

## Research article

# Experimental evidence for stronger cacao yield limitation by pollination than by plant resources

Janna H. Groeneveld<sup>a</sup>, Teja Tschardt<sup>a</sup>, Gerald Moser<sup>b</sup>, Yann Clough<sup>a,\*</sup>

<sup>a</sup> Agroecology, Department of Crop Sciences, University of Göttingen, Waldweg 26, 37073 Göttingen, Germany

<sup>b</sup> Ecology and Ecosystem Research, Albrecht-von-Haller-Institute for Plant Sciences, University of Göttingen, Untere Karspüle 2, 37073 Göttingen, Germany

## ARTICLE INFO

## Article history:

Received 8 July 2009

Received in revised form

17 February 2010

Accepted 22 February 2010

## Keywords:

Agroforestry

Drought

Fruit:flower ratio

Nitrogen

*Theobroma cacao*

Water availability

## ABSTRACT

Both pollination and resource limitation may cause low fruit:flower ratios in plants, but pollen and resource limitation have never been contrasted in commercially important crop species. Here we experimentally investigated the relative effect of pollen limitation and resource limitation in *Theobroma cacao*. In Central Sulawesi, Indonesia, we applied different relative levels of hand pollination (10%, 40%, 70% and 100% of available flowers up to 2 m height) to mature cacao trees in two separate experiments encompassing (1) different light (shade roofs) and nitrogen (fertilizer application) treatments, and (2) water availability (throughfall displacement) treatments. None of the resource availability treatments had a significant effect, while number of mature pods and yield increased non-linearly with pollination intensity up to 200% of current yield levels. The largest benefits were reached by increasing pollination from 10% to 40%, with non-significant increases beyond that level. Despite an increase of fruit abortion with pollination intensity, *T. cacao* yield is determined, at least on the short term, by the number of flowers pollinated. This suggests pollination deficit in crops can be very large and that a better knowledge of pollen and resource limitation to devise adequate pollinator management strategies may be critical for increasing production.

© 2010 Rübél Foundation, ETH Zürich. Published by Elsevier GmbH. All rights reserved.

## Introduction

Numerous plant species produce more flowers than fruits (Charlesworth, 1989; Sutherland and Delph, 1984; Ayre and Whelan, 1989). In species as different as *Mandevilla pentlandiana*, *Hesperoyucca whipplei* and *Asclepias speciosa*, only a few percent of the flowers lead to mature fruits (Udovic, 1981; Bookman, 1983; Torres and Galetto, 1999). Several hypotheses have been proposed as the ultimate cause for low fruit:flower ratios, including increased pollinator attraction by large floral displays, improvement of female fitness through the selective maturation of high quality fruits (wider choice or selective abortion hypothesis), increase in male fitness through higher pollen dissemination (male function hypothesis), insurance against flower losses due to herbivory, weather or mechanical accidents, or against variations in resource and pollen availability (Stephenson, 1981; Ayre and Whelan, 1989). According to the last hypothesis, excess flower production is equivalent to a “bet-hedging strategy”, allowing plants to produce more offspring without flowers being the limiting factor in conditions of plentiful resources and/or pollen transfer (Stephenson, 1981; Udovic, 1981; Sutherland, 1986;

Burd, 1988; Brown and McNeil, 2006; Bos et al., 2007b). The proximate causes of low fruit:flower ratios are still poorly understood for most plant species.

A review of pollen supplementation studies suggests pollen limitation is common in wild plants (Ashman et al., 2004), but little information is available for economically important crop species. The role of varying availability of pollination for crop yield is often neglected or questioned (Ghazoul, 2005). Evidence suggests passion fruit (*Passiflora edulis*), musk melon (*Cucumis melo*) and pumpkin (*Cucurbita maxima*) are limited by pollinators (Bos et al., 2007b; Maciel Sousa et al., 2009; Nicodemo et al., 2009). Only recently the importance of pollinators for yield has been shown in a major cash crop species, highland coffee *Coffea arabica* (Ricketts et al., 2004; Veddeler et al., 2008). However, to our knowledge there are no published studies simultaneously investigating resource and pollen limitation and their interaction in a crop species (Bos et al., 2007b), although several studies are available for wild plants (Galen et al., 1985; Horvitz and Schemske, 1988; Ackerman and Montalvo, 1990; Vaughton, 1991; Ehrlén, 1992; Campbell and Halama, 1993; Brookes et al., 2008).

