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Article *in* Basic and Applied Ecology · January 2017

DOI: 10.1016/j.baae.2017.01.004

2 authors:

Thijs Fijen
Wageningen University & Research

David Kleijn
Wageningen University & Research

BAAE-51015; No. of Pages 8
ARTICLE IN PRESS

GfÖ
GfÖ Ecological Society of Germany, Austria and Switzerland
Basic and Applied Ecology xxx (2017) xxx–xxx
Basic and Applied Ecology
www.elsevier.com/locate/baae

How to efficiently obtain accurate estimates of flower visitation rates by pollinators

Thijs P.M. Fijen*, David Kleijn

Plant Ecology and Nature Conservation, Wageningen University & Research, Droevendaalsesteeg 3a, 6708 PB Wageningen, The Netherlands

Received 19 September 2016; accepted 20 January 2017

Abstract

Regional declines in insect pollinators have raised concerns about crop pollination. Many pollinator studies use visitation rate (pollinators/time) as a proxy for the quality of crop pollination. Visitation rate estimates are based on observation durations that vary significantly between studies. How observation duration relates to the accuracy of the visitation rate estimate is, however, unknown. We studied this relationship using six day-long observations (06:00 h–19:00 h) in leek-seed production fields (totalling 78 h). We analysed beyond which point in time observing longer did not significantly improve the accuracy of the visitation rate estimate (minimum observation duration). We furthermore explored the relationship between the minimum observation duration and visitation rate, time of day and temperature. We found that the minimum observation duration (mean ± SD: 24 ± 11.9 min) was significantly related to visitation rate, where the observation time required to obtain accurate estimates decreased with increasing visitation rate. Minimum observation duration varied greatly between days and between fields but not within days. Within days, the visitation rates differed significantly only between the hour-intervals 06:00 h–07:00 h (lowest visitation rate) and 09:00 h–11:00 h (highest rate). Minimum observation duration decreased up to around 22 °C beyond which it remained fairly stable. Surprisingly, even after three day-long observations on the same plant we found new pollinator species visiting the flowers, suggesting that species-richness estimates based on plant observations alone probably underestimate true species richness. Because especially between-day variation in visitation rate on single plants can be large, reliable estimates of the pollinator visitation rate during the plant's flowering time require observations on multiple days. Standardising the number of pollinators rather than the time to observe (standardised pollinator timing approach: time to *n*-pollinator visits) may provide more consistent accurate assessments of visitation rate, especially for studies that use gradients in visitation rates to examine the contribution of pollinators to crop pollination.

Keywords: Minimum observation duration; Visitation rate; Pollination; Crop systems; Observation protocol; Time of day; Weather; Species richness

Introduction

Regional declines in insect pollinators have raised interest in pollination limitation of insect-pollinated crops (Allen-Wardell et al. 1998; Potts et al. 2010). Seed or fruit set of

*Corresponding author.
E-mail address: Thijs.Fijen@wur.nl (T.P.M. Fijen).

http://dx.doi.org/10.1016/j.baae.2017.01.004
1439-1791/

Please cite this article in press as: Fijen, T. P. M., & Kleijn, D. How to efficiently obtain accurate estimates of flower visitation rates by pollinators. *Basic and Applied Ecology* (2017), http://dx.doi.org/10.1016/j.baae.2017.01.004

an estimated 70% of the world crops benefits at least partially from pollinators (Klein et al. 2007). Because of that, an increasing body of literature has studied the relationship between crop yield and pollinators (Klein et al. 2007; Garibaldi et al. 2013, 2016), how this is influenced by the effects of landscape structure (Ricketts et al., 2008) and what the relative contribution is of managed versus wild pollinators (Winfree, Williams, Gaines, Ascher, & Kremen 2008; Garibaldi et al. 2013; Winfree, Fox, Williams, Reilly, & Cariveau 2015). All these studies have in common that they link the number and diversity of pollinators visiting crop flowers per unit of time to some measure of crop yield. Such visitation rate estimates can be made at the scale of the wider landscape, agricultural fields, individual plants or even individual flowers. In the case where individual plants or flowers are harvested, the most accurate assessment of the contribution of pollinators to production comes from observations that directly link the number of pollinators visiting a plant to the fruit or seed set of that plant. Such an approach was used by 21 of the 41 studies in a crop pollination meta-analysis by Garibaldi et al. (2013).

