Pollination benefits are maximized at intermediate nutrient resource levels

Giovanni Tamburini, Francesco Lami, Lorenzo Marini

ELECTRONIC SUPPLEMENTARY MATERIAL

Summary

(a) **Experimental setup**
(b) **Meteorological conditions during flowering**
(c) **Flowering pattern and pollination treatment**
(d) **Flowering phenology analysis**
(e) **Yield model predictions**

(a) Experimental setup

Figure S1. Overview of the experimental location (*a*) and examples of pollination treatment: flower temporally closed (*b*) and flowers temporally open (*c*; on the right). Pollinator exclusion was performed by means of tulle bags (mesh size 1 mm) placed over sunflower heads. Bag removal and placement was performed daily between 08:00 and 10:00 am. Since flower heads expand during the flowering, bags were periodically adjusted to avoid contact with florets.

(b) Meteorological conditions during flowering

Meteorological conditions during the flowering period (from the 3rd to the 24th of July) were optimal for pollinator activity (figure S2; average temperature, 24.6 °C; one day with precipitation more abundant than 1 mm; average solar radiation, 25.4 MJ/m^2; average wind speed, 2.2 m/s). The meteorological station is located in the Experimental Farm of the University of Padova, (45°20'49''N; 11°57'07''E). Meteorological data are available at http://www.arpa.veneto.it/bollettini/storico/Mappa_2017_TEMP.htm .

Since plants belonging to the same pollination treatment started flowering on different dates (figure S3), temporarily unfavourable weather conditions are not expected to have impacted differently the pollination treatments.

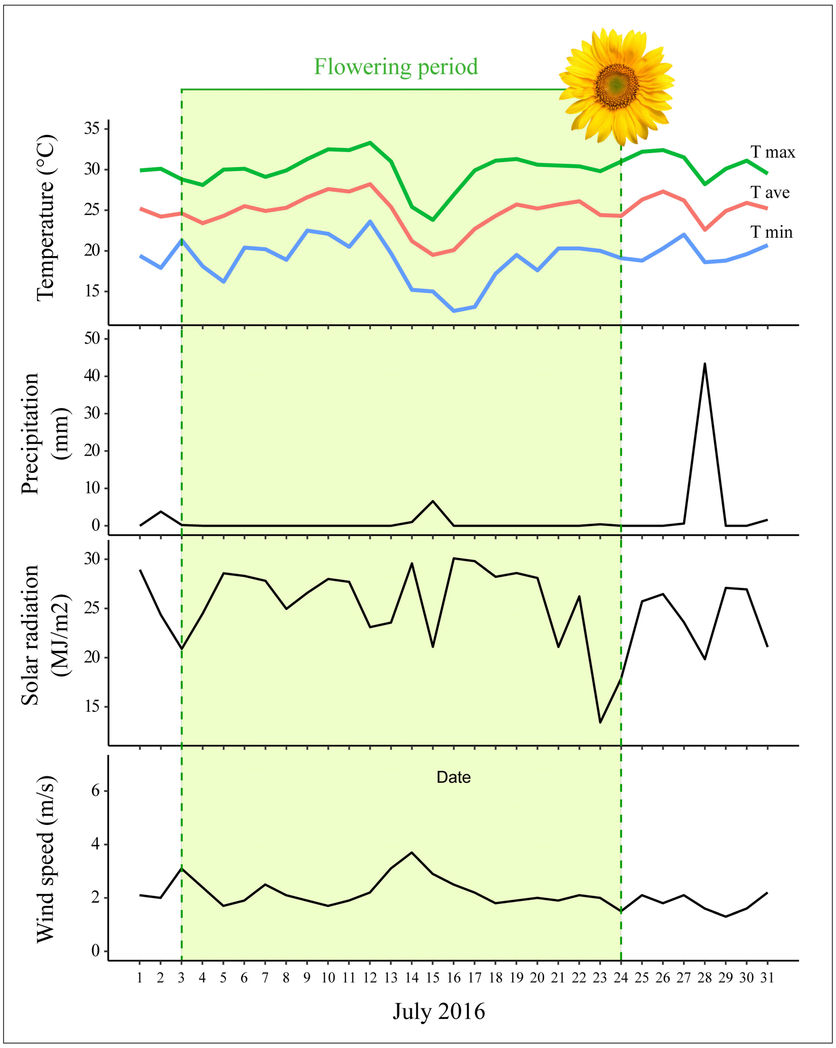

Figure S2. Meteorological conditions (mean temperature, precipitation, solar radiation and wind speed) during the crop flowering period.