*Theobroma cacao* L. (Malvaceae; Fig. 1) is a cauliflorous tree and produces, like many other tropical plants, a surplus of flowers. Most cacao varieties are self-incompatible, and are thus dependent on cross-pollination, mainly by midges (Diptera: Ceratopogonidae;

\* Corresponding author. Tel.: +49 (0) 551 39 22358; fax: +49 (0) 551 39 8806.  
E-mail address: [yclough@gwdg.de](mailto:yclough@gwdg.de) (Y. Clough).

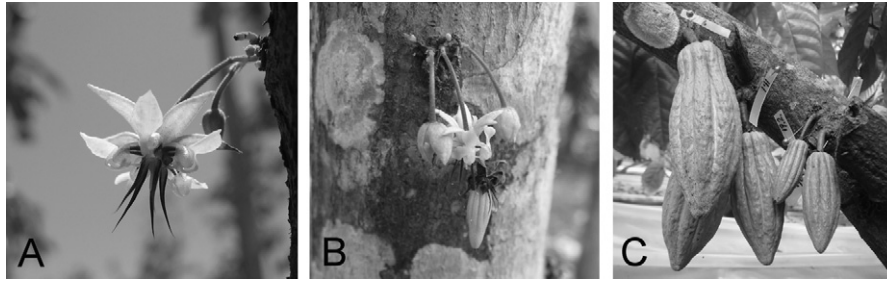


Fig. 1. *Theobroma cacao* flower (A), young fruit (cherelle) with open and unopened flowers (B) and maturing fruit (C); labels on panel C indicate pollination date.

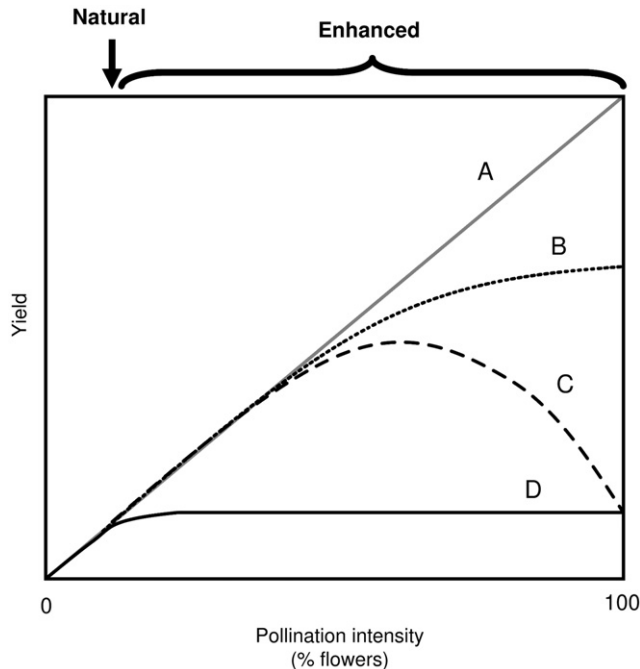


Fig. 2. Hypothetical relationships between pollination intensity and number of mature fruits. Scenario A indicates no limitation other than pollination, scenarios B, C and D indicate limitation due to incomplete fruit set and/or fruit abortion, with: buffering of pollination effects (B), decrease of yield at high pollination levels, e.g. through excessive competition by (aborted) young fruits (C), and natural pollination fully sufficient, with resources the only limiting factor (D).

