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

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## 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

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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