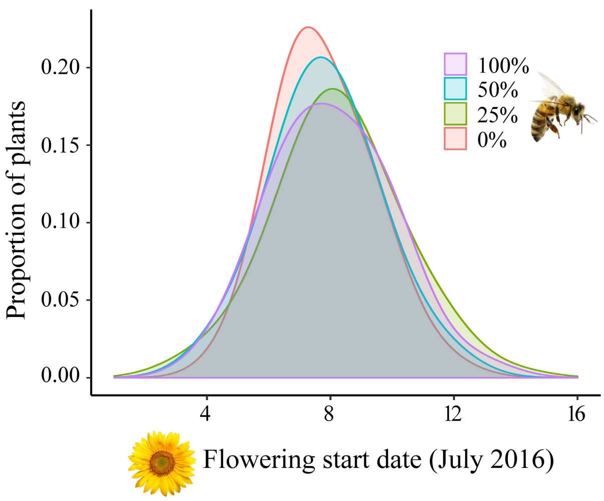

Figure S3. Density plot displaying the proportion of sunflower plants starting flowering at each day (from the 3rd to the 16th of July) in relation to pollination treatment.

(c) Flowering pattern and pollination treatment

Florets in the sunflower capitulum are arranged in a spiral and geometric pattern. Each head flowers for about 6–10 days. The outer whorl of disk florets open first. Successive whorls of one to four rows of florets open daily for 5 or more days. Each floret is open for at least two days: on the first day, the stamens elongate above the top of the corolla and the anthers release pollen. By the second day, the stigma has elongated and its inner receptive surfaces open to receive pollen. Florets are therefore male first and then female [1]. However, florets wither quickly if pollination is optimal. If not florets stay fresh for up to two weeks ([2,3]; figure S4). Our results regarding flower phenology showed the same pattern: pollinator exclusion increased flowering duration according to the level of exclusion treatment (see below, "Flower phenology" paragraph).

The different pollination levels were achieved by manipulating the number of days during which pollinators had access to flowers: complete exclusion (0% pollination), 1 out of 4 day access (25%), 1 out of 2 day access (50%) and full open pollination (100%). Exclusion was performed by means of tulle bags (mesh size 1 mm) placed over sunflower heads after one day of potential pollinator visits to female florets (1-2 whorls of female-phase florets open for one day). Since female florets remain receptive for several days if pollination does not occur, all the florets have been exposed at least two days to insect visitation (figure S4).

One consequence of the flowering pattern in sunflower is that the centremost florets have a lower probability of being pollinated because the ratio of male to female florets decreases with time [3]. Nevertheless, given the small scale of the experiment and the interspersed spatial arrangement of plants at different flowering stages, pollinators easily moved from one plant to the other, favouring the flow of pollen also towards those plants with low number of male florets.

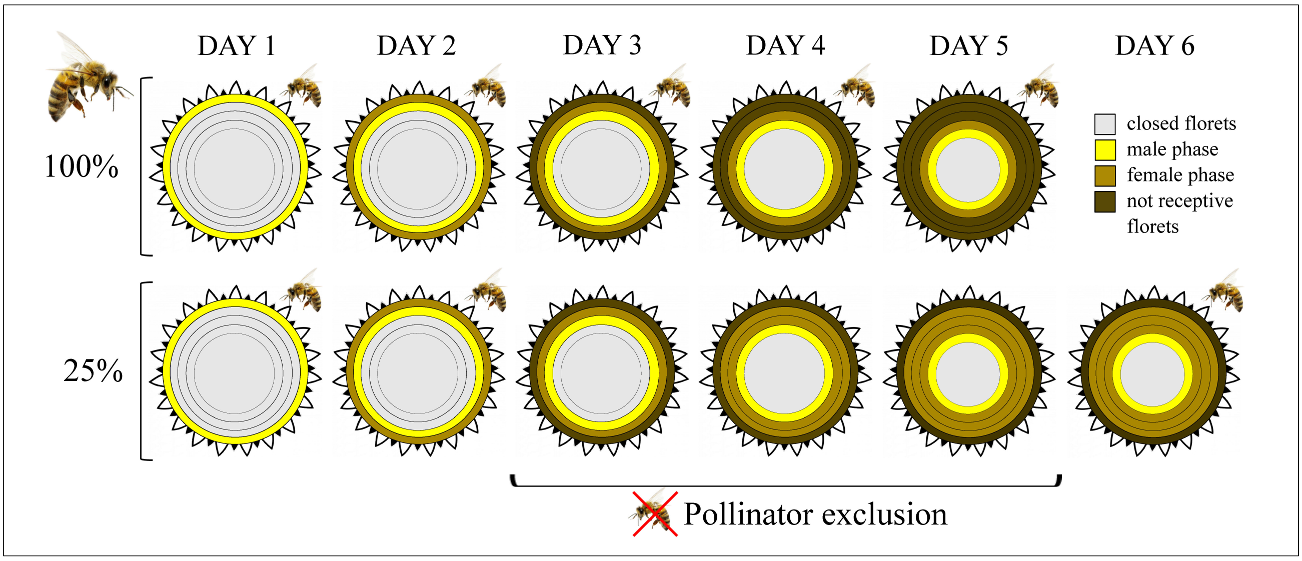

Figure S4. Flowering pattern in sunflower under optimal pollination (100% pollination) and under experimental conditions (25% pollination). If not pollinated, female florets stay open several days.