Glendenning, 1972). Only about up to 10% of flowers, and often much less, are naturally pollinated and even fewer (up to ~5%) develop into mature fruits, due to fruit abortion (Falque et al., 1995; Bos et al., 2007b; de Almeida and Valle, 2007). Unpollinated flowers abscise after 24–36 h (de Almeida and Valle, 2007). Abortion, or cherelle wilt, affects almost exclusively young pods (the “cherelles”) up to 12 weeks of age. Wilted cherelles darken and dry out but remain on the tree.

Young (1982, 1994) ascribes the low fruit:flower ratio in cacao to pollinator limitation. However, Valle et al. (1990) conclude from a hand pollination experiment in which they compared fruit set, cherelle wilt and yield between naturally pollinated, continuously hand pollinated, and depodded trees that fruit set in cacao is regulated by assimilate production, with cherelle wilt being the mechanism whereby the tree adjusts production. At high pollination levels, the energetic cost of producing young pods is very high, leading to a dramatic increase in relative cherelle wilt and resulting in similar yields between natural and enhanced pollination. The question of the existence of a pollination optimum above natural

pollination remains unanswered since their study did not allow for the detection of non-linear responses of yield to pollination intensity, which may occur if too much plant resources are spent on forming cherelles at high pollination intensity levels. Intermediate hand pollination intensity could result in higher fruit set when compared to natural pollination, but lower relative cherelle wilt when compared to full hand pollination, thus maximising yield (scenario C in Fig. 2).

Furthermore, the pollination optimum is unlikely to be fixed, but is likely to depend on resources available to the plant. Limitation in nutrients, light and water can be expected to reduce the carrying capacity of cacao trees (Cunningham and Lamb, 1958; Cunningham and Arnold, 1962; Ahenkorah et al., 1987; Beer, 1988; Egbe and Adenikinju, 1990; Johns, 1999; Zuidema et al., 2005), and thus reduce the effective pollinator limitation (scenario D in Fig. 2).

In this study, we hand pollinated mature cacao trees at near-natural and enhanced intensities (10% and 40%, 70%, 100% of flowers below 2 m stem height). These pollination intensity treatments were combined with different levels of resource availability in two experiments. In the first, cacao trees were subjected to nitrogen fertilizer and experimental shade treatments. In a second experiment, we performed a large-scale replicated and controlled drought experiment (Schwendenmann et al., 2010), to our knowledge the first of its kind. Fate of individual flowers and fruit was followed until abortion/harvest. We used these data to answer the following questions: (1) are cacao trees pollen and/or resource limited and (2) does the pollination optimum depend on resource availability?

## Material and methods

### The study area

Both experiments were conducted from December 2007 to August 2008 in Central Sulawesi, Indonesia, at the western margin of the Lore Lindu National Park. All plots were located in the sub-districts Kulawi and Kulawi Selatan. The mean monthly precipitation of the area is  $143.7 \pm 22.74$  mm, with the heaviest rains between December and April, but no pronounced wet or dry season. The annual mean temperature is  $24.0 \pm 0.16$  °C. Cacao is the main cash crop grown in the study area. Hybridization between out-crossing genotypes is common in smallholder cacao, and cacao trees in this study, as well as most of the other trees in the area, originate from hybrids between Forastero and Trinitario types.

