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Bombus terrestris: a more efficient but less effective pollinator than *Apis mellifera* across surveyed white clover seed fields

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ABSTRACT

White clover (*Trifolium repens* L.) seed crops require insects for pollination. *Apis mellifera* Linnaeus, 1758 are normally placed within fields but other insects might also be contributing additional pollination. To understand their potential contribution, we evaluated abundances across fields during peak flower; measured loose body pollen on four bee and two fly species and compared the rate of movement between inflorescences and florets for *A. mellifera* and *B. terrestris* (Linnaeus, 1758). *A. mellifera* were the most abundant verified flower visitor, although *B. terrestris* and long-tongued *Bombus* species also visited fields. Other insects recorded included *Lasioglossum* bees and two hover fly species, but these were considered ineffective pollinators (either low abundances or carried few pollen grains). *B. terrestris* were considered a more efficient pollinator than *A. mellifera* moving faster between florets but were less effective due to low abundances. Many *Bombus* spp. will forage under weather conditions unfavourable to *A. mellifera*, therefore, providing complimentary pollination.

Introduction

New Zealand, particularly the Canterbury region, is a significant producer of white clover seed worth $25 million in 2015 (10.6% of the total arable export value) (Chynoweth et al. 2015). White clover florets are typically self-incompatible (Sareen 2003) and bee pollination is considered an essential requirement (Pearson 1982; Thomas 1996). In New Zealand, a lack of pollination has been considered the key reason for poor seed yield in a less intensively managed pasture (termed high country) (Pearson 1985) and may be an important factor influencing yields in seed crops.

The European honey bee (*Apis mellifera*) has proven to be an effective pollinator of white clover (Rodet et al. 1998; Goodwin et al. 2011) and in New Zealand may be the only reliable pollinator for seed crops (Palmer-Jones et al. 1962). However, unmanaged pollinators might also have the potential to contribute to pollination. To our knowledge, there are no published studies estimating the efficiency of non-honey bee pollinators within white clover seed fields in New Zealand. A limited study by Palmer-Jones et al.

(1962) noted much higher numbers of *A. mellifera* than bumblebees (*Bombus* spp.) in fields suggesting than *A. mellifera* was probably the most effective pollinator. However, encouraging a diversity of pollinating species, within many crop species can have significant yield benefits (Garibaldi et al. 2013). This may result from differing but complimentary foraging activity between pollinating species either diurnally (Rader et al. 2013; Howlett et al. 2017a), across days (Mesa et al. 2013) and in response to weather. *Bombus* spp. and certain species of Diptera are more active than honey bees under conditions of lower light intensity and temperature (Howlett 2012; Howlett et al. 2013) and interactions between pollinators can improve their efficiency as pollinators by altering their behaviour (Brittain et al. 2013).

The presence of non-honey bee pollinators may also be beneficial for providing additional pollination to white clover should honey bee pollination effectiveness decline through the spread of the Varroa mite (*Varroa destructor* Anderson & Trueman, 2000). To our knowledge, the relative contribution of feral honey bees to the pollination of New Zealand white clover seed fields has not been assessed but Varroa is known to decimate feral honey bee numbers (Donovan 2007). Therefore, even if managed honey bees are retained in fields at the recommended hive stocking rate of 4–6 ha (Goodwin 2012), the loss of feral honey bees could negatively impact seed yields.

Besides *A. mellifera*, *Bombus* spp. (particularly *Bombus terrestris*) are the most studied pollinators of white clover, but, there remains a lack of published research comparing their relative efficiencies. When caged with white clover, both *B. terrestris* and *A. mellifera* have been shown to increase seed yields similarly compared to control cages with no bees present (Cecen et al. 2007). This study, however, did not compare the relative efficiency of the two species. Cages with *B. terrestris* contained half the number of bees compared to *A. mellifera* and no comparison was made of their rates of movement between florets and inflorescences (a key element for comparing pollinator efficiency) (Bohart and Nye 1960). In New Zealand, both bee species are considered important pollinators of white clover, particularly in non-intensively managed pasture (Palmer-Jones et al. 1962; Pearson and Braiden 1990) and are among the most common flower visitors of many other New Zealand crops including blueberry (Macfarlane 1992), onion (Howlett et al. 2009), brassica (Rader et al. 2012; Mesa et al. 2013), carrot (Howlett et al. 2015) kiwifruit (Howlett et al. 2017a), avocado (Read et al. 2017) and plums (McBrydie et al. 2017).

