Can insect body pollen counts be used to estimate pollen deposition on pak choi stigmas?

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Abstract Measuring pollen deposition onto stigmas by insects is one technique used to assess pollinator effectiveness, but it can be unpredictable and time-consuming as insects must visit test flowers. This study examined whether a measurement of pollen grains from flower-visiting insects could be used to predict pollen deposited on stigmas. Individuals were collected from four bee and six fly species as they visited pak choi flowers in commercial and trial seed fields to assess their body pollen. Pollen was removed from insects by pressing their bodies (excluding actively-collected pollen on bees' hind legs) with a cube of gelatine-fuchsin. In this study, there appears to be a strong correlation between mean estimated pollen counts for each insect species and previously published data recording mean number of pollen grains deposited on stigmas for the same species. Therefore, the measurement of pollen grains directly from flower-visiting insects shows potential as a quicker and easier technique to assess pollinator effectiveness as insects can be directly collected from flowers.

Keywords *Brassica rapa* var. *chinensis*, pollinator effectiveness, Diptera, Apoidea, Brassica, pollination, pollen, New Zealand.

INTRODUCTION

Unmanaged insects can provide a significant and valuable contribution to crop pollination (references cited in Rader et al. 2009). New Zealand crops often attract a diverse range of unmanaged flower-visiting insect species (Macfarlane & Ferguson 1984; Howlett et al. 2005; Howlett et al. 2009a; Howlett et al. 2009b; Rader et al. 2009), but in most cases little is known about their effectiveness as pollinators. Pak choi (*Brassica rapa* var. *chinensis* (L.) Hanelt) is one

of few crops where the pollination effectiveness of unmanaged insect species has been explored in New Zealand (Walker et al. 2009; Rader et al. 2009). Rader et al. (2009) documented the effectiveness of eight insect species (four bees and four flies) as pollinators. These represent only a small proportion of insect species recorded visiting pak choi flowers (Howlett et al. 2009).

Time and labour constraints make it difficult to ascertain the amount of pollen deposited on

stigmas by numerous individuals, particularly in diverse pollinator assemblages. This is because the technique requires exposing virgin flowers to insect visits, the results of which can be highly unpredictable, both in the time taken and the type of species that visit. In contrast, the estimation of loose pollen (pollen not actively collected into the corbiculae or scopae on hind legs) is less labour intensive (Dafni 1992) and might be used to assess pollinator effectiveness. It has the advantages that the researcher can specifically select different pollinating species from flowers, allowing greater numbers of samples and species to be obtained.

In this study flower-visiting insects representing four bee and six fly species were collected from within commercial and trial pak choi seed crops. The aim was to investigate whether mean estimated body pollen carried by these insects could potentially be used as a predictor of the amount of pollen they transfer onto pak choi stigmas. This was assessed by comparing body pollen counts taken from flower visiting insect species collected from 19 fields (published here) with counts of pollen deposited onto stigmas from published data (four bee and four fly species (Rader et al. 2009)) and unpublished data (two fly species). All pollen deposition data were collected from one field at Gore and two fields at Lincoln in 2006. Insects observed depositing pak choi pollen onto stigmas were not collected for the assessment of body pollen.

METHODS

Pollen loads on flower visitors

To estimate the amount of loose pak choi pollen grains carried by different flower visitors, insects were collected while foraging on flowers from 19 different commercial and trial pak choi fields (Table 1). All crops were the same cultivar and at peak flower (flower density > 1000/m²) (Howlett et al. 2009b) when insects were collected.

Insects were captured whilst visiting pak choi fields using plastic vials (height 55 mm × diameter 42 mm) containing two layers of paper tissue. The bottom tissue had been soaked in ethyl acetate to act as a rapid killing agent. The second

Table 1 Number of pak choi crops from which flower visiting insects were sampled – tabulated by location and year. All insects were collected during December.

Location	2004	2005	2006
Gore (46°06'S, 168°54'E)	2	1	1
Lincoln (43°38'S, 171°28'E)	2	0	2
Methven (43°41'S, 171°40'E)	4	0	1
Pukekohe (37°12'S, 174°55'E)	2	1	0
Hastings (39°39'S, 176°52'E)	2	1	0

tissue (placed above the first and positioned to cover the walls of the vial to prevent the insect from touching the ethyl acetate soaked tissue) allowed removal of the insect with minimal loss of pollen. Dead insects were removed from the vial using forceps 1–2 min following capture.

Pollen was removed from the surface of an insect's body by swabbing it with a cube (approximately 7 × 7 × 1 mm³) of gelatine-fuchsin using clean forceps. The hind legs of bee species possessing pollen collecting structures (e.g. corbiculae, scopae) were removed because pollen from these structures is not available for pollination (Thorpe 2000). Slides were prepared in the field by melting the gelatine-fuchsin cube containing the pollen sample on microscope slides with each cube overlaid with a cover slip. The insect specimen was placed in a numbered Eppendorf® microcentrifuge tube with 70% ethanol for further identification to species level if possible.

