems. For example, the effective mutualistic support of hummingbird populations by sequential flowering of *Delphinium nelsonii* Greene (Ranunculaceae) and *Ipomopsis aggregata* (Pursh) V.Grant (Polemoniaceae) is most likely to be a result rather than a cause of divergence in flowering times (Waser & Real 1979).

Competition and facilitation may operate at the same time (Rathcke 1983). This was illustrated by the work of Campbell & Motten (1985) who found that the presence of *Claytonia virginica* L. enhanced the pollinator visitation rate to *Stellaria pubera* Michaux (Caryophyllaceae) but reduced its seed set due to the loss of *S. pubera* pollen by interspecific pollen transfer. Most studies have focussed on interspecific interactions, but intraspecific interactions may also be important (Rathcke 1983; Sih & Baltus 1987; Jennersten *et al.* 1992). By studying patches composed of a single plant species, density effects can be examined in isolation, without the intrinsic differences between plant species confounding pollinator behaviour.

5.1.3 Pollinator responses to floral density

The response of a pollinator to variation in patch size can be likened to predator-prey interactions (Holling 1959), where the pollinator is the 'predator' and the flowers the 'prey'. Two types of response can be identified: a functional response where more prey items are consumed with increased prey density, and, an aggregative numerical response where there is an increase in predator density with increased prey density. A reproductive numerical response can also occur, but this is outside the scope of the current study. For a factor to act in a density-dependent manner, a super-proportional response to increased prey density is required.

For plant-pollinator interactions, a larger patch of flowers may receive more visitors (aggregative numerical response) and have more flowers visited (functional response) than smaller patches. In terms of plant reproductive, success it is the balance of these two responses, the net visitation rate per flower, that is important. Rathcke (1983) proposed a parabola-shaped relationship between flower visitation rates and flower density (Fig. 5.1). At low floral densities, visitation rates are disproportionately low, but as flower density increases so too does the visitation rate due to enhanced attraction and support of pollinators. At very high floral densities, the visitation rate declines, due to saturation of the available pollinator population. Thus plant-plant interactions may shift between facilitation and competition, depending on floral density.

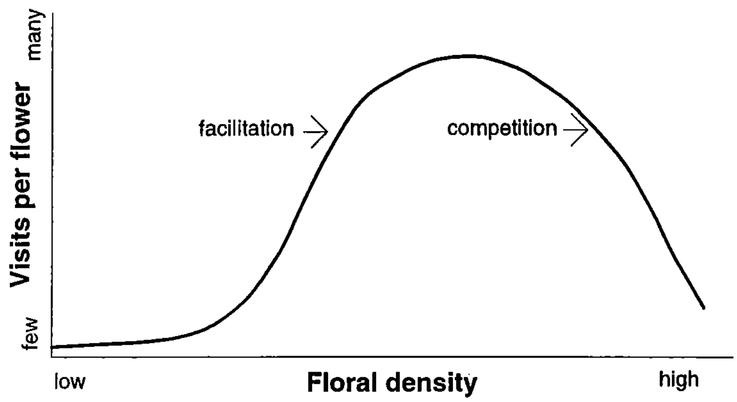

Figure 5.1. Model of the relationship between floral density and pollinator visitation (from Rathcke 1983).

Only one paper has explicitly tested this theoretical model to see if visitation rate is dependent on floral density. Sih & Baltus (1987) found that there were density-dependent effects of catnip (*Nepeta cataria* L., Labiatae) flowers on visitation rate but they differed over time and with different pollinator species. Further experiments are needed to determine if these patterns of behaviour are common among plant-pollinator systems.

The purpose of this study was to quantify the effects of flower density on pollinator abundance and flower visitation rates. The hybrid lavender *Lavandula* x *intermedia* Lois. cv. Grosso (Labiatae) was used. This plant was chosen for its attractiveness to bees (pers. obs.; Free 1993) and its ability to tolerate hot, dry conditions. Also, all plants were from the same genetic source (grown from cuttings), negating any genetic differences in their physiology and morphology that might affect pollinator behaviour.

5.1.4 Objectives

- To determine if bees show a numerical aggregative response to increased floral density.
- To determine if bees show a functional response to increased floral density.
- To determine if the overall effect of the above responses on flower visitation is density-dependent.

5.2 Methods

5.2.1 Study plant

L. x *intermedia* cv. Grosso is a hybrid of *Lavandula angustifolia* Mill. and *Lavandula latifolia* Medik. and is sometimes referred to as lavandin. It is grown commercially for the production of essential oils. Flowers of *L. angustifolia* and *L. latifolia* are readily visited by bees for nectar and pollen (Herrera 1989; Free 1993). Lavandin plants showed a 16-20 % increase in their essential oil content when visited by bees (Barbier 1958, cited in Free 1993). For this experiment, 31 plants of Grosso lavender were each planted in separate 300 mm diameter plastic tubs (Plate 5.1). The lavender plants were approximately 150 mm high. The plants were watered every second day.

5.2.2 Experimental design

Three different sized patches of lavender were created using the above pots. These patches were designated as small (one plant), medium (five plants) and large (25 plants). The three patches were placed approximately 150 m apart in an organic pasture at the Biological Husbandry Unit, Lincoln University.