The duration of pollinator observations on crop flowers varies greatly between studies and crops, and ranges from three minutes (e.g. Tamburini, Berti, Morari, & Marini 2016) to 3.5 h (e.g. Hoehn, Tscharntke, Tylianakis, & Steffan-Dewenter 2008). How this observation duration relates to the accuracy of visitation rate estimates is generally unknown. Observations of flower visitations by pollinators are usually made under more or less standardised environmental conditions to avoid results being influenced by inclement weather. Observations generally take place on sunny days without rain and temperature and wind speed thresholds are being used below which observations cannot be made (Kleijn et al. 2015). Nevertheless, weather conditions may vary greatly above these thresholds. Whether and how such variation influences the accuracy of visitation rate estimates is also unknown. Ideally, the observation duration should be as long as the shortest time period required for a visitation rate estimate that does not significantly deviate from the true visitation rate. Too short observations may lead to inaccurate estimates that are not representative for the observed plant, which in turn could lead to inaccurate conclusions on the effects of pollinators on crop yield. Too long observations would be inefficient and this time could better be invested in increasing sample size. What observation duration is most efficient for estimating pollinator visitation rates probably also depends on the visitation rate itself, as it is likely that a minimum number of encounters must exist for accurately estimating visitation rate (Burnham, Anderson, & Laake 1980). But also the relationship between the visitation rate and observation duration is unknown.

To examine how the accuracy of visitation rate estimates is related to observation duration and whether this is influenced by weather conditions, we observed pollinators visiting leek plants in seed production fields in southern Italy. This crop is well suited for this kind of studies, as it is well visited by a wide variety of insect pollinators (Kleijn et al. 2015). The landscape of southern Italy is diverse which makes it possible to choose sites that differ in habitat suitability for pollinators and thus pollinator richness and abundance (Ricketts et al. 2008). We observed plants for full days to determine the true daily visitation rate. We then subdivided these days into intervals of different length (1–12 min) to determine at which observation duration the accuracy of the visitation rate estimate ceased to improve significantly (minimum observation duration). For each observation day, on both fields, we used the data to analyse the relationship between observation duration and estimated visitation rate. We then used this relationship to explore how time of day and weather conditions influence the minimum observation duration. Based on these results we discuss survey strategies that most efficiently produce reliable estimates of pollinator visitation rates.

Materials and methods

Study system

Commercial leek (*Allium porrum*) is mainly produced in Europe and comes in several hybrid varieties (Brewster 2008). Leek seeds are produced in hybrid seed production systems (Wright 1980). In these systems, a fully fertile inbred (male) line is crossed with a male sterile inbred (female) line to produce a high-yielding hybrid variety. Because the pollen of the male line have to be transferred to the female line and wind pollination plays no role (Brewster 2008), these systems fully rely on pollinators for pollination. Leek forms one primary umbel (flower head) and, depending on the line, one to three secondary umbels. Primary umbels can have up to 4000 flowers each of which can produce up to six seeds, like other *Allium* species (Brewster 2008; Simon & Jenderek 2010). The primary umbel contains open flowers for approximately three weeks, in which individual flowers open irregularly for a few days (Brewster 2008).

We selected two commercial leek-seed production fields in southern Italy that potentially attracted low or high amounts of pollinator individuals and species. The two fields were located about 40 km apart (field A & B) and were used to produce seeds of the same leek variety (i.e. the same male and female lines in both fields). Field A was located in a predominantly flat area, close to a small river, but otherwise surrounded by agricultural production fields, mainly wheat. Field B was located in hilly terrain. This area contained much more semi-natural habitat and was characterized by small-scale agriculture.

Observation protocol

In June 2015 we observed flower visitation by pollinators in both fields on three days from 6:00 h until 19:00 h (total observation time 78 h). Sunrise in this area and time of year was around 5:30 h and sunset at around 20:30 h. This time period covered the full daily activity period of pollinators, as our observations showed that pollinator activity started only well after 6:00 h and ceased before the end of observations. Within the fields, we selected a representative female plant before the start of flowering, approximately 20 m from the edge of the field. We observed the same, individually marked, primary umbel over the three observation days and recorded each pollinator that touched the umbel. We identified the species in the field when possible, or caught and stored the pollinator to identify the species to the best possible taxonomic level otherwise. For each pollinator, we noted the landing time on the umbel to the minute.