Other insects may also contribute to white clover pollination. For example, the long-tongued bumblebees ((*B. hortorum* (Linnaeus, 1761), *B. ruderatus* (Fabricius, 1775) and *B. subterraneus* (Linnaeus, 1758)) were introduced to New Zealand to pollinate red clover (Howlett and Donovan 2010). Some native bees have also been observed visiting white clover florets (Palmer-Jones et al. 1962; Quinn 1984) with the *Leioproctus* species *L. boltani* Cockerell, 1904, *L. imitatus* Smith, 1853, *L. metallicus* Smith, 1853, *L. paahaumaa* Donovan, 2007, *L. pango* Donovan, 2007 and the *Lasioglossum* species *L. sordidum* (Smith, 1853) and *L. cognatum* (Smith, 1853) having been recorded in white clover pasture fields (Malone et al. 2010). Although not verified as pollinators, the active collection of white clover pollen by *Leioproctus vesitus* (Smith, 1876) and *L. pango* Donovan, 2007 suggest they are likely to contribute to pollination (Palmer-Jones et al. 1962; Donovan 2007). Non-bee insects have been assumed to play little role in the pollination of white clover and their occurrence and potential contribution appears to have been largely ignored.

The aim of this study was to evaluate the likely efficiency and effectiveness of *A. mellifera*, and other non-honey bee pollinators of white clover. Pollinator efficiency has been defined previously by Bohart and Nye (1960) and Rader et al. (2009) as the estimated rate at which viable pollen is deposited onto stigmas. In our study, we similarly assess the rate of likely movement of pollen to stigmas within florets. We used a combination of measures including loose body pollen and the rate of between-floret movements to compare the relative efficiency between pollinator species. We then estimated relative pollinator effectiveness by multiplying the calculated pollination efficiency of each species with their relative abundances in each field (Rader et al. 2009) across eight flowering white clover fields. We used these findings to identify whether there were other pollinating species present within fields that potentially added to or complemented *A. mellifera* pollination of white clover, which could provide a focus for future management strategies.

Material and methods

White clover fields

Surveys were conducted during January 2005, when fields were at peak of flowering, to assess the efficiency and effectiveness of insect flower visitors. The study sites consisted of eight commercially grown hybrid clover seed crops of 6–9 ha located in the Canterbury region within a radius of 100 km from Rakaia (43.7561°S, 172.0226°E). Five fields each contained a different white clover cultivar whilst a further three fields contained the same cultivar. All fields were stocked with honey bees at the recommended rate of 4–6 hives/ha (Goodwin 2012).

Pollen on insects

Individuals of four bee species *A. mellifera*, *B. terrestris*, *B. ruderatus*, *L. sordidum* and the flies *Melanostoma fasciatum* (Macquart, 1850) and *Melangyna novaezelandiae* (Macquart, 1855) observed visiting clover flowers were captured singly using plastic vials containing paper tissue soaked with ethyl acetate as a rapid killing agent. After capture, the insect was removed after 2–4 minutes and placed into individual marked Eppendorf tubes. Pollen was removed separately from the head, upper body and lower body by rubbing each specific area of the insect on 2 mm^3 cubes of Gelatin-Fuchsin (Dafni 1992). Cubes were then placed on glass slides and the gelatin melted using a small flame. Cover slips were then placed over the melted gelatin and left to set. For bees, the hind legs were removed to avoid mixing loose body pollen with actively collected pollen contained on the corbiculae of *A. mellifera* and *Bombus* spp. or scopae of *L. sordidum*. Slides were numbered and the captured insects retained, numbered and placed in a small vial with 70% ethanol for identification. In the laboratory, the slides were examined using a compound microscope. The number of clover and non-clover pollen grains was estimated on each slide by counting the number of grains lying within 10 μm of a reference line that was marked across the slide. Eight equally spaced reference lines were used to estimate the total numbers of grains collected from each insect. Where pollen grain densities were particularly high, that is, where counts of several hundred grains per line were present, then only the grains lying within 2 μm of the reference line were counted. Total pollen grains

were then estimated by multiplying the number of pollen grains counted within the observed area of the reference line relative to the total area of the slide.

Movement of A. mellifera and B. terrestris between florets and inflorescences

Within-floret and between-inflorescence movements were observed at a distance of 20–40 cm and recorded for 23 *A. mellifera* and 15 *B. terrestris* individuals within a single white clover field. Behavioural observations were recorded using an audio recorder over a three-day period between 09:00 h and 15:00 h. The time an individual flower visitor spent on each inflorescence and the number of flowers visited per inflorescence was counted. The distance each bee moved between each inflorescence was also measured.

To estimate the numbers of seed set per minute by *A. mellifera*, we use a demonstrated linear relationship between the number of florets visited by *A. mellifera* within an inflorescence and resulting numbers of seed set per floret (average 1.24 seeds/floret) (Goodwin et al. 2011). We also use this relationship to tentatively estimate seed set per minute for *B. terrestris*.