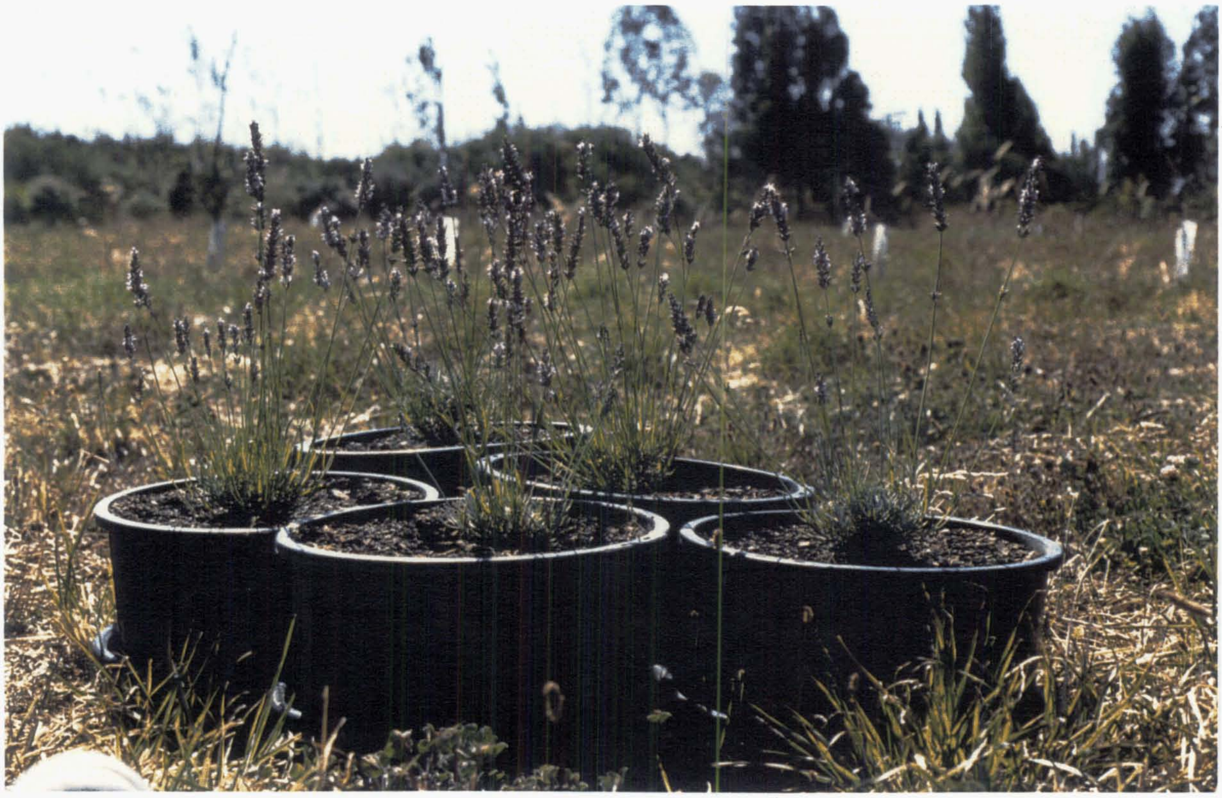

Plate 5.1 Medium sized 'patch' of *Lavandula* x *intermedia* cv. Grosso.

Despite a lack of replication in space, the confounding effects of location were removed by rotating the patches daily (keeping the same groups of pots together) so over three days (19-21 February 1998), each position was occupied once by each patch size (Fig. 5.2). The 'patches' were then shifted to new sites in the paddock and this rotational process was repeated over another three days (25-27 February 1998) (Fig 5.2). On each observation day, the number of flowers per patch was counted directly in the small and medium patches. The number of flowers was estimated in the large patch by counting the number of flowering inflorescences and multiplying this by the mean number of flowers per inflorescence from 10 randomly selected inflorescences.

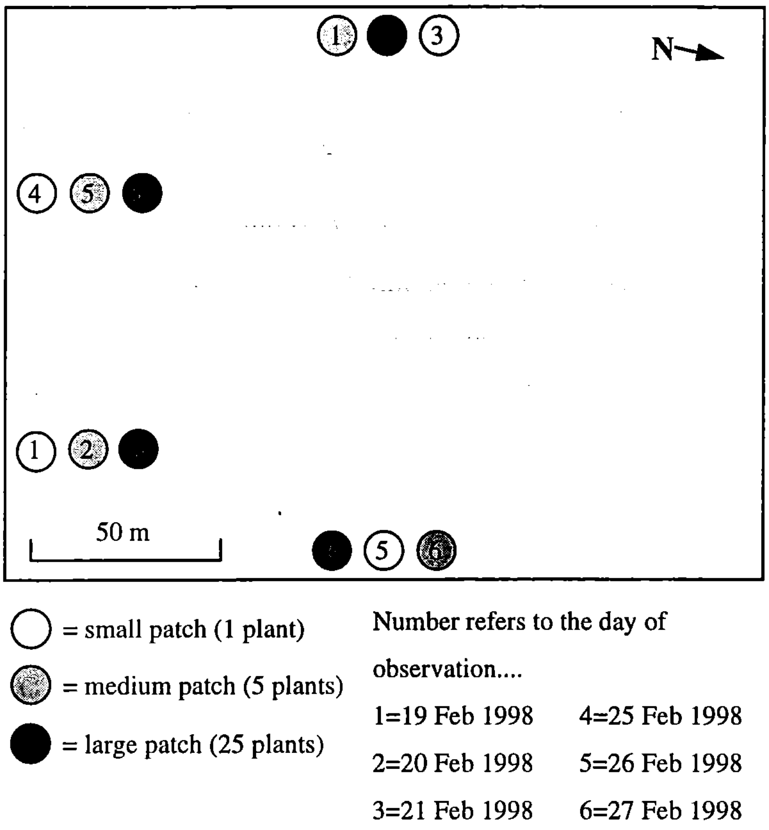

Figure 5.2. Experimental layout of artificial *Lavandula* x *intermedia* cv. Grosso patches within a 4.5 ha organic paddock, Biological Husbandry Unit, Lincoln University.