We observed field A on 13, 16 and 22 June 2015 and field B on 19, 25 and 29 June 2015. In field A, the observed umbel was 80% flowering (20% of the flowers still closed), 90% flowering (10% setting seed) and 70% flowering (30% setting seed) on the respective observation days. In field B, the observed umbel was 80% flowering (20% of the flowers still closed), 100% flowering, and 70% flowering (30% setting seed), respectively.

We observed only on days without rain and with wind speeds below 8 m/s (<5 Bft). During the observations, we recorded temperature (°C), relative humidity (%) and wind speed (m/s) every half hour with a handheld recorder (Digital Meter 50302).

Analyses

All data calculations and statistical analyses were done in R version 3.3.1 (R Core Team 2016). To analyse the relation between observation duration and the accuracy of the estimation of visitation rate we subdivided our day-long observations in time intervals of lengths ranging from 1 to 120 min. For each observation day, we then calculated the standard deviation (SD) of the visitation rates based on each time interval (i.e. 780 one-minute intervals, 390 two-minute intervals). SD is independent of sample size and this allows us to compare SDs from time intervals, with different sample sizes, with the SDs of the visitation rate of the day-long observations. The SD of the day-long observations was calculated as the mean SD from the time intervals from 80 to 120 min, as at those intervals the SD had always reached an asymptote. We then analysed at which observation duration (i.e. time interval) the SD of the estimated visitation rate no longer differed significantly from the SD of the actual visitation rate based on the day-long observation to determine the minimum observation duration. We tested for this by bootstrapping the 95% confidence interval (CI) for the SD of the estimated visitation rate for each observation interval, following Anderson and Santana-Garcon (2015) in the R-package 'boot' (Canty & Ripley 2015) with 10,000 bootstrap replicates. Subsequently, we identified the first observation duration for which the bootstrapped CI overlaps with the SD of the day-long visitation rate.

To examine whether minimum observation duration differs with more or less pollinators visiting the flowers per unit of time, we analysed how the calculated minimum observation duration was related to the day-long visitation rate using an ordinary least squares regression.

To analyse if visitation rate varies between different parts of the day, we analysed effects of time of day on hourly visitation rates. We regard time of day as a proxy for the complex interactions between the environment and pollinator activity patterns. We used a linear mixed effect model, with standardised hourly visitation rates as response variable, hour as independent fixed variable, and observation day nested within field as random variable to correct for nestedness of the data (function 'lmer' in R-package 'lme4') (Bates et al. 2015). We performed pairwise comparisons between the hours using function 'glht' in R-package 'multcomp' (Hothorn, Bretz, & Westfall 2008). To allow comparison between observation days with different variation, we standardised visitation rates using Z-transformation by subtracting the mean day-long visitation rate and dividing by the SD of the day-long visitation rate.

To illustrate the implications of differences in time of day for the minimum observation duration, we used the relationship between visitation rate and minimum observation duration. We averaged observed hourly visitation rates for each of the six observation days and subsequently calculated the minimum observation duration for each hour. We then fitted a orthogonal polynomial regression to the second degree using ordinary least squares regression.

Similarly, we illustrate the implication of differences in weather for the minimum observation duration. As the weather variables were highly correlated with each other (Spearman's r; see results), we only used temperature in this analysis, as this is the most easy variable to measure in the field. Firstly, we interpolated our temperature data linearly to 1 min resolution using the function 'approx' in R. Secondly, we used the interpolated temperature data to calculate the observed visitation rate for each temperature unit (rounded to the nearest °C) and we used these visitation rates to estimate the minimum observation duration per temperature unit. Lastly, we fitted an orthogonal polynomial regression to the third degree using ordinary least squares regression.

Results

Observations

In field A, the total number of pollinators observed on a single umbel during an entire day was remarkably stable with 81, 87 and 77 visitors on the three observation days respectively (Fig. 1). In field B, the range was much larger with our single observed umbel receiving 166, 610 and 367 visitors on the three observation days respectively (Fig. 1). In both fields, we observed a steady increase in cumulative abundance throughout the day (Fig. 1).