The pak choi pollen grains sampled from each insect were quantified using a compound microscope by counting the number of grains laying on six equally spaced 20 µm lines that were marked across each cover slip. Counts were then extrapolated across the total sample by multiplying with the factor 7.33 (= total sample area of the slide divided by the examined area).

Pollen deposition on stigmas

The data from Rader et al. (2009) for eight species was used in this analysis along with new information for a further two taxa (*Calliphora*

stygia and *Odontomyia* sp.). The data for *C. stygia* and *Odontomyia* spp. were collected alongside Rader et al.'s (2009) published data (same fields, methods, time period and researchers). However, these data were not published within Rader et al. (2009) due to limited information on other variables required to assess pollinator efficiency. Methods detailing the measurement of pollen deposition on pak choi stigmas are provided in full by Rader et al. (2009), and are briefly summarised here. Virgin inflorescences in bud were bagged in fine mesh to exclude insect pollinators. When the inflorescence commenced flowering, the bag was removed and a single insect allowed to visit a flower. Following the visitation, the stigma was removed by severing the style with forceps and placed on a cube of gelatine-fuchsin. The stigma was then fixed between a slide and coverslip by melting the gelatine fuchsin. Pollen grains surrounding the stigma were then counted under a compound microscope. Rader et al. (2009) also assessed virgin stigmas from similarly bagged flowers that were not visited by insects to assess any background pollen movement (e.g. through air movement or very small arthropods that may have moved through the mesh, such as thrips and mites) and found that few grains were

transferred (63% of virgin flowers had no pollen on stigmas while the remaining 37% of flowers had a mean of 1.96 pollen grains on stigmas, $n = 456$).

Data analysis

Data were primarily analysed informally through graphing and tabulation. However, to allow stigma pollen count to be estimated from body pollen, linear regression of stigma pollen means on the estimated means of body pollen was carried out. This was done not to model the underlying relationship, but purely for predictive purposes. The regression analysis was also carried out using the medians, but since the results of these were very similar to those for the means, they are not presented here.

RESULTS

The mean estimated number of pak choi pollen grains sampled from the bodies of insects was greatest for the bees, *B. terrestris*, *A. mellifera* and *Leioproctus* sp., and the flies, *D. nigro stigma*, *E. tenax* and *C. stygia* (Table 2). In contrast, the flies *M. novaezealandiae* and *M. fasciatum* carried relatively few pak choi pollen grains while the mean pollen count from the bodies of

Table 2 Mean (Standard deviation in parentheses) estimated number of pak choi pollen grains per individual from insects collected while visiting flowers and mean count of pak choi pollen grains per stigma deposited onto pak choi stigmas by the same insect species.

Insect	Body pollen	No. insects	Stigma pollen	No. stigmas
<i>Bombus terrestris</i> (Linnaeus, 1758) ^{B3}	11,295 (8558)	24	237 (175) ¹	16
<i>Apis mellifera</i> (Linnaeus, 1758) ^B	10,940 (6391)	64	123 (93) ¹	32
<i>Leioproctus</i> sp. ^B	8400 (5923)	22	153 (128) ¹	10
<i>Lasioglossum sordidum</i> (Smith, 1853) ^B	3800 (3560)	12	30 (32) ¹	7
<i>Dilophus nigro stigma</i> (Walker, 1848)	8440 (6330)	39	68 (76) ¹	24
<i>Eristalis tenax</i> Linnaeus, 1758	8183 (5501)	22	107 (93) ¹	22
<i>Calliphora stygia</i> (Fabricius, 1794)	6256 (6033)	11	61 (43) ²	17
<i>Odontomyia</i> sp.	3321 (1869)	18	58 (73) ²	11
<i>Melangyna novaezealandiae</i> (Macquart, 1855)	1136 (1590)	18	16 (22) ¹	11
<i>Melanostoma fasciatum</i> (Macquart, 1850)	417 (534)	12	6 (8) ¹	11

¹Stigma data are from Rader et al. (2009). ²Unpublished data.

^{3B} indicates bee species.

the fly *Odontomyia* sp. and the bee *L. sordidum* was intermediate (Table 2). A similar trend was also reported for pak choi pollen deposition onto stigmas. *Bombus terrestris*, *Leioproctus* sp., *A. mellifera* and *E. tenax* individuals deposited a mean of more than 100 pollen grains while *M. novaezelandiae* and *M. fasciatum* deposited relatively few grains (Table 2). The mean number of pollen grains deposited onto stigmas by *D. nigrostigma*, *Odontomyia* sp., *C. stygia* and *L. sordidum* was intermediate (Table 2).

There was high variation within species in body pollen counts (Figure 1). For example, pollen sampled from *B. terrestris* ranged from a maximum of 32,022 grains on one individual to just 990 on another (Figure 1). For *M. fasciatum* and *M. novaezelandiae*, the majority of individuals carried relatively few grains (66.7% of *M. fasciatum* and 61.1% *M. novaezelandiae* carried less than 600 grains), while just a few individuals carried a moderate number (11.1% of *M. fasciatum* and 27.8% *M. novaezelandiae* carried more than 1000 grains) (Figure 1).