### Shade, fertilizer and pollination intensity plots

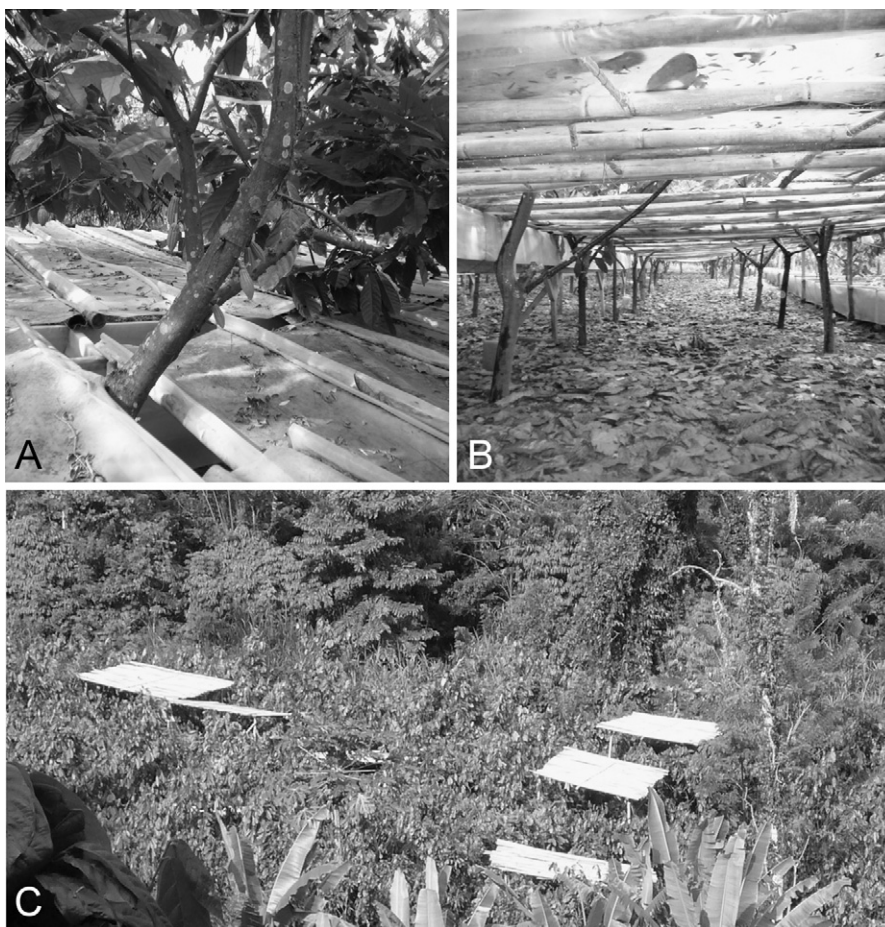
Previous studies suggested both shade (Steffan-Dewenter et al., 2007) and nitrogen, but not other nutrients (Dechert

et al., 2005), are limiting cacao production in the highlands of Central Sulawesi. We established shade and nitrogen fertilization treatments on three predominantly unshaded  $40 \times 40$  m cacao plots situated several kilometres apart. Each plot was divided into a fertilized and an unfertilized subplot of size  $20 \times 40$  m; the fertilized subplots were treated with  $200 \text{ kg N ha}^{-1}$  in December 2006, July 2007, December 2007 and July 2008. Fertilizer was applied in concentric circles around the trees. Analyses from leaves collected in October 2007 revealed significantly higher C/N ratios in unfertilized plots than in fertilized plots, which confirmed that nitrogen was successfully taken up by the cacao trees (YC, unpublished results). Tree height ranged from 2.8 to 5 m, with an average height of 3.4, 4.1 and 3.8 m, respectively, for each plot. In November 2007 four shade-treatment and four control trees per subplot were randomly selected from the subset of unshaded, flower-bearing trees and bamboo roofs for shading were constructed (Fig. 3C). The roofs were completed in December 2007 with a vertical light interception of around 70%, which corresponds to a moderate to high shade level within the range of current shading practices (Steffan-Dewenter et al., 2007). In total this experiment thus included 48 trees: 16 trees per plot, comprising four shaded and four unshaded trees on the fertilized and unfertilized subplots, respectively. Per plot and treatment combination, one tree was randomly assigned to a hand pollination treatment (10%, 40%, 70% and 100%). The trees were hand pollinated daily for 60 days starting on December 29th, 2007. In one fertilized subplot a single, and in one unfertilized subplot two trees did not bear flowers during the experiment, reducing the total number of trees to 45.