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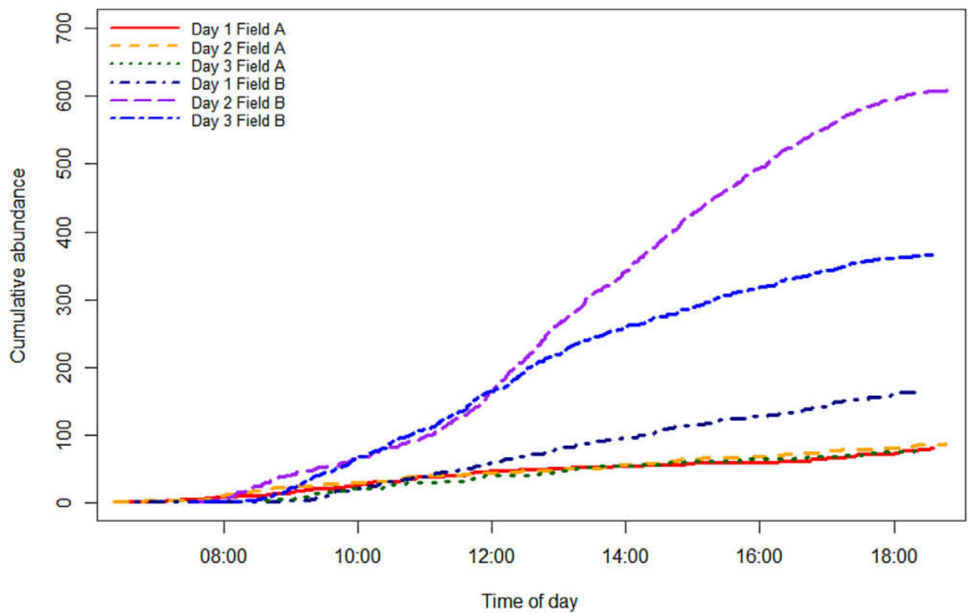

Fig. 1. The cumulative abundance of pollinators visiting the focal plants (leek) in the two study sites on three observation days. Each visitor that landed on the umbel was recorded.

We recorded in total 47 species in the two fields, with the most dominant species being *Apis mellifera* (993 individuals, 71.6% of total), *Lasioglossum malachurum* (150 individuals, 10.8% of total), *Bombus terrestris*-group (43 individuals, 3.1% of total) and *Andrena flavipes* (42 individuals, 3.0% of total). Cumulative species richness over the three days followed the same pattern in both fields. In field A we recorded 36 species, with 20 species on the first observation day, eight additional species on the second day and another eight additional species on the third day. In field B we found only 28 species despite the much larger number of visitors than on field A. Eighteen species were observed on the first day, five additional species on the second day and five more new species on the third observation day.

Minimum observation duration

On all observation days, increasing the observing duration resulted in a rapid decrease of the SD of the estimates of visitation rate towards an asymptote (Fig. 2). The minimum observation duration ranged from 7 to 36 min (mean 24.0 min ± 11.9 SD) between the observation days. In field A the minimum observation duration was 32, 36 and 28 min on the three different days on the same umbel, while in field B the variation was much larger with minimum observation durations of 29, 7 and 11 min on the three observation days. The minimum observation duration was negatively related to the number of pollinators visiting the umbels per minute. With a visitation rate of 0.6 pollinators per minute, 11.7 min were needed to accurately estimate visitation rate, but with a visitation rate of 0.2 pollinators per minute 30.3 min were needed (LM test $\beta = -40.602 \pm 7.479$ SE, Adj. $R^2 = 0.85$, $p < 0.01$, $n = 6$; Fig. 4A).

Time of day

Visitation rates increased rapidly between 6 am and 9 am after which they decreased gradually during the remainder of the day (Fig. 3). The multi-comparison test revealed that visitation rates only differed significantly between the hourly observation intervals with the lowest (06:00h–07:00 h) and highest visitation rates (09:00h–10:00 h & 10:00h–11:00 h; Tukey pairwise comparison, mean difference 9–6 h = 2.20 ± 0.61 SE, $z = 3.316$, $p = 0.048$ & mean difference 10–6 h = 2.20 ± 0.61 SE, $z = 3.635$, $p = 0.017$).

The relationship between minimum observation duration and time of day follows a clear U-shaped curve ($F_{2,10} = 83.24$, adj. $R^2 = 0.93$, $p < 0.001$; Fig. 4B), with an optimum (i.e. lowest minimum observation duration) at 12:00 h with a minimum observation duration of approximately 18 min.