The data for pollen deposition on stigmas by the different insect species also showed a great deal of variation (Figure 2). For example, an individual *B. terrestris* visit to a flower deposited as many as 502 pollen grains to the stigma or as few as 36 grains (Figure 2). For *M. fasciatum* and *M. novaezelandiae*, a large proportion of individuals delivered few pollen grains to the stigma (63.6% *M. fasciatum* and 50.0% *M. novaezelandiae* transferred 5 or less grains) (Figure 2).

Mean estimated body pollen was well correlated with pollen grains deposited on stigmas (Figure 3, $r = 0.875$). For this data-set, the tentative linear predictive equation is:

$$y = -10.4 \text{ (SE=22.1)} + 0.01552 \text{ (SE=0.00304)} x$$

where x =mean estimated body pollen and y =pollen grains deposited on stigmas ($R^2 = 76.5\%$).

DISCUSSION

The apparent strong correlation between mean estimated body pollen and mean pollen

deposition onto pak choi stigmas by the bee and fly species examined in this study offers promise as an alternative and potentially more efficient method for the assessment of pollinator effectiveness. Using the measurement of body pollen as opposed to pollen deposited onto stigmas would allow a larger range of flower visiting insect species to be assessed, thereby increasing the potential to evaluate a higher proportion of species that visit pak choi.

However, a limitation of this study is that insect individuals sampled for body pollen were not those that were recorded visiting the virgin flowers from which pollen deposition onto stigmas were subsequently measured. Ideally, measuring body pollen and pollen deposition onto stigmas for the same individuals would provide a more rigorous method for exploring and modelling the relationship between the two variables due to the direct relatedness of the two variables. However, this technique is likely to be difficult as insects would need to be captured as soon as they leave the test flower and before landing on another flower. Such a technique would require extreme care not to disturb the test flower and an ability to capture the insect with minimal body pollen loss.

The amount of body pollen and pollen delivered to stigmas was found to be highly variable between individuals within a species, particularly for those capable of carrying large amounts of pollen, such as *B. terrestris*, *Leioproctus* sp. and *A. mellifera*. Sampling must therefore be adequate to obtain an accurate mean estimate. We recommend at least 10 and preferably more than 30 individuals be sampled to obtain this (standard error for the mean of 10 samples would be approx $x = SD/\sqrt{10}$), where SD is the SD in Table 2; this provides an approximate 95CI for the mean of $\pm 2x$). The sampling of too few individuals of a particular insect species increases the risk of developing an ecological fallacy. That is, the insect body pollen counts lead to an erroneous conclusion about the number of pollen grains they place onto stigmas. The factors responsible for these large variations were not explored in this study but may be

Figure 1 Dot histogram showing the estimated number of pollen grains on individuals of each species caught visiting pak choi flowers.

Figure 2 Dot histogram showing the number of pollen grains deposited onto pak choi stigmas by individuals of each species. Data are from Rader et al. (2009) with the exception of *C. stygia* and *Odontomyia* sp., which were collected at the same time and locations as Rader et al. (2009).

Figure 3 Relationship between the mean number of pollen grains found on the body of an insect species and the mean number of pollen grains deposited on the stigma of a pak choi flower visited by that species. The linear regression line is indicated.

influenced by the time spent within the crop (i.e. insects newly arrived to the crop may have low body pollen counts) and factors influencing a pollinator's within-field floral preferences, such as flower age or preferences for specific floral traits, e.g. variation in floral and visual cues emitted that may have been associated with the stage of pollen development and dehiscence.

Although the predictive equation, could be useful in assessing the contribution of different insect species to crop pollination, its use should be limited to *B. rapa* crops and to bee and fly species. Firstly, the relationship between mean estimated body pollen and mean pollen deposition is likely to be very different for other crop species. Floral morphology is highly variable between different crops (Free 1993) and may have a large effect on the amount of body pollen collected and the ability of insects to deposit pollen onto stigmas. Secondly, only species of bees and flies were assessed in this study and the relationship may be quite different for other flower-visiting insect orders such as Lepidoptera and Coleoptera. For

pak choi, flies and bees were found to be the most abundant flower visitors (>95% of individuals) of pak choi across 11 fields (Howlett et al. 2009).

An ability to simplify the assessment of unmanaged flower-visiting insects as crop pollinators should assist in quantifying the role and economic value of pollinator assemblages. This will help quantify the economic value of both agricultural and natural ecosystem services to crop pollination as well as provide a framework to develop techniques for predicting or building pollinator populations for improved crop production. Such information could help offset the potential decline in honey bee pollination if populations become increasingly threatened by the spread of bee-specific diseases (Cunningham et al. 2002; Cox-Foster et al. 2007; Winfree et al. 2007).

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