5.2.3 Forager behaviour

The abundance of bumble bees and honey bees foraging on each patch was estimated by making 'instantaneous' (approx. 5 sec) counts of the number of bees per patch. This method was chosen because preliminary observations showed that it was impossible for one or two observers to keep track of the number of bees arriving and departing from the large patch within a defined observation period. Instantaneous counts were made 10-14 times throughout the day, between 0900 and 1700 h (NZ Summer Time). The foraging bouts of individual *B. terrestris* were recorded using a stopwatch to time bout length and a hand-held counter to record the number of flowers visited per bout. A bout was considered to be finished when a bee left the patch and did not return within 5 s. The foraging bouts of 25, 29 and 31 individual *B. terrestris* were recorded on the small, medium and large sized patches respectively over the 6 days of the experiment. Also, over the course of this experiment, 27 *B. terrestris* were collected while foraging on the lavender and were individually marked by placing them in a marking tube (Kwak 1987) then gluing a honey bee queen number tag (Opalith-Plättchen) to their thorax. The foraging bouts of these marked individuals were recorded only once to avoid pseudoreplication in the flower visit data.

5.2.4 Analysis

Because of the low number of honey bees and *B. hortorum* visiting the lavender, analysis was confined to the *B. terrestris* data. Numerical and functional relationships were estimated using regression analysis (linear and log-linear models). Data was log-transformed to improve normality and homogeneity of variances and randomised block ANOVAs were used to partition the effects of day/location and patch size on pollinator abundance and behaviour.

5.3 Results

5.3.1 *B. terrestris* on *L.* x *intermedia* cv. Grosso

B. terrestris was the dominant bee species visiting the lavender, comprising 96.6 % of all bees observed. Individually marked bees showed site loyalty, returning to forage on the lavender throughout the day and between days (even though the patch size and the plants had changed). *B. terrestris* was often observed visiting the same flower twice or more during a foraging bout, especially on the small patches. This was also evident by records of flower visits per bee, which exceeded the number of lavender flowers available.

5.3.2 Aggregative numerical responses

Patch size had a significant effect on the number of *B. terrestris* visiting the patch (ANOVA on log-transformed data; F=69.3, df=2, P<0.001) and the day of observation/patch location had no effect on *B. terrestris* abundance (F=2.3, df=5, P=0.120). There was a strong positive relationship between the number of *B. terrestris* visiting the patch and patch size (linear regression; R=0.850, P=<0.001, y=0.336+0.002x; Fig. 5.3).

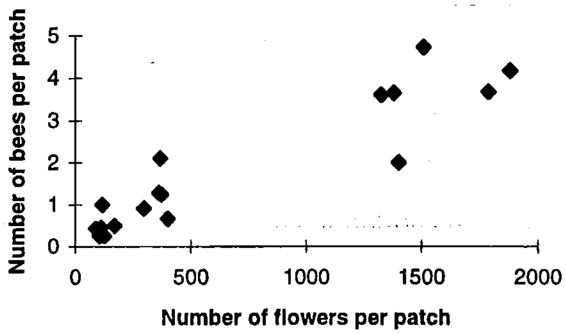

Figure 5.3. Relationship between *Bombus terrestris* abundance and *Lavandula* x *intermedia* cv. Grosso patch size (data points are means for each day of observation).

However, this numerical response was not super-proportional; patch size was not a good predictor of the number of bees per flower (best fit was log-linear; R=0.205, *P*=0.066, y=0.007-0.001*log(x); Fig. 5.4).

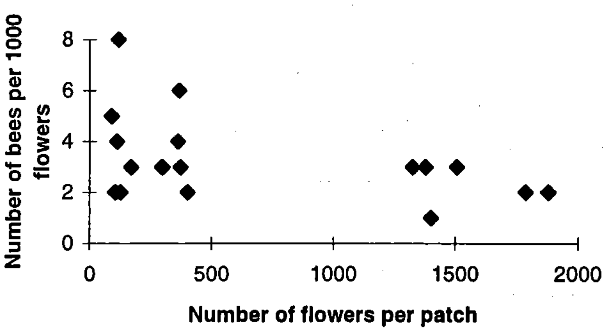

Figure 5.4. Relationship between the number of *Bombus terrestris* per 1000 flowers and *Lavandula* x *intermedia* cv. Grosso patch size (data points are means for each day of observation).

5.3.3 Functional responses

There was a significant positive relationship between the number of flowers visited within each patch and patch size (log-linear regression; R=0.427, P=0.003, y=-314.8+78.5*log(x); Fig. 5.5). The mean proportion of flowers visited by *B. terrestris* was calculated by dividing the mean number of flowers visited per patch by the number of flowers available per patch. When this variable was regressed against patch size there was a significant but weak decline in the proportion of flowers visited with increasing patch size (log-linear regression; R=0.364, P=0.008, y=1.2-0.13*log(x); Fig. 5.6), i.e., an inverse density-dependent relationship.

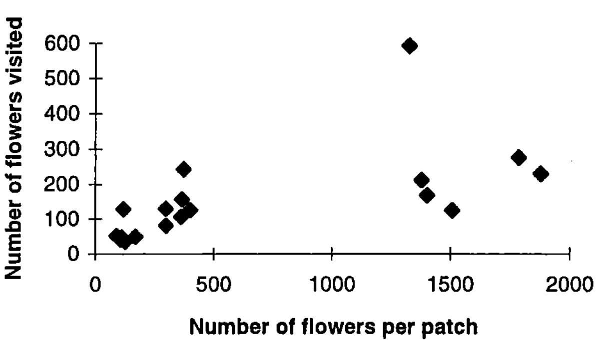

Figure 5.5. Relationship between the number of flowers visited per foraging bout by *Bombus terrestris* and *Lavandula* x *intermedia* cv. Grosso patch size (data points are means for each day of observation).