Survey design

Three survey points (two in diagonally opposing corners and one in the centre) were established within each field consisting of a window trap and a 15.75 m circular transect along which the observer slowly walked over a period of 10 minutes recording flower-visiting insects. Single-day observations were conducted within each field during fine weather conditions (temperature 15–25°C in all cases) whereas traps were set to capture insects across five consecutive days. Further details of the survey methods are described in (Howlett et al. 2013) and window trap design in (Howlett et al. 2009). One of the fields was certified organic (see Asurequality n.d.).

Number of open flowering inflorescences and florets within fields

The number of flowering white clover inflorescences was estimated at the three sample points by counting their numbers within three 1 m^2 quadrats placed randomly within 5 m of each window trap. Thus, nine quadrat counts were conducted per field. Inflorescence surveys were conducted on the same day as flower visitor observation surveys. An estimate of the number of open florets per inflorescence was also determined by randomly selecting eight inflorescences per quadrat and counting the number of open florets. The counts were then used to estimate the number of *A. mellifera* visiting inflorescences and florets in each field.

Data analysis

To assess whether the estimated number loose pollen grains varied between head, thorax and abdomen of *A. mellifera*, *B. terrestris*, *B. ruderatus*, *L. sordidum*, *M. novaezelandiae* and *M. fasciatum* we conducted an analysis of variance of estimated total pollen counts between the different parts of a pollinators body. Post hoc Tukey's tests were then conducted to compare total pollen counts between the different parts of a pollinator's

body. We also conducted an analysis of variance to compare the estimated total number of loose pollen grains on the head of the insect for *A. mellifera*, *B. terrestris* and *B. ruderatus*. Pollen loads were log transformed prior to analysis and we assumed a normal distribution to test for differences between bee species. The fit of the model and equality of variances was assessed visually through diagnostic plots and we saw no evidence for violation of any assumptions of a general linear model.

We compared the behavioural parameters: time on inflorescences, florets visited per minute, florets visited per inflorescence and distance moved between inflorescences, for *A. mellifera* and *B. terrestris* using a mixed effects generalised linear model or mixed effects generalised linear model of the effect of insect and time (as a continuous variable) (Bates et al. 2015). Repeated observations by an individual bee were treated as a random effect. For distance moved, florets/inflorescence, and time on inflorescences models were fitted assuming a negative binomial distribution as these data represented counts. We also tested the data against a model with a Poisson error distribution however we found evidence for over dispersions (ie variance much greater than the mean) which violated the assumptions of the poisson distribution. Estimators of model quality (eg AIC and BIC) suggested that the negative binomial distribution models fit better for these data. Florets per minute was considered a normally distributed variable and visual inspection of diagnostic plots revealed no obvious violation of the assumptions for a general linear mixed model. Time was removed from the final models as there was no strong influence on any behaviour measure. *P*-values were based on the hypothesis that *B. terrestris* is different from *A. mellifera* in each behaviour parameter (*t*-statistic used for florets per inflorescence, time on inflorescence and distance moved between inflorescences and *F* statistic used for the number of florets visited per minute).

Counts of insects within flowering white clover fields obtained using observation and trap surveys were examined using descriptive statistics (counts, means, percentages). Data comparing counts of bees between fields are presented as boxplots.