Weather

Temperature, relative humidity and wind speed were strongly correlated. With an increasing temperature, relative humidity dropped ($r = -0.853$, $p < 0.001$) and wind speed increased ($r = -0.337$, $p < 0.001$), while with increasing relative humidity wind speeds were lower ($r = -0.478$, $p < 0.001$).

The relationship between temperature and the estimated minimum observation duration showed an optimum (i.e. lowest minimum observation duration) around 29 °C and minimum observation duration becomes steadily lower from 17 °C to 22 °C ($F_{3,20} = 18.37$, Adj. $R^2 = 0.69$; $p < 0.001$). When the temperatures are above 29 °C, minimum observation duration increases again (Fig. 4C).

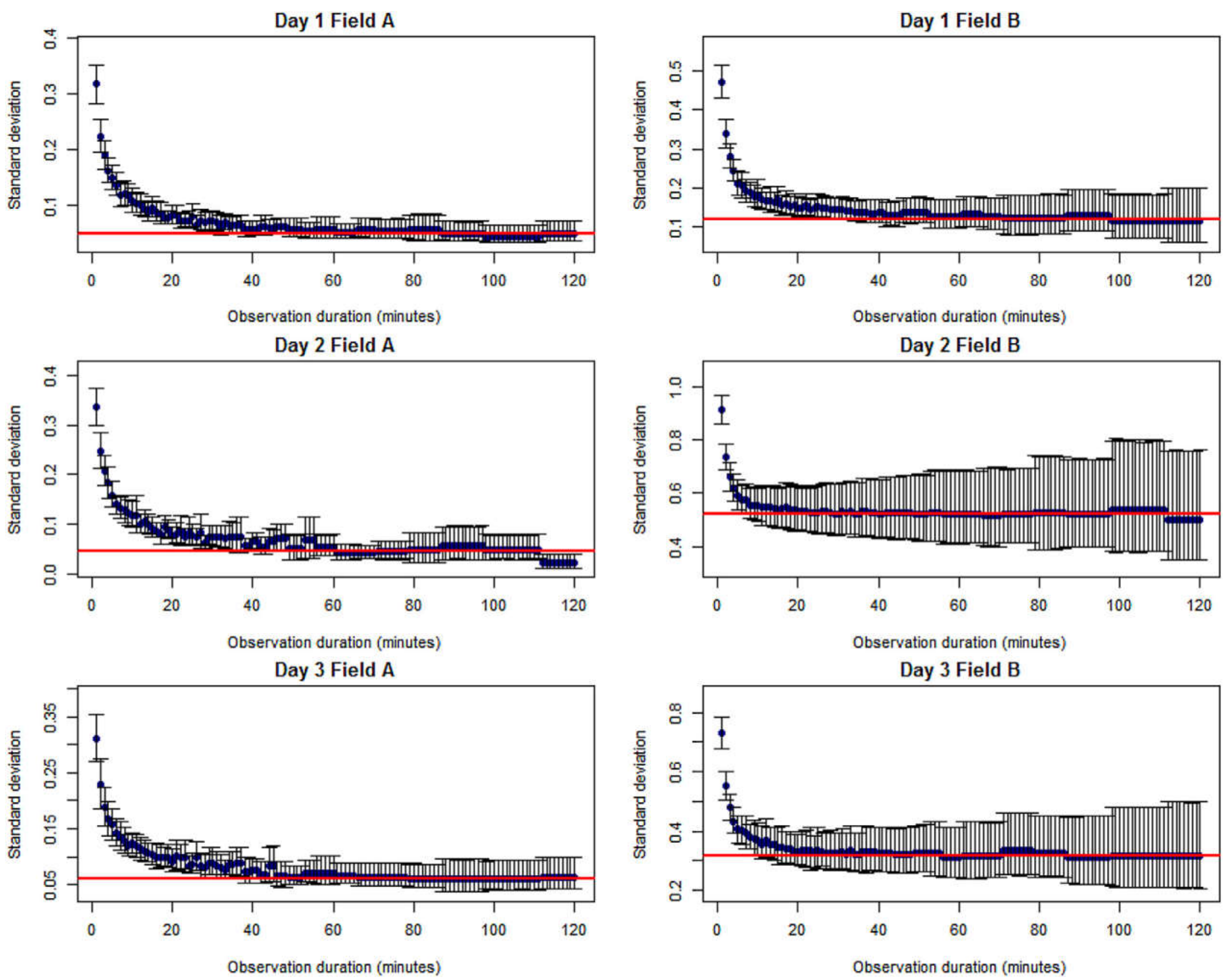

Fig. 2. Decrease in standard deviation (SD) of visitation rate (pollinators/minute) with increasing observation durations. Points show bootstrapped SDs with 95% confidence interval. The mean SD was calculated for observation interval 80–120 min and is indicated by the solid red line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