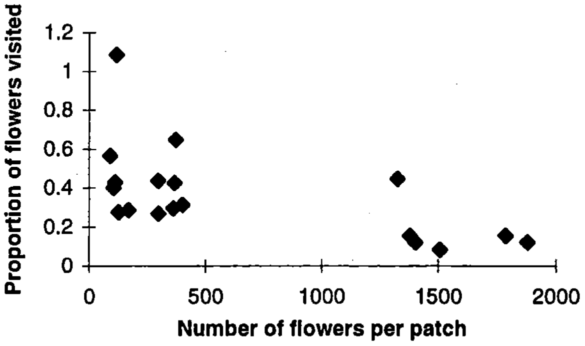

Figure 5.6. Relationship between the proportion of flowers visited per foraging bout by *Bombus terrestris* and *Lavandula* x *intermedia* cv. Grosso patch size (data points are means for each day of observation).

Patch size had a significant effect on the time spent by individual *B. terrestris* foraging on the patch; more time was spent on the larger patches (ANOVA on log-transformed data; F=25.27, df=2, *P*=<0.001; Fig 5.7). However, the number of flowers visited per second (flower handling time) was similar over all patch sizes (ANOVA; F=0.31, df=2, *P*=0.736). The mean (±SE) flower handling time was 0.658 (±0.031), 0.652 (±0.04), and 0.679 (±0.036) flowers per second for the small, medium and large patches respectively.

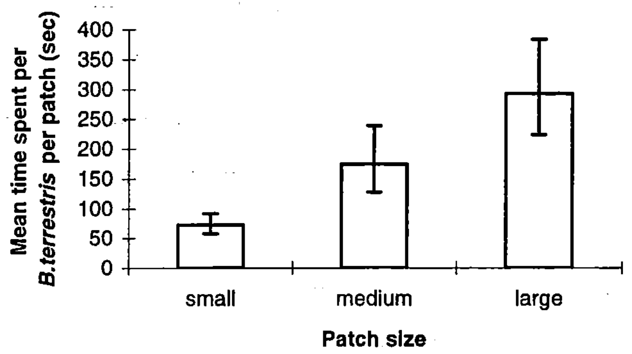

Figure 5.7. Mean time spent by individual *Bombus terrestris* foraging on different sized patches of *Lavandula* x *intermedia* cv. Grosso (back-transformed means and 95 % confidence intervals shown).

5.3.4 Net visitation per flower

The effects of patch size on pollinator abundance and the number flowers visited can be combined to estimate the net visitation rate per flower (Rathcke 1983; Dafni 1992). In the current study, instantaneous counts of bee abundance were made, so the calculation of a rate of visitation would be invalid because the number of bees arriving per unit time was not known. Instead an index of visitation (IV) per flower was calculated:

$$IV = (B^*F_v)/F_a$$

where B is the mean *B. terrestris* abundance per patch, F_v is the mean number of flowers visited per patch and F_a the number of flowers available per patch. When the index of visitation was plotted against patch size, no relationship could be found (best fit was log-linear; R=0.112, *P*=0.174, y=-0.252+0.122*log(x); Fig. 5.8).

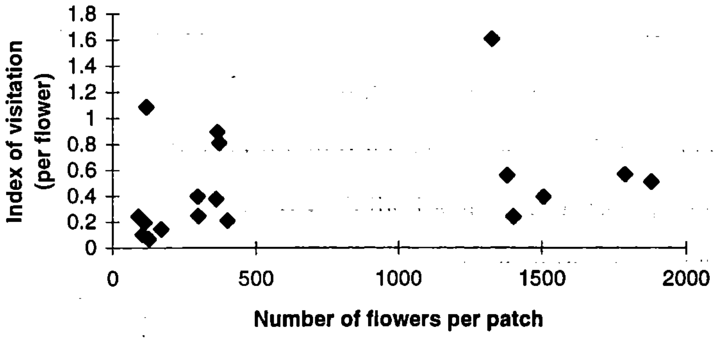

Figure 5.8. The relationship between visitation per flower by *Bombus terrestris* and patch size of *Lavandula* x *intermedia* cv. Grosso (data points are means for each day of observation).

5.4 Discussion

5.4.1 Pollinator responses to floral density

In this simple plant-pollinator system, the number of consumers showed a positive linear function with resource density. This increase was less than proportional; the number of bees per flower was not directly related to flower density. Therefore the aggregative numerical response was density-independent. The number of flowers visited on a foraging bout increased with patch size, but the proportion of flowers visited declined with patch size (inverse density-dependence). Flower handling time was not reduced at higher floral densities. Therefore, the number of flowers visited (irrespective of patch size) was probably limited by honey crop and/or corbiculae load capacities. The possible consequence of this for the flowers is that at higher floral densities there will be intraspecific competition between flowers for a limited number of individual pollinator visits.