Results

Pollen present on bees and other insects

We assessed the estimated number of loose white clover pollen grains on the body of 47 bees (25 *A. mellifera*, 8 *B. terrestris*, 5 *B. ruderatus*, 9 *L. sordidum*) and 24 flies (17 *M. fasciatum* and 7 *M. novaezelandiae*). The number of loose pollen grains was higher for *A. mellifera*, *B. terrestris* and *B. ruderatus* than for *Lasioglossum sordidum* (Figure 1). For honey bees, the estimated total number of loose pollen grains on their heads was significantly larger than on their thoraces and abdomens (post hoc Tukeys test: $p < .01$; $p = .02$, respectively). For *B. terrestris* and *B. ruderatus*, the mean and the median estimated total number of pollen grains were also higher on their heads than on their abdomens or thoraces (Figure 1), but these differences were not statistically significant (post hoc Tukeys tests *B. terrestris*: head–abdomen $p = .08$, head–thorax $p = .30$; *B.ruderatus*: head–abdomen $p = .25$, head–thorax $p = .51$). Overall, there was no significant difference between the estimated total numbers of pollen grains on the heads of *A. mellifera*, *B. terrestris* or *B. ruderatus* ($F_{(2, 35)} = 0.74$, $p = .49$). For the two hover fly species, *M. fasciatum* and *M. novaezelandiae*, very few pollen grains were found on their bodies and these were evenly distributed across the body segments (Figure 1).
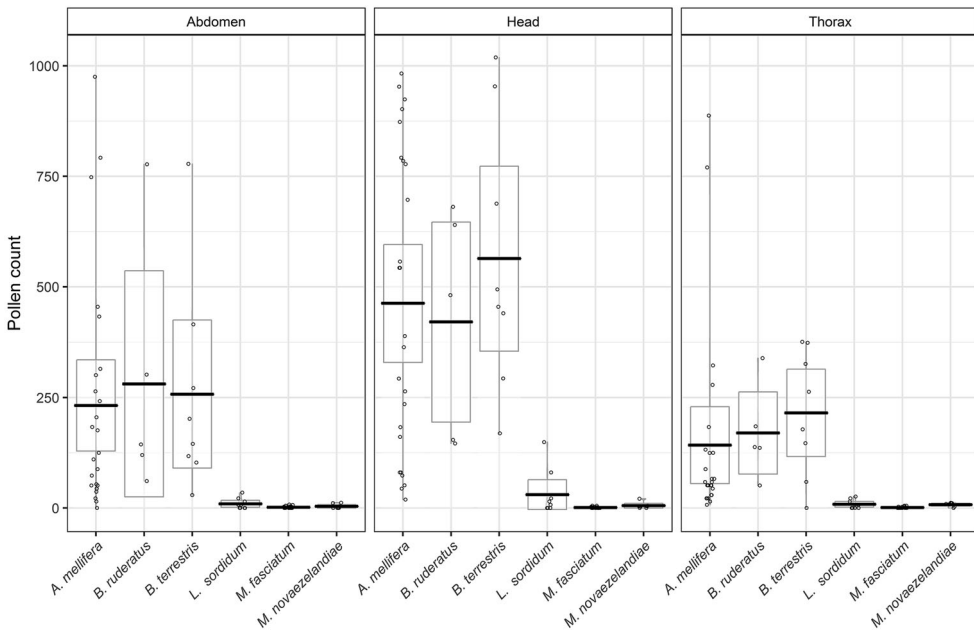
Figure 1. White clover pollen collected from the head, thorax and abdomen of captured bees from the genera *Apis mellifera* (*n* = 25), *Bombus terrestris* (*n* = 8), *B. ruderatus* (*n* = 5) and *Lasioglossum sordidum* (*n* = 9) and the syrphid flies *Melanostoma fasciatum* (*n* = 17) and *Melangyna novaezelandiae* (*n* = 7) captured in fields. Boxplots indicate quartiles, with median marked as a horizontal line, and means as a cross; points are outliers.

Between floret and inflorescence movement by *A. mellifera* and *B. terrestris*

Both *A. mellifera* and *B. terrestris* were observed to access nectar or pollen within each floret by opening the fused keel then inserting their lower head (particularly tongue and mouthparts) inside the corolla tube (Figure 2). *B. terrestris* visited significantly more florets per minute than *A. mellifera* (Figure 3A, Table 1), but the rate of movement between inflorescences was similar (Figure 3B, Table 1). The distance moved between inflorescence was also similar for the two bee species (Figure 3C, Table 1).

Using Goodwin et al.'s (2011) data, we estimated that an individual *A. mellifera* visiting white clover florets would result in the average production of 19.9 seeds/minute. If this relationship is consistent for *B. terrestris,* then this species would be more efficient because of its higher floret visitation rate. Theoretically, visitation of white clover florets

Table 1. Behavioural measure comparisons between *Apis mellifera* and *Bombus terrestris* visiting flowering white clover inflorescences using a mixed effects generalised linear model or mixed effects linear model (florets/minute) of the effects of insect and time (as a continuous variable).

Behaviour measures	*A. mellifera* Mean ± S.E.	*B. terrestris* Mean ± S.E.	d.f. residual	Statistic (t/F)	P-value
Distance moved (cm)	13.7 ± 0.5	14.5 ± 2.2	595	0.06 (*t*)	.95
Florets/inflorescences	3.0 ± 0.2	5.4 ± 0.3	595	4.21 (*t*)	<.001
Time on inflorescence (sec)	10.1 ± 0.5	12.0 ± 0.7	595	1.37 (*t*)	.17
Florets/minute	21.7 ± 0.6	28.2 ± 0.7	595	18.74 (*F*)	<.001

Note: Degrees of freedom are denoted as d.f.

Figure 2. The stigma and stamens in white clover florets are positioned together above the nectaries (located at the base of the corolla tube). Bees access pollen and nectar by opening the floret keel (consisting of two fused petals). This exposes the bee's lower head to contact with dehiscing pollen and the stigma.

by *B. terrestris* would result in 27.5 seeds/minute, that is, 1.38 times more efficient than *A. mellifera*.

Bee abundance, distribution and diurnal visitation

Two hundred and twenty-one bees (all species combined) were captured in the window traps, while 995 were observed across the survey points within all fields. Just three bee species (*A. mellifera*, *B. terrestris*, *B. ruderatus*) were observed visiting white clover florets, while four species (*A. mellifera*, *B. terrestris*, *B. ruderatus*, *L. sordidum*) were collected within traps (Figure 4). Of these, *A. mellifera* were the most abundant, representing 85% of individuals trapped (*n* = 188) and 99% of individuals observed.