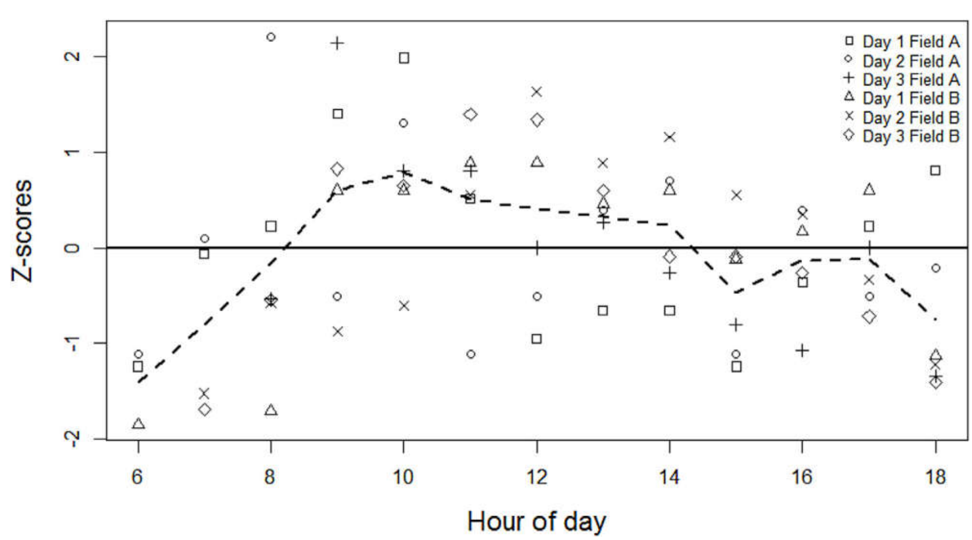

Fig. 3. Standardised visitation rates (pollinators/minute) showing variation within observation days. Visitation rates were standardised using Z-transformation by subtracting the mean day-long visitation rate and subsequently dividing by the SD of the day-long visitation rate. Symbols with a Z-score of −1 indicate visitation rates 1 SD-unit below daily average visitation rate, and symbols with a Z-score of 1 indicate visitation rates 1 SD-unit above daily average visitation rate. The average Z-score for all observation days combined is indicated by the dashed line.

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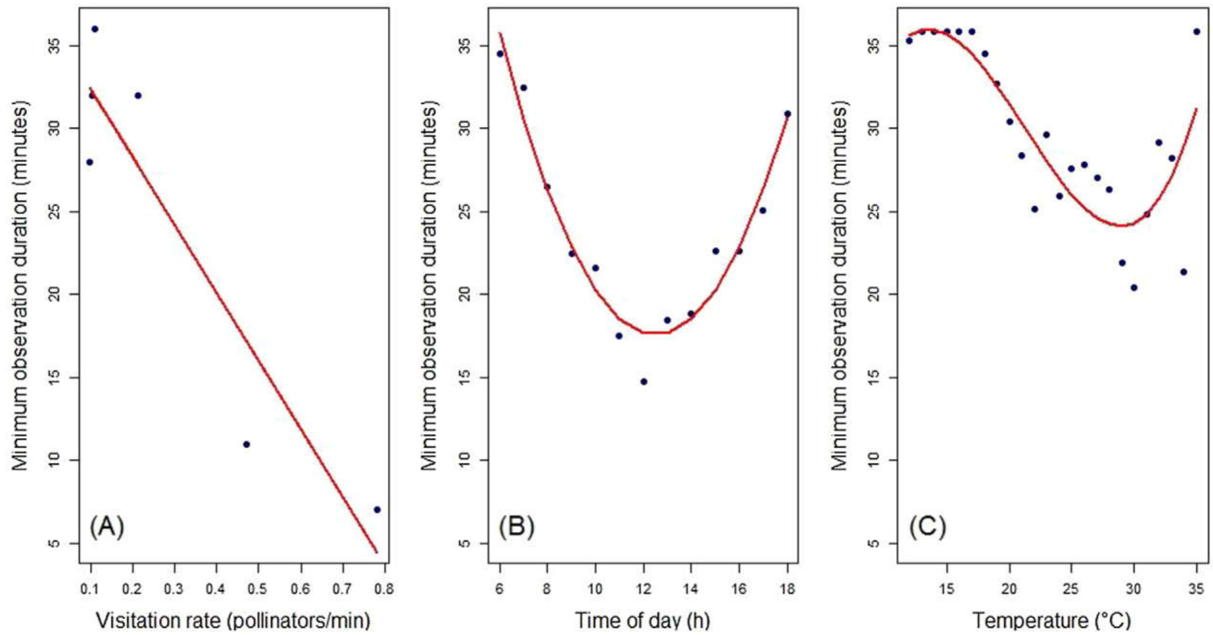