#### Experimental drought and pollination intensity plots

The study was part of the Sulawesi Throughfall Displacement Experiment (Moser et al., unpublished, Schwendenmann et al., 2010). The experiment was conducted on six adjoining seven-year-old cacao plots, each  $35 \times 40$  m. The site was located in the Kulawi valley 560 m a.s.l. on a slope ( $8\text{--}12^\circ$  inclination) more than 30 m above the water level of the main river and was naturally shaded by overstorey shade trees (*Gliricidia sepium* L.; shade tree cover mean  $\pm$  SE =  $51.8 \pm 2.0\%$ ). Canopy rain interception was around 20%. The plots were fertilized twice a year until June 2006, before the experiment started.

Three of these plots were roofed below the cacao tree canopies with plastic-covered bamboo frames (each  $5 \times 0.5$  m) intercepting and draining off 80% of the throughfall (Fig. 3A, B). The remaining three plots served as controls. The roof was established on March 1st, 2007 and maintained until April 10th, 2008. The soil volumetric water content down to 2 m depth decreased continuously during the induced soil desiccation between March and August 2007 from about  $0.45 \text{ m}^3 \text{ m}^{-3}$  to values below  $0.30 \text{ m}^3 \text{ m}^{-3}$ , which was close to the theoretical permanent wilting point, and remained at this level until December 2007 (Schwendenmann et al., 2010). During a natural dry period in January and February 2008, the soil water storage fell below 300 mm, and was then refilled to about 320 mm until the end of March. When the roofs were opened on April 10th, 2008 the soil water storage of the roof plots increased rapidly to a level around 400 mm, but stayed below the control plots until the end of the study period (July 2008). Root excavation of 6 cacao trees per



**Fig. 3.** View above (A) and below (B) throughfall displacement panels at drought experiment site in cacao; one of the three sites of the cacao shade and fertilizer experiment (C).

treatment after the roof opening revealed a maximum length of tap roots to 1.6 m soil depth, found in control plots, and the excavation of 12 soil pits down to 3 m depth revealed a maximum fine rooting depth to 2.4 m.

In December 2007 eight trees per plot were randomly selected, resulting in a total of 48 trees (24 on the roofed plots, 24 on the control plots). Per plot, two trees were assigned randomly to each of the four hand pollination intensity levels (10%, 40%, 70% or 100%). The trees were hand pollinated daily for a period of 60 days starting on January 11th. In one roofed plot, a single tree did not flower at all during the experiment, reducing the total number of trees to 47.

#### Hand pollination

Before starting with manual cross-pollination, all fruits and open flowers were taken off to ensure no old flowers would be pollinated. In *T. cacao*, pods are concentrated on the trunk and main branches, while flowers are also produced in high numbers on secondary branches. Flowers developing within the cacao canopy above 2 m height were out of reach and were not hand pollinated. The flowers below 2 m represented 14% of the total number of flowers (based on counts conducted on all experimental trees on 19–20th January). Fruits developing from natural pollination above 2 m height were continuously removed during the period of hand pollination to rule out competition and compensation effects from fruits developing outside of the experiment.

To determine the number of flowers needed for the respective pollination level (10%, 40%, 70% and 100% of open flowers), all open flowers up to 2 m height were counted daily. The flowers for subsequent hand pollination were then chosen randomly. As pollen donors, fresh, open flowers of three different non-experimental trees were collected. This was done to maximise cross-pollination success and minimise pollen source/quality effects (Ayre and Whelan, 1989; Aizen and Harder, 2007). One open anther from each of these three flowers was rubbed against the stigma of the flower to be pollinated, as described by Falque et al. (1995). Pollination was done once per flower, and several hundred pollen grains were deposited on each flower, thus maximising fruit set probability (Falque et al., 1995). All hand pollinated flowers were marked using clear plastic strips featuring the pollination date, attached to the bark by a pin, while the remaining unpollinated open flowers were removed. The procedure was repeated daily for 60 days. Given the heterogeneity in tree architecture, the high quantity of pollen transferred to each flower and the removal of non-pollinated flowers, it was decided not to prevent pollinator access to the tree.