Abundance of other insects

With the exclusion of micro-insects (body length less than 3 mm), Diptera were the only common non-bee insect trapped (*n* = 803) and observed visiting inflorescences (*n* = 172). At least 14 dipteran species were captured belonging to at least 9 different families. Of these, *Delia platura* (Meigen, 1826) (Anthomyiidae) was by far the most frequently trapped representing 79% (*n* = 638) of all individuals but just 6% of those observed (*n* = 10). In contrast, the hover fly, *M. fasciatum*, represented just 0.4% of Diptera trapped, but 62% of Diptera observed (*n* = 106). The only other flies commonly observed were the hover fly, *M. novaezelandiae*, representing 20% of dipteran specimens (*n* = 35), and *Anthomyia punctipennis* Wiedemann, 1830 (Anthomyiidae), 8.7% (*n* = 15) which were also quite commonly captured in traps, representing 6.1% of individuals. The striped flesh fly *Oxysarcodexia varia* (Walker, 1836) (Sarcophagidae) was the only other dipteran with an abundance greater than 5% from trap specimens, representing 7% of specimens (*n* = 59) but just two specimens were observed on inflorescences.

Estimated effectiveness of A. mellifera and Bombus spp. as pollinators

Although the two bee species carried similar amounts of loose pollen, *B. terrestris* visited more florets per minute than *A. mellifera*. Despite *B. terrestris* being possibly more
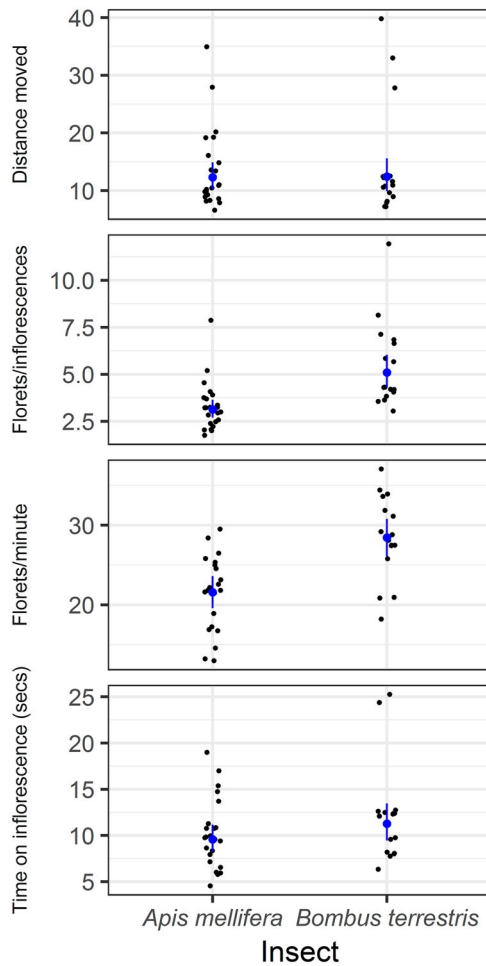
Figure 3. Scatterplots comparing behaviours of individual *Apis mellifera* (*n* = 23) and *Bombus terrestris* (*n* = 16) bees visiting white clover inflorescences. These are, (A) distances moved between consecutive inflorescences, (B) florets visited per inflorescence, (C) florets visited per minute and (D) time spent on inflorescences. Vertical lines indicate 95% confidence intervals, circles are means and crosses are individual bees.

efficient pollinators as individual foragers; the much greater abundance of *A. mellifera* resulted in this species being collectively a more effective pollinator. Even if we group all *Bombus* spp. together (assume similar efficiency for *B. hortorum* as for *B. terrestris*) then *Bombus* spp. were still ineffective pollinators relative to honey bees across most fields (Table 2).