Fig. 4. Relationships between (A) visitation rate (pollinators/minute), (B) time of day (h) and (C) temperature (°C) with the minimum observation duration needed to accurately estimate visitation rate. The linear relationship between visitation rate and minimum observation duration (panel (A)) is used for calculating the minimum observation duration in panels (B) and (C). In all plots, predicted values are indicated by a solid red line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Discussion

There is a rapidly increasing body of literature on pollination based on studies that survey the number of pollinators visiting individual plants or even flowers of crops. The durations of these plant observations vary greatly between studies and it is generally unknown how this affects the accuracy of the visitation rate estimates. Our study suggests that the minimum observation duration for efficiently and accurately estimating visitation rate may differ by a factor of about five between fields. Furthermore, even for one and the same umbel, fourfold differences in minimum observation duration were observed on different days. This variation was mainly due to differences in the number of pollinators visiting the flowers during our observations, as the minimum observation duration decreased significantly with increasing visitation rate. New species were visiting the observed umbel even at the end of the 39 h long observation period which indicates that plant observations are not the best method for accurately estimating the pollinator species pool.

Even at the high visitation rate of 0.78 pollinators/min we needed to observe the plant for 7 min to accurately assess visitation rate. Minimum observation durations of up to 36 min were found at visitation rates of 0.1–0.2 pollinators/min which are commonly observed in other studies (e.g. (Ricketts 2004; Chacoff & Aizen 2006; Boreux, Krishnan, Cheppudira, & Ghazoul 2013). Many studies make use of gradients in visitation rates to determine the effects of the contribution of pollinators to seed or fruit set of crops (Garibaldi et al. 2013). Because these studies make use of a fixed observation duration, our results suggest that estimates from plants with low visitation rates are structurally less accurate than estimates from plants with high visitation rates. This could affect the results of studies as it reduces the power of analyses to find statistically significant patterns. This problem can be avoided by determining the standardised observation duration based upon plants receiving the lowest visitation rates (i.e. least attractive plants, variety or crop). The disadvantage of this approach is that this would make the visitation rate estimates on plants with higher visitation rates (i.e. highly attractive plants, variety or crop) inefficiently long. A more elegant approach may be to scale the observation duration by the visitation rate, which can be done for example by measuring the amount of time it takes before a certain number of pollinators have visited the plant.

We found that visitation rates varied considerably between days within one field, but not in the other (Fig. 1). If we had focussed on only one day per field, but instead increased the number of fields, we might have under- or overestimated the average number of pollinators visiting umbels during the flowering period of leek in field B. If, for example, a pollination study focusses on a large gradient in visitation rates in a large geographic region, observing more fields (i.e. increasing sample size) may be of larger interest than improving accuracy within fields. However, our results suggests that studies that estimate visitation rate on plants based on single day observations might contain a lot of environmental noise which could significantly influence the outcomes (Fig. 1). This should be taken into account when designing studies.