The net effect of these functional and numerical responses to the flower (as measured by the visitation index) was to cancel each other out, i.e., the presence of more flowers in a patch attracted more bumble bees but decreased the probability of a flower being visited, so the IV was similar over a wide range of patch sizes. This contrasts with the work of Sih & Baltus (1987), who found that there was a density-independent aggregative numerical response and density-dependent functional response of bumble bees to increased catnip flower density, resulting in an overall density-dependent effect on visitation rates at larger patch sizes. The aggregative numerical response detected by instantaneous counts in the current study could have been driven by the functional response of pollinators spending more time on larger patches and/or an increased rate of pollinator visitation to the patch, i.e., more pollinators arriving per unit time on larger patches. An increased rate of pollinator arrivals could have been caused by the bigger stimulus (flower density, colour and odour) and enhanced detection by pollinators of larger patches of flowers. Patch retention times were measured in the current study but visitor arrival rates were not (for logistical reasons, see Methods section). It is possible that visitor arrival rates were higher at larger patches (as Sih & Baltus 1987 found) and that some undetected density-dependent effects were operating.

5.4.2 Limitations of the data

Regression analyses require the dependent variable values to be independent of each other (Zar 1984), but this was not the case in the current study. Data points were measurements of the same patches over different days and in different locations (repeated measures). Ohashi & Yahara (1998) found that although the shape of the functional relationship between pollinator visits and flower number was similar between observation days the magnitude of the response differed. Thus the plant-pollinator relationships in the current study could have been confounded by environmental effects due to different locations or days of observation.

There was no evidence of the type of relationship proposed by Rathcke (1983) (Fig. 5.1), but visitor arrival rates were not measured. The relationship between IV and floral density may have been clearer in this study if a greater range of patch sizes had been used. There was a gap in the 500-1000 flower patch size range and one can only speculate whether IV would have peaked in this range as in Rathcke's (1983) model.

5.4.3 Other factors affecting visitation

It must be stressed that this study was very limited in its scope, looking at only one bee and one plant species. Visitation with regard to patch size was examined in isolation but many other variables will affect pollinator behaviour, such as microclimate conditions, isolation of patches, nectar volumes and distribution, other flowering plants in the area, location of pollinator nest sites and competition with other pollinators. Density-dependence in time as well as in space can also occur, where the visitation rate is higher at peak flowering (Thomson 1982; Campbell 1985; Sih & Baltus 1987).

Waser (1983) concluded that visitation is only one component of pollination success, pollinator quality also being important. Pollinator quality can also be affected by patch size. Jennersten *et al.* (1988) found that seed set was lower in smaller patches of *Viscaria vulgaris* (Bernh.) than in large patches, even though the visitation rate by bumble bees was the same. He suggested that this was due to the 'major and minor' foraging patterns exhibited by bumble bees (Heinrich 1979a). In larger patches, the bumble bees were probably 'majoring' on *V. vulgaris* resulting in high conspecific pollen transfer, whereas in small patches *V. vulgaris* would have been a minor species and would have received more interspecific pollen thereby reducing its seed set. Thus in small isolated populations of plants, small patch size may cause two negative effects on pollination success; reduced pollinator visitation and increased interspecific pollen transfer (Rathcke & Jules 1993).

The plant used in this study was chosen for its availability and agronomic characteristics; it may be more relevant to use a species with a higher economic or conservation status in future studies. However *L.* x *intermedia* was chosen to withstand the exceptionally high temperatures of the New Zealand 1997/98 summer, *P. tanacetifolia* not having met this need (see Chapter 4). The experiment should be repeated with a greater range of patch sizes to test the hypothesis of Rathcke (1983). The next step would be to measure pollen movement and pollination success to determine if pollination is limited in different sized patches and what the mechanisms are (Campbell 1985; Campbell & Motten 1985) provided a thorough methodology for doing this).

CHAPTER SIX: GENERAL DISCUSSION AND CONCLUSIONS

6.1 Introduction

This thesis has looked at bumble bee management options for crop pollination and spatial aspects of their foraging behaviour. It has highlighted some of the problems with their management and the need for a better understanding of their biology and ecology. In particular, bumble bee movement in the landscape is poorly understood. Where and how far bumble bees forage in relation to their nest has important implications for both agricultural and conservation applications and warrants further study. The development of harmonic radar technology offers a greatly improved method to track bumble bee movement (Riley *et al.* 1996).

Pollination ecology is a multi-discipline science, practised by botanists, apiculturists, entomologists, ecologists, physiologists, plant breeders, ethologists, horticulturists and geneticists (Torchio 1987). As a result, there are many independent studies at many different levels of analysis but few studies actually link the behaviour of the pollinator to pollen movement to fruit set in the plant (Rathcke 1992). The failure of most studies (including this one) to elucidate these linkages is a major obstacle to the understanding of such a critical ecological process. Because pollination is a landscape scale process, studies of plant-pollinator "communities", "guilds" or "functional groups" are recommended (Bronstein 1995; Cane 1997; Corbet 1997). Conservation of these pollinator guilds is desirable and can be thought of as an insurance policy for pollination - if one species undergoes a population crash, another species may provide compensatory pollination services (Cane 1997).

There is a need for standardised methods and terminology (Inouye *et al.* 1994) so comparisons may be made between the relative efficacies of different pollinators (Torchio 1987). A problem with such comparisons, however, is that insect numbers and behaviour vary greatly over time and space as a result of multiple extrinsic and intrinsic variables, so such studies are likely to be site- and time-specific. The use of plant-pollinator models (e.g., Plowright & Hartling 1981; Ingvarsson & Lundberg 1995) that incorporate these multi-variates are thus likely to become more important in the future.

Below is a review of the conclusions of each experimental chapter and a discussion within the broader context of pollination ecology, as well as an analysis of the problems encountered and ideas for future work. Pollinator management in New Zealand is also discussed.