Number of flowering inflorescences, florets and associated A. mellifera

The field with the highest mean density of inflorescences (Field 5) contained approximately twice as many flowers as the field with the lowest density of inflorescences (Field 4) (Table 3). The mean number of open florets per inflorescence was highest in Field 6, having nearly ten more open florets per inflorescence than Field 2 (Table 3).
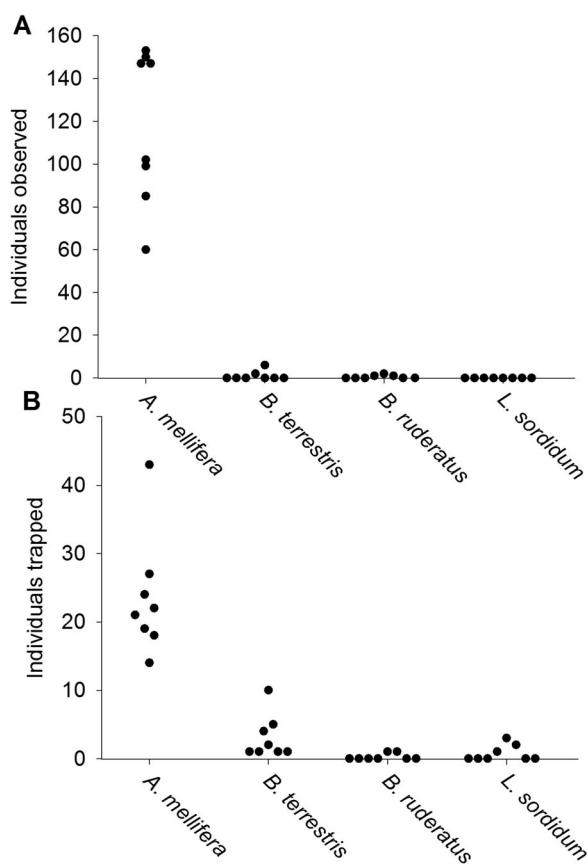
Figure 4. Numbers of bees from the genera *Apis*, *Bombus* and *Lasioglossum* in eight peak flowering white clover seed fields in Canterbury, New Zealand, recorded using observation surveys (A), and window traps (B). Each circle represents the total number of individual bees recorded in a single field.

Table 2. Estimated relative pollination effectiveness of *Apis mellifera* over *Bombus* spp. (*A. mellifera/Bombus* spp.) across eight white clover fields (applying *A. mellifera* to *Bombus* spp. efficiency ratio 1:1.38 florets visited/minute).

Field	Observation count (*n*)		Trap Count (*n*)		*A.mellifera* to *Bombus* spp. relative effectiveness (*n A. mellifera*)/(*n Bombus* spp. ×1.38)	
	A. mellifera	*Bombus* spp.	*A. mellifera*	*Bombus* spp.	Observation	Trap
1	147	0	43	2	No *Bombus*	15.6
2	99	0	22	2	No *Bombus*	8.0
3[a]	60	0	27	4	No *Bombus*	4.9
4	147	1	18	2	106.5	6.5
5	102	8	24	10	9.8	1.7
6	85	0	19	1	No *Bombus*	13.8
7	153	3	14	5	37.0	2.0
8	150	0	21	1	No *Bombus*	15.2
Mean	117.8	1.5	24.6	3.4	56.9	5.0

Note: Estimates are based on counts obtained from single-day observational surveys and from window traps that were activated over five consecutive days.
[a]Certified organic field (Asurequality n.d.).

Table 3. Mean (±Std. Err.) number of white clover flowering inflorescences (infl.) and florets per inflorescence across eight fields and estimated number of *Apis mellifera* per 1000 inflorescences and per 10,000 florets.

Field	Infl./m^2	Florets/infl.	*A. mellifera*/1000 infl.	*A. mellifera*/10,000 florets
1	216.0 ± 16.8	20.1 ± 1.4	4.8	9.7
2	199.3 ± 14.8	13.0 ± 0.9	3.5	4.6
3[a]	216.0 ± 23.3	16.2 ± 1.0	2.0	3.2
4	126.2 ± 16.7	19.0 ± 1.0	8.2	15.7
5	268.1 ± 52.9	21.9 ± 1.4	2.7	5.9
6	149.6 ± 10.5	22.9 ± 1.9	4.0	9.2
7	206.1 ± 17.8	18.2 ± 1.1	5.3	9.6
8	203.0 ± 21.2	20.2 ± 1.6	5.2	10.6

[a]Certified organic field (Asurequality n.d.).

Using the counts of *A. mellifera* observed foraging on inflorescences in each field, the estimated number of bees per 1000 flowering inflorescences was 4.1 times higher in Field 4 than in Field 3. The difference was even more pronounced between these same fields in terms of bees per 10,000 florets, with 4.9 times more bees visiting florets in Field 4 than in Field 3 (Table 3).

Discussion

Our findings indicate that *B. terrestris* is likely to be a more efficient pollinator of white clover than *A. mellifera*, due to a faster rate of movement between florets. However, their relatively low abundances (as with most other flower-visiting insects) indicated that they were overall much less effective pollinators across most fields compared to *A. mellifera*. Our findings agree with an earlier study by Palmer-Jones et al. (1962) conducted on white clover seed fields near Timaru (South Canterbury). Despite their paper noting the presence of bumble bees within fields (count data not provided) they concluded, these bees were of no importance for pollination because of low abundances.

The efficiency of different pollinating species

Measuring single-visit stigmatic pollen deposition by insects on white clover florets is particularly problematic because stigmas and anthers are enclosed inside the fused petals of the keel and are only exposed when visited by a pollinator. Moreover, it is necessary to remove the anthers to prevent movement of self-pollen to the stigma during a pollinator visit (Rodet et al. 1998). Usually, because of the difficulty of measuring single-visit stigmatic pollen deposition by insects, researchers look for and often substitute other measures that may, or have been found to correlate, for example, pollen carried on the flower visitor's body (Howlett et al. 2013) and the hairiness of the insect (Stavert et al. 2016). In doing so, an assessment of a broader range of pollinating species may be more feasible.