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Many studies make sure that pollinator observations are equally distributed over different parts of the day to account for pollinator activity peaks (Herrera 1990). In our study, only the period from 06:00 h to 07:00 h, which is well avoided in pollination studies (Kleijn et al. 2015), differed significantly in visitation rates from the most visited hours (09:00 h to 11:00 h). Within the period from 07:00 h to 19:00 h we found no significant differences in visitation rates. Additionally, when we look at the period from 09:00 h to 17:00 h, a generally accepted time frame within which pollinators can be surveyed (Kleijn et al. 2015), variation in the predicted minimum observation duration is small (Fig. 4B). Our results are based on full-day plant observations, allowing us to distinguish between within-day variation and between-day variation in visitation rates and the associated minimum observation durations. This suggests that variation in minimum observation duration is larger between days (Fig. 4A) than within days (Fig. 4B). If our results are representative for other study systems, this indicates that accounting for differences in pollinator activity across the day is less important than is generally assumed.

Many pollinator studies use the rule of thumb that pollinators can only be surveyed at temperatures of 15 °C or higher (Kleijn et al. 2015). We found a sigmoid relationship between minimum observation duration and temperature, with a sharp decline in minimum observation duration between 17 °C and 22 °C and an optimum at around 29 °C (Fig. 4C). When temperatures rose further, minimum observation duration increased again. This relationship undoubtedly differs between plant-pollinator systems. For example, in warmer climates pollinator activity peaks will probably occur at higher temperatures than in colder climates and pollinator communities dominated by cold-tolerant bumble bees have lower activity peaks than pollinator communities dominated by solitary bees (Fründ, Dormann, Holzschuh, & Tscharntke 2013; Kühsel & Blüthgen 2015). The number of days with weather conditions that are really suitable for surveying pollinators usually limits sample size of pollinator studies and raising the generally accepted 15 °C temperature threshold below which no observations can be made might make it altogether impossible to perform well-replicated pollinator studies, especially at higher latitudes or altitudes. However, the influence of marginally suitable weather conditions could be incorporated better in study designs, for example, by making sure that days with better or worse conditions are evenly distributed over the experimental treatments or gradients.

Surprisingly, even after observing one and the same umbel for three full days, we found no saturation in the number of species visiting it. This means that regardless of the minimum observation duration, we would always have highly underestimated the total number of species visiting our plants during the receptive period of the flowers. Stigmata of onion flowers (*Allium cepa*, a close relative of leek) are receptive for two to five days (Moll 1954). If this is also the case for leek, even the last new pollinator species at the end of our third observation day could have increased seed set, for example through functional complementarity (Hoehn et al. 2008). But also for flowers that are only receptive for one day, pollinator species richness by plant observations alone would be underestimated. A better estimate of the total pollinator species pool in the system could be obtained with transect counts (Westphal et al. 2008). Transect counts can also be used to estimate visitation rate. However, because transect counts are done at a larger spatial scale than plant observations they less precisely describe the pollinators to which individually harvested plants have been exposed. Estimating both the total number of individuals and species to which a plant has been exposed during its flowering period can therefore probably be better done by a hybrid approach: estimating visitation rate by means of plant observations and species richness by standardised transect counts that cover more surface area, more flowers and therefore more readily detect less abundant species.

Evaluating the methods that are used to collect the data that are at the basis of scientific studies, even if they are generally accepted and widely used, is essential to uphold scientific quality in research (Elphick 2008). The lengths of the observation periods used to estimate the visitation rates that are at the core of the rapidly increasing number of pollination studies are largely based on general assumptions and rules of thumb. Our results suggest that more accurate and consistent estimates can be obtained by taking into account the effect visitation rate itself has on the reliability of its estimate. Standardising the number of pollinators rather than the time to observe may be both a more consistent and efficient approach. Determining the amount of time it takes to record a certain number of pollinators visits to the plant of interest ensures that observation duration is not too short in sites with low pollinator abundance and not too long in sites with high pollinator abundance. Accuracy of the estimates obviously increases with the number of pollinators that is used to time the visitation rate. In our study, timing the period until five pollinators visited the leek umbels gave on average estimates equal to those of the minimum observation duration. Such a standardised pollinator timing approach can easily be expressed in traditional units for visitation rate estimates (pollinators/time) allowing for easy comparisons with previous studies.

Acknowledgements

This work is part of the research programme NWO-Groen, which is jointly funded by the Netherlands Organisation for Scientific Research (NWO) and Bayer Vegetable Seeds (Bayer VS) under project number 870.15.030. Bayer VS kindly provided access to the field sites and assisted in the logistics of this study. Bayer VS has no influence on the scientific output or on the conclusions and recommendations. DK was additionally funded by the EC FP7 project LIBERATION (311781; www.fp7liberation.eu).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.baae.2017.01.004.

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