#### Fruit development

Trees were checked for wilted flowers and fruits every day starting on the day following the first hand pollination. The check continued for another two weeks after the 60 days and was repeated on April 15th and May 15th, as well as during harvest. From April onwards, we separately noted diseased pods. Before this date, dead fruits obviously not resulting from cherelle wilt were also recorded. Due to a high incidence of black pod disease *Phytophthora palmivora* Butl. (37% of all maturing fruits), it was not possible to reliably weigh all harvested pods, so the fresh weight of healthy harvested pods was recorded and these data used together with the number of harvested pods to extrapolate total yield per tree (see following section). Starting from May 6th and May 26th on the shade/fertilizer and rain interception plots respectively, the trees were harvested every two weeks. Ripe

fruits were counted and weighed, whole fruits and fruit interior only, per tree. Monitoring was completed on the drought experiment plots on August 4th and on the shade and fertilizer plots on August 13th, 2008.

#### Data analysis

Analyses were conducted on the data pooled per tree. Fruit set was calculated as the number of flowers pollinated minus the number of flowers wilted within 4 days. The number of wilted cherelles was obtained from the number of wilted flowers older than 4 days (after 5 days ovary enlargement can be seen) plus the number of wilted fruits. Under the assumption that diseased fruits would have matured under healthy conditions, we calculated the sum of mature fruit as a sum of fruits harvested, plus diseased pods.

Dry cocoa yield was extrapolated from harvested fresh yield in two steps. In the first step, the fresh weight of individual healthy harvested pods was modelled separately for both experiments with the respective plant resource treatments and number of pods as explanatory variables. We used a linear mixed model with the random grouping variables plot and tree in plot to account for the non-independence of pods within tree and trees within plots. Only the number of pods per tree were found to have a significant effect on fruit weight (see results). An analysis on the combined pod yield data of both experiments showed no effect of experiment on slope or intercept of the relationship between individual pod fresh yield and number of pods on the tree, so the model estimates based on the combined data were used in the calculation. In the second step, the dry yield per tree was calculated from the number of pods per tree, correcting for the model-predicted, linear negative effect of number of pods per tree on yield per pod, as well as for the water content of the pods (dry weight = fresh weight/3.00; YC and GM, unpublished results).

We used linear mixed models to analyze the yield and the number of harvested pods, and generalized linear mixed models (GLMMs) for proportion data to analyze the fruit set relative to number of pollinated flowers and cherelle wilt relative to number of fruits set. The use of mixed models is made necessary by the presence of random variables, i.e. plots in the case of the throughfall displacement experiment and plots as well as subplots within plots in the case of the shade and fertilizer experiment. In addition, the maximum and restricted maximum-likelihood based estimation used in these models is robust to the unbalanced design caused by non-flowering trees. Proportion data can be handled by GLMMs without losing information on the number of cases, which is not the case when transforming the data (Crawley, 2002). We refer to Pinheiro and Bates (2000) and Bolker et al. (2009) for introductions to linear mixed models and GLMMs, respectively. Linear mixed models were fit using REML with the function *lme* from the package nlme (Pinheiro et al., 2008) and GLMMs with Laplace approximation with the function *lmer* from the package lme4 (Bates et al., 2008), both packages for R (R Development Core Team, 2008; Version 2.8.1.).

For the analyses of the shade and fertilizer experiment, we specified random intercepts for each of the 6 subplots, as nested random factors, i.e. plot and subplot within plot, caused failure to converge during model fitting. Fixed-effects were the main effects shade, fertilizer and pollination intensity as factors, as well as the two-way interactions between pollination intensity and the two resource variables. Number of open flowers (log-transformed) was included as a covariate.

For the analyses of the throughfall displacement experiment random intercepts were specified for each plot. Fixed-effects were the main effects rain interception and pollination intensity as

factors, and number of open flowers (log-transformed) was included as a covariate. Final models included interactions only when significant differences between levels were found.