Because of the structure of the white clover flower, a visiting pollinator must open the keel to access nectar and pollen. The close positioning of the anthers, just behind the stigma within the keel and above the nectaries, maximises the chance that pollen will be transferred between florets via the pollinator's head as it collects pollen or nectar. Our assessments of pollen on the bodies of flower visitors indicated that significantly

more white clover pollen was found on the head of *A. mellifera* than on the thorax and abdomen. A similar but not significant distribution of pollen grains was found on the bodies of *B. terrestris* and *B. ruderatus*. The counts of pollen on the heads of these species did not differ and we consider that all three species may deliver a similar amount of pollen to stigmas when visiting florets. The production of similar white clover seed yields by *B. terrestris* and *A. mellifera* in cage experiments (Cecen et al. 2007) suggests this is true.

Our assessment of *L. sordidum* found this bee to carry far fewer pollen grains on its body than the other species, and further assessments are required to determine whether the species is capable of pollinating white clover. Our assessment of the hover flies *M. fasciatum* and *M. novaezelandiae* found that both species carried very few pollen grains and they were more evenly distributed across the body, suggesting they were not opening the keel to make contact with the anthers with their head. The low numbers of pollen grains on the body of these insects support the general observation that flies are insignificant pollinators of white clover.

The frequency of pollinator movements between inflorescences is another key element for evaluating pollination efficiency. Goodwin et al. (2011) found a positive linear relationship between the number of florets visited in an inflorescence by an individual *A. mellifera* and seed set. Although *A. mellifera* can move large amounts of self-pollen between florets, this does not appear to reduce seed set in subsequent florets visited in the same inflorescence (Goodwin et al. 2011). Our observations found *B. terrestris* visited more florets per minute than *A. mellifera*. If we assume both bees deposited similar numbers of pollen grains to the stigma per visit (ie if body pollen can be used as a substitute measure, as found to be the case in *Brassica rapa* (Howlett et al. 2011)), and the relationship between seed set and floret visitation was similar, then *B. terrestris* should be the more efficient pollinator, based on faster movements between florets (our estimates are 1.38× more efficient). We did not examine the movement of the other bee species, but Plowright and Plowright (1997) found short-tongued bumble bees to be faster at moving between florets than long-tongued species. Based on the Plowright and Plowright (1997) findings, we therefore predicted *B. terrestris* (a short-tongued species) is probably a more efficient pollinator of white clover than the long-tongued species (*B. ruderatus*, *B. hortorum* or *B. subterraneus*) that occur in New Zealand. However, we were unable to estimate the relative efficiency between the bumble bee species because of lack of data.

The effectiveness of different pollinating species

Within all white clover fields observed in this study, *A. mellifera* was a more effective pollinator compared to other insect species due to their far greater densities. Based on our observation data, *Bombus* spp. were not recorded as contributing to pollination in five of the eight fields, while their greatest contribution within a field was about one-tenth that of *A. mellifera* in terms of estimated rate of pollen delivery to florets. However, *Bombus* spp. may have been more actively foraging within the white clover fields outside the observation times. *Bombus* spp. are known to forage on other crops earlier and later in the day than honey bees (Miñarro and Twizell 2015) and under cooler or lower light intensity weather (Howlett et al. 2013). The trap captures demonstrated the presence of *Bombus* spp. across all fields, and counts were consistently higher than for

observation data. If the counts from traps reflected relative numbers of foraging bees, then *Bombus* spp. could have provided about one-third of the bee pollination in two of the eight fields. However, the trap design is more efficient at capturing *Bombus* spp. relative to *A. mellifera* (Howlett et al. 2009) and the resulting counts may overestimate the relative effectiveness of *Bombus* spp.

The only other insect we recorded that may be a potential pollinator was the native bee *L. sordidum*. However, it was low in abundance (only trapped, not observed) and based on body pollen loads it is likely to be a much less efficient pollinator than *A. mellifera* and *Bombus* spp.