6.2 Provision of nest and forage sites to enhance bumble bee numbers

- Domicile occupancy rates increased substantially over three years as the domiciles became more attractive and/or were discovered with time.
- There was no association between previous use by mice and subsequent occupancy by bumble bees.
- There was a positive association between the presence of a bumble bee nest in one year and occupancy in the following year and the "seeding" of domiciles with pieces of old bumble bee nests is recommended to enhance occupancy rates.
- The provision of *Phacelia* attracted bumble bees to field margins but its effect on occupancy rates and nest productivity could not be ascertained. However, areas with higher floral diversity and abundance (i.e., the Biological Husbandry Unit) had higher domicile occupancy rates.

This experiment highlighted the logistical problems of habitat manipulation at a landscape scale; better communication and coordination with land mangers is essential for the establishment and maintenance of habitat refuges. The experiment rested on the assumption that nest sites and forage were limiting bumble bee populations. Although not explicitly tested, the results of this and other studies (Donovan & Wier 1978; Pomeroy 1981) suggest that this is a valid assumption. The choice of plants for habitat refuges is important; it is recommended that hardy perennials or shrubs are used. This is because perennial plants are preferred over annuals by bumble bees, they require less maintenance and once established are more resistant to grazing and competition from grasses. Future studies could repeat this experiment (with a different forage plant) to determine if the provision of a forage resource increases occupancy and nest productivity. The next step would be to see if bumble bees move from habitat refuges into crops and what size refuge is needed to support the desired bumble bee population.

Habitat manipulation is a low-cost, low-input management technique but it may take some years for bumble bee populations to build up. Torchio (1987) expressed doubts about the viability of habitat management programmes in very large, intensively managed agricultural areas because of high agrochemical inputs, asynchrony between crops and pollinators, costs of management and the possibility of refuges acting as pest reservoirs. However, farming in New Zealand is not as large scale as in the western USA, so habitat management is a more practicable option here. The potential of habitat refuges to harbour pest and weed populations should be investigated further. However, Corbet (1995b) argued that the abundance of insect pests and annual weeds is low in an established perennial sward and a floristically diverse vegetation also supports other beneficial insects such as parasitoids and predators of insect pests (Root 1973; Altieri 1991).

6.3 Foraging behaviour of **Bombus hortorum** *within a red clover seed crop*

- All *B. hortorum* observed foraging on the crop were pollinating the flowers. Most *B. terrestris* and *Apis mellifera* were nectar robbing, but their densities were three and 14 times greater respectively than those of *B. hortorum.*
- Pollen analysis showed most pollen-collecting bees from the supplied nests were visiting the crop and this did not change as flowering progressed.
- Reobservation rates of nest-marked bees were too low to compare statistically numbers at different distances from the nest but most were observed over 50 m away from their nests. Bees from one nest were flying over 200 m from the nest to forage on non-crop flowers.
- Supplementation with one *B. hortorum* nest per hectare increased the number of *B. hortorum* foraging on the crop by only 2 %. Supplied nests may therefore be cost-effective only if natural populations are low or out of synchrony with crop flowering.

One of the aims of this study was to find out where and how far bumble bees from supplied bests were foraging. This was achieved to a limited extent by pollen analysis and mark-reobservation, but these methods had their limitations. Research on the foraging ecology of bumble bees is often restricted to observations of individual behaviour, studies on whole colony foraging are scarce; this may be related to the ease of gathering data for individuals compared with colonies. In honey bees, the waggle dance of scout bees may be correlated with the location of forage patches to which they recruit other foragers (Visscher & Seeley 1982). Because bumble bees have no recruitment communication, this method cannot be used and correspondingly, because bumble bees are individual foragers, they are likely to be more randomly dispersed over an area. Piecing together information about whole colony foraging will therefore require a range of methods. Possibilities include radioactive isotopes (Lecomte & Pouvreau 1968), magnetic tags (Gary *et al.* 1972), genetic markers (Kennet 1995), harmonic radar (Riley *et al.* 1996) as well as pollen analysis and mark-reobservation.

An interesting observation in this and other studies (Synge 1947; Brian 1951; Free 1970; Free & Williams 1974; Waddington *et al.* 1994) is that bee colonies in the same area utilise the available resources in different ways. Waddington *et al.* (1994) hypothesised that this phenomenon in honey bee colonies was due to incomplete sampling of resources, different nutritional needs of the colonies or competition from other bees making some patches not profitable. Competition was probably not important on the experimental crop because bee densities were not as high as have been reported elsewhere for tetraploid red clover. Michener (1974, cited in Visscher & Seeley 1982) found there was a positive correlation between colony size and foraging range. The colony not collecting red clover pollen was the largest and at a different growth phase from the other three nests, so colony size and nutritional status could have some affect and could be experimentally tested in the future.

In retrospect, nectar quantities and seed set should have been measured within the crop to provide some information about the relationships between resource availability, pollinator densities and seed yields. The calculation of a stocking rate relies on such information. The low percentage that the supplied nests contributed to the total pollinator population suggests that some previous calculations on the value of supplied nests may be misleading because they overestimate the number of supplemented bees likely to be working on the crop. R.C. Plowright (pers. comm.) suggested that it is better to consider the marginal value (i.e., economic benefit to the grower) of each supplemented colony to the crop. To do this the gross value of a bee colony placed on a particular crop is calculated from bee working speed, foraging trip duration and forager number data (assuming there are no other pollinators present, i.e., an "empty field"). The pollination deficit is calculated from difference between maximum possible yields under perfect pollination and actual realised yields in the field. These two values (empty field value and pollination deficit) are multiplied together to give the marginal value of a bumble bee colony, which can then be reconciled with the cost of the colony. Hence the value of supplied nests is conditional on bee densities already present. A different approach was suggested by N. Pomeroy (pers. comm.) who compared the economic value of one "pollination unit" (in this case a red clover seed) to the cost of generating one "pollination event". Both approaches are likely to give more realistic estimates of the profitability of using bumble bee colonies for field crop pollination than simplistic flower/bee density counts.