(d) Flowering phenology

Methods. Flower phenology was checked daily in order to detect the beginning and the end of the anthesis for each plant and to calculate the flowering duration (data available online as supplementary material, “flowering phenology.xlsx”).

Statistical analysis. We used a generalized additive mixed model (GAMM, [4]) to test the effect of pollination, fertilization and their interaction on flowering duration. Both pollination and fertilization were considered as continuous variables. GAMMs were fit using the “gamm” function in the “mgcv” package [5]. Cubic regression spline smoothers with “shrinkage” were applied for each explanatory variable in the GAMMs. The model included block and plot ID as random factors.

Results and discussion. We found that flowering duration strongly decreased with pollination, but its effect was slightly modulated by fertilization (table S1, figure S5). These results confirm that the plants belonging to the lowest level of pollination (25%) still had high chances to receive pollinator visits, being receptive for a longer period than those exposed to insects 100% of the time. Flower longevity is known to increase with decreasing pollinator visitation as a mechanism to increase chances of reproduction success [6-8].

Table S1. Summary of the results of generalized additive mixed model testing the effects pollination and nitrogen fertilization (N fert) and their interactive effect on the duration of the flowering period. Degrees of freedom (d.f.) for each variable refer to the complexity of the additive curve. P-values in bold are statistically significant (*p*<0.05).

	d.f.	**F-value**	***p*-value**
flowering duration			
pollination	2.73	90.18	**<0.0001**
N fert	1.72	3.81	**<0.0001**
pollination x N fert	2.71	0.78	**0.0335**

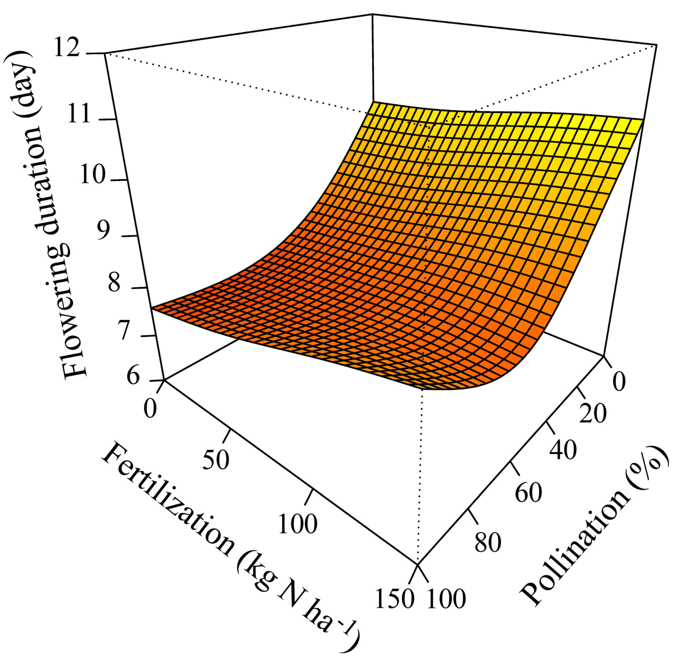

Figure S5. Spline fit of flowering duration (day) per plant in relation to pollination and fertilization treatments.

(d) Yield model prediction

Table S2. Model predictions of yield (g) (from GAMM) at each pollination (%) and fertilization (kg N ha^{-1}) level.

	pollination (%)			
fert (kg N ha^{-1})	0	25	50	100
0	83.30	90.04	94.21	95.89
15	84.10	92.13	97.65	100.21
30	85.09	94.37	101.08	104.33
45	86.44	96.84	104.35	107.82
60	88.15	99.46	107.28	110.44
90	92.50	104.48	111.80	113.18
120	98.44	109.37	115.19	113.92
150	105.48	114.24	117.95	113.61

References

1. Free J. 1993 *Insect pollination of crops*. Academic Press.

2. Roubik DW. 1995 *Pollination of cultivated plants in the tropics*. Food & Agriculture Org.

3. Delaplane KS, Mayer DR, Mayer DF. 2000 *Crop pollination by bees*. Cabi.

4. Wood SN. 2006 *Generalized additive models: an introduction with R*. CRC press.

5. Wood SN. 2011 Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *J. R. Stat. Soc. Ser. B Stat. Methodol*. 73, 3–36.

6. Primack RB. 1985 Longevity of individual flowers. *Annu. Rev. Ecol. Evol. Syst.* 16, 15–37.

7. Ashman TL, Schoen DJ. 1996 Floral longevity: fitness consequences and resource costs. In *Floral biology* (pp. 112–139). Springer US.

8. Van Doorn WG. 1997 Effects of pollination on floral attraction and longevity. *J Exp. Bot*. 48, 1615–1622.