Comparing A. mellifera pollinator service between fields

Previous studies have attempted to estimate the number of *A. mellifera* visits required to optimise pollination of white clover. These estimates have used different measures and varied somewhat in their consideration of abundances and visitation rates, measures of inflorescence and floret density per unit of field area, and resulting field seed set (Green 1956, 1957; Palmer-Jones et al. 1962; Free 1993; Goodwin et al. 2011). Limited studies have attempted to measure pollen deposition onto stigmas (Rodet et al. 1998) or seed set following flower visitation by single honey bees (Goodwin et al. 2011). The use of different methods between authors can result in wide fluctuations in the estimated number of honey bees required to pollinate white clover seed fields optimally. For example, estimates of numbers of honey bees required per hectare by Free (1993) are about three times greater than those of Goodwin et al. (2011). We did not collect seed set data in this study and therefore do not offer a suggestion on the numbers of honey bees required for optimal seed set in the fields we studied. However, our estimated numbers of bees per 1000 flowers were within the ranges recorded by Goodwin et al. (2011) (mean for two fields of 5.3 and 9.5) and Palmer-Jones et al. (1962) (mean variation across seven fields of 2.7 and 7.6). Despite our finding that bee counts varied by up to 2.7 times between fields, when we considered the number of bees per 1000 flowering inflorescences, the variation between fields was greater (up to 4.1 times between fields). It was even greater still when considering the number of bees per 10,000 florets (up to 4.9 times between fields). Therefore, the variation in field flowering intensity is likely to be an important consideration in determining the numbers of hives required to optimise pollination.

Our surveys were conducted before the discovery of the *Varroa destructor* mite in the South Island (Donovan 2007) and its subsequent throughout the island (Iwasaki et al. 2015). As we did not assess the number of feral honey bees present within fields, we could not determine their relative contribution to pollination. Further field surveys may determine whether the loss of feral honey bees has significantly affected overall honey bee pollination effectiveness within white clover seed fields and whether recommended hive stocking rates require revision.

Limitations of the study

Our study used established techniques to compare pollinator efficiency and effectiveness between flower-visiting species (Rader et al. 2009; Ne'eman et al. 2010). However, we used

a comparison of loose body pollen between the different flower-visiting species as a proxy for probable single-visit stigmatic pollen deposition. Our use of loose body pollen as an indirect measure of probable single-visit stigmatic pollen deposition follows several studies that have also adopted this procedure in their assessment of pollinator efficiency (Freitas 1997; Woodcock et al. 2013; Huda et al. 2015; Orford et al. 2015). For white clover, we argue that the use of this method could potentially be a more accurate measure for determining pollinator efficiency. That is because a direct measure requires the removal of anthers before flower opening (Rodet et al. 1998), potentially altering normal pollinator–floret interactions (Howlett et al. 2017b). Moreover, other studies have shown a correlation between loose body pollen and single-visit pollen deposition onto stigmas by *B. terrestris* and *A. mellifera* for *Brassica rapa* (Howlett et al. 2013) and *Gelsemium sempervirens* L. (Adler and Irwen 2006).

Our counts of flower-visiting insects (or potential visitors) varied greatly for certain species depending on whether we observed flowers or used window traps. This may be a reflection of several factors, including the timing of our observational surveys compared with the trapping period. Observational surveys were conducted on a single day in each field under fine weather condition between 09:00 h and 15:00 h, whereas window traps were left to capture insects both day and night over four consecutive days. Therefore, insects that may have been more active outside the period of the observation surveys could have been more abundant, as reflected in the counts from the traps. *Bombus* spp. can be more active over longer periods of the day and therefore their abundances may have been higher than observations indicated. Other insects, such as the fly *Delia platura* have been observed to be particularly abundant during the early morning and evening on the flowers of other crops (Howlett et al. 2013; Rader et al. 2013) and it is likely that observations did not account for their actual abundance, as reflected in the trap counts. The traps can also capture certain flower-visiting species more effectively than others. A similar window trap design placed in flowering *Brassica rapa* and onion fields was less efficient at capturing syrphid flies than *Bombus* spp. or bibionid flies (Howlett et al. 2009). Many studies use just one survey method and have the same issues regarding the true representation of potential pollinating species. However, the use of both methods in this study provided an awareness of the potential of broader diversity of flower-visiting species within the fields, as well as an ability to identify more flower visitors to species level. Our study also did not consider the roles of nocturnal or of very small insects (body length <3 mm) as pollinators, and we are unaware of any other studies that have examined these insects in white clover.

Conclusion

To our knowledge, this is the only study to have assessed the comparative pollination efficiency of *A. mellifera* with other wild species in pollinating New Zealand white clover seed fields. Although *A. mellifera* was the most effective and reliable pollinator within the surveyed fields, *B. terrestris* appeared to be a more efficient pollinator. The development of cost-effective management strategies to promote *B. terrestris* as a white clover pollinator would complement the role of *A. mellifera* because of its ability to forage under weather conditions not preferred by *A. mellifera*. We did not find evidence that any other species provided effective pollination. The native *Leioproctus* bee species, *L. vestitus* and

L. pango, are considered to be likely pollinators of white clover in some pasture locations in New Zealand and it may be possible to establish populations on arable farms through the transfer of soil cores containing their pre-pupae (Donovan et al. 2010). However, further research is required to determine whether they are efficient pollinators.

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