6.4 Foraging distances of **Bombus terrestris** *from the nest*

- There was no difference between the number of marked bees foraging at 20 or 200 m from their nest; however, it was not known where more than 95 % of the bees were foraging.
- Colonies for field crop pollination should be placed upwind of the target crop.

This experiment did not achieve its aim because the pots of *Phacelia* did not attract enough bees and/or the differences between the distances (20 vs 200 m) were too small for the bee to discriminate energetically between. Mean outgoing flight distances of foragers from two *B. terrestris* nests recorded using harmonic radar were 339 ($\pm$26.2) m and 201 ($\pm$18.7) m from the nest, but these data did not include bees flying over 650 m, i.e., beyond the range of the radar (J. Osborne unpub. data). These are larger distances than the often quoted "...within a few hundred metres from their nest..." (Alford 1975, p. 88) and include cases where patches of apparently rewarding flowers close to the nest are 'passed over'. These observations do not fit those predicted by optimality models which state flight distances should be minimised.

Bumble bee optimality models use rate of energy (nectar) gain as the 'currency' of fitness because nectar is their sole source of energy, it is easily quantified and when foraging for nectar, bees are not distracted by other 'constraints' such as predator avoidance or finding a mate. However, pollen is essential to brood rearing, only pollen is collected from some flowers and at the height of colony growth a large proportion of foragers are collecting it. Therefore, quantification of nectar-only forager energetics would not be representative of colony foraging as a whole. Heinrich (1983a) criticised the application of a theory designed to answer evolutionary (ultimate) questions to provide proximate mechanisms for bumble bee behaviour and says that in an attempt to provide *post-hoc* explanations of already observed phenomena, they often ignore other, simpler mechanisms. He suggested that if the primary goal is to understand an animal's behaviour, researchers should concentrate on investigating proximate mechanisms, rather than worrying if the bees' behaviour is optimal or not according to criteria predetermined by the researcher.

Roubik (1989) was the first to take a mechanistic approach (as opposed to a functional approach) to honey bee colony foraging distances. He fitted a probability density function to the flight data of Visscher & Seeley (1982) and Vergara (1983, cited Roubik 1989). Wenner *et al.* (1991) argued that data from these and other studies were better described by a log-normal distribution, although this would be modified by wind direction and forage density and distribution (Meade 1991; Schneider & McNally 1993). Given that a lognormal distribution represents a random distribution of bees from their colony, then bumble bee colonies would be expected to exhibit this pattern. However, problems associated with training bumble bees to visit feeding stations make it difficult to test this hypothesis.

The higher re-observation rate of bees when pots of *Phacelia* were upwind of the nest was an unexpected outcome of this experiment. Wenner *et al.* (1991) documented a similar phenomenon in feral honey bees on Santa Cruz Island, USA; most bees were foraging upwind of the colony regardless of the type or quality of forage downwind. As Wenner *et al.* (1991) stated, it is surprising that that this phenomenon has not been studied further given that it is grounded on two indisputable facts: flower odour acts as a signalling cue for pollinators and odour can travel only downwind.

6.5 Effects of patch size on **Bombus terrestris** *abundance and behaviour*

- The number of *B. terrestris* visiting the lavender increased with patch size but this increase was less than proportional (a density-independent aggregative numerical response).
- The number of flowers visited per foraging bout increased with patch size but the proportion of available flowers visited decreased with patch size (an inverse-density dependent functional response).
- Combining these two responses, the net visitation per flower was independent of patch size.

Floral resources are patchily distributed in nature and it is important to recognise how the spatial distribution of these affects pollinator behaviour and pollinator-mediated gene flow. It is important for conservation goals to predict the effects of fragmentation on plant-pollinator communities. In agriculture, this information can be used to determine plant spacings, seed isolation distances, and the geometry of habitat refuges for crop pollinators. This experiment used a simple, one-to-one, plant-pollinator relationship to investigate density dependent foraging in *B. terrestris*. Both functional and numerical responses were examined but no density dependent effects were operating. A wider range of patch sizes should be used in future studies to provide more information on the relationship between patch size and pollinator abundance and behaviour. Density dependence may not have been detected because the visitation rate could not be measured directly and instead was calculated from bee abundance and foraging bout parameters. This method can introduce temporal errors because bee abundance and bee foraging rates will vary with time (Osborne 1994). However, measurements of these parameters were done within quick succession of each other so this temporal heterogeneity would have been minimal. The use of this method also meant that rejections (in which the bee approaches a flower but does not visit it) were not recorded. Other studies have shown that bees can assess patch/flower quality remotely (Corbet 1984; Marden 1984) and this appraisal may be affected by patch geometry (size, shape, density).

Observation of marked individuals showed that movement between patches was minimal. This apparent patch fidelity would mean that there would be little pollen flow between the patches. Studies on pollinator-mediated gene flow (via pollen) have shown that gene flow between populations is restricted in space (Levin & Kerster 1974). Increased isolation of patches could further limit pollen flow as isolated patches receive fewer visits from social bees (Sih & Baltus 1987). This has important implications for the genetic diversity of small isolated patches of entomophilous plants, particularly if they are dependent on a specialised pollinator. Pollen flow and the degree of pollinator limitation should be measured in future studies of this kind, although this is not easy. The development of isoenzyme markers, such as those in white clover (Michaelsonyeates *et al.* 1997), is a powerful new technique that allows the pollen carryover by insect vectors to be detected by analysing the paternity of seeds produced from these pollination events.

6.6 Pollinator management in New Zealand

In the Northern Hemisphere there has been a growing awareness of the importance of pollination for agricultural sustainability and for the conservation of biodiversity. The motivating factors have been an increased demand for pollination services coupled with a decline in the abundance and diversity of bee species (in particular honey bees) as well as concern about the effects of fragmentation on plant communities. Active and integrated pollinator programmes are recommended that incorporate honey bee management, habitat management to increase pollinator species diversity and population size, and the development of alternative pollinators for specific crops (Parker *et al.* 1987; Torchio 1987; Kevan *et al.* 1990; Corbet *et al.* 1991; Kearns & Inouye 1997; Allen-Wardell *et al.* 1998).

In New Zealand, honey bees are relatively free of the pests and diseases affecting the Northern Hemisphere honey bee industry and they are still the most widely used pollinator. Because of this reliance on honey bees, if a parasite like varroa mite did get into the country, the effects on crop yields could be disastrous. Apart from honey bee disease monitoring, New Zealand has no active pollinator management programmes and there is presently no government funding into the research of alternative pollinators. Department of Science and Industrial Research (DSIR) programmes in the late 1970s and early 80s resulted in the introduction of the lucerne leafcutting bee (*M. rotundata*) and the alkali bee (*N. melanderi*) for lucerne pollination but their potential has been limited by unsuitable climatic conditions. More recently, attempts to introduce the orchard bee (*O. cornifrons*), which is a superior pollinator to honey bees for pip fruit pollination, have failed because of a lack of funding (B.J. Donovan pers. comm.). Management of New Zealand native bees is not considered to be a viable option because of their small size and small populations (Gurr 1974) (although *Leioproctus* spp. have been identified as potential effective pollinators of kiwifruit (Donovan 1987)). Therefore, the only option available is to manage more effectively bees such as *Bombus* spp. that have already been introduced to New Zealand.

Management of bumble bees in New Zealand is purely an economic objective (increasing crop yields), whilst in the Northern Hemisphere, where bumble bees are endemic, there are also related conservation objectives. Bumble bees are not a conservation threat in New Zealand because they confine their visits mainly to exotic plants, although they may help maintain weed populations such as gorse on offshore islands (Macfarlane & Gurr 1995). The use of bumble bees for pollination of glasshouse tomatoes is a widespread and cost-effective practice and a thriving bumble bee rearing industry has developed in New Zealand to meet the demand. At present, the cost effectiveness of purchasing bumble bee colonies for field crop pollination is unproven and habitat management techniques are recommended. The use of bumble bees in the field may become more cost effective as the commercial rearing process becomes more refined and more cost competitive. An increase in honey bee colony prices due to additional disease control costs could also swing the balance in the favour of bumble bees.

Alternative pollinators such as bumble bees are often not considered to be as reliable pollinators of crops as are honey bees because their population densities vary from year to year. However, as Torchio (1987) pointed out, this is a flawed comparison, because honey bees are intensively managed whilst non-*Apis* populations are not. The status of alternative pollinator management today has been compared with that of biological control 20 years ago - showing potential, with some successes, but still requiring more theoretical and empirical work (Parker *et al.* 1987). Likewise the key to overcoming obstacles to pollinator management will be more research into their biology and behaviour. Management of biocontrol and pollinating insects can also be integrated. The use of bumble bees in glasshouses went hand in hand with the biological control of greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) by a parasitic wasp (*Encarsia formosa* Gahan) because both practices require insecticide levels to be reduced (Dijkgraaf 1994). The management of beneficial insects such as bumble bees will play a key role in the future development of a more diverse and sustainable approach to agriculture.

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APPENDICES

Appendix 3.1.

Video recordings of *B. hortorum* nests

Nest	Date	Time (NZ summer time)
2	28/1/97	7:13-14:48
2	29/1/97	16:00-17:26
2	14/2/97	7:00-18:30
2	18/2/97	7:04-21:00
2	27/2/97	12:45-16:45
4	12/3/97	7:01-20:42

Appendix 3.2.

Corbiculae pollen samples collected from *B. hortorum* foragers

Nest	Date *	Time (NZ summer time)	Number of samples
1	20/1/97	10:20-11:20	3
2	21/1/97	15:00-16:00	6
2	23/1/97	15:20-16:20	1
2	26/1/97	15:10-16:00	6
1	27/1/97	10:20-11:20	3
2	30/1/97	15:45-16:45	5
2	31/1/97	15:15-16:05	6
1	1/2/97	10:05-11:05	5
2	6/2/97	14:20-14:48	6
1	7/2/97	10:20-11:18	2
2	8/2/97	15:20-16:15	6
2	10/2/97	14:05-14:45	6
2	13/2/97	14:13-15:10	5
1	14/2/97	10:30-11:32	1
2	17/2/97	14:05-15:01	6
2	19/2/97	14:25-15:25	5
4	20/2/97	10:30-11:20	6
4	25/2/97	10:00-11:00	5
4	26/2/97	10:05-10:44	6
2	26/2/97	14:15-15:03	6
2	28/2/97	14:05-15:05	3
3	3/3/97	15:03-15:55	1
3	9/3/97	14:00-15:00	2
4	10/3/97	10:25-11:25	4

* Does not include occasions where sampling was attempted but no pollen collectors were caught

Appendix 3.3.

Pollen types identified from corbiculae loads of *B. hortorum*

Carduus spp.

Convulvulaceae

Cucurbitacece

Ericaceae

Eucalyptus spp.

Honeysuckle (*Lonicera periclymenum* L.)

Mallow (*Malva sylvestris* L.)

Red clover (*Trifolium pratense* L.)

Rosaceae

Unidentifiable type

White clover (*Trifolium repens* L.)