## Results

The fresh weight per fruit did not differ between experimental resource treatments (all  $P > 0.30$ ), but decreased linearly with number of mature fruits on the tree both in the shade and fertilizer experiment (yield =  $134.21 - 0.987 \times N$  pods tree<sup>-1</sup>; df=27,  $P=0.056$ ) and in the throughfall displacement plots (yield =  $138.32 - 1.058 \times N$  pods tree<sup>-1</sup>; df=29,  $P=0.057$ ). In models based on the combined data from both experiments neither slope nor intercept significantly differed between experiments (likelihood-ratio test  $P=0.916$  and  $0.7216$ , respectively) and thus we used a single model (dry cocoa yield =  $135.76 - 0.993 \times N$  pods tree<sup>-1</sup>; df=52,  $P=0.0059$ ) to extrapolate yield per tree from number of pods harvested in analyses of both experiments (see below).

**Table 1**

Experiment manipulating pollination intensity (10%, 40%, 70% and 100% of open flowers), shade and nitrogen fertilization in *Theobroma cacao* stands: fixed-effects of mixed models explaining dry yield (a), number of harvested pods (b), relative fruit set (c) and relative fruit abortion (d) per tree. Error distribution normal (a) or binomial (b, c). For pollination intensity, successive difference contrasts are used. Abbreviations are PI: pollination intensity; S: shaded; Us: unshaded; F: fertilized; Uf: unfertilized.

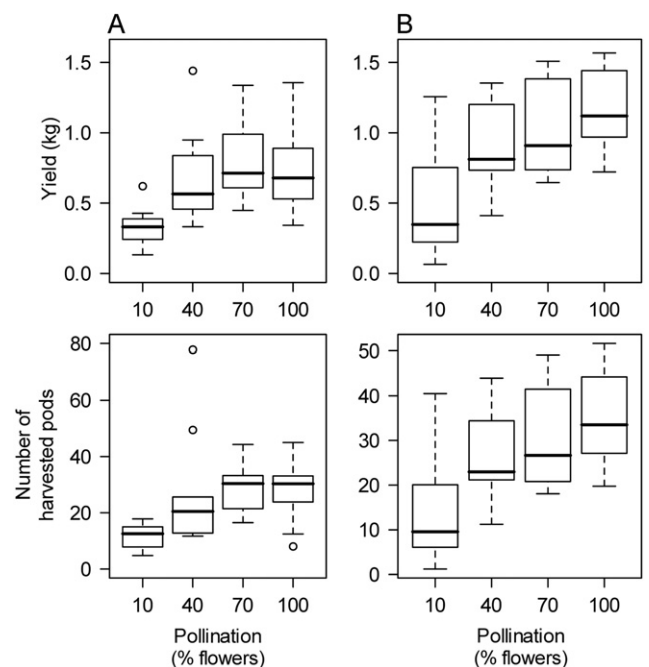
	Coefficient	SE	DF	<i>t</i>	<i>P</i>
<b>(a) Yield</b>					
Intercept	-560.45	233.54	34	-2.40	0.0220
Log( <i>N</i> open flowers)	196.91	45.09	34	4.37	0.0001
PI 40–10	330.12	109.35	34	3.02	0.0048
PI 70–40	153.48	110.44	34	1.39	0.1736
PI 100–70	-66.46	103.40	34	-0.64	0.5247
Shaded–unshaded	-76.67	75.73	34	-1.01	0.3185
Fertilized–unfertilized	72.95	149.57	4	0.49	0.6512
<b>(b) Harvested pods</b>					
Intercept	-39.27	9.87	34	-3.98	0.0003
Log( <i>N</i> open flowers)	10.51	1.95	34	5.39	< 0.0001
PI 40–10	15.18	5.56	34	2.73	0.0100
PI 70–40	2.06	5.60	34	0.37	0.7156
PI 100–70	-0.76	5.24	34	-0.15	0.8854
Shaded–unshaded	1.27	3.82	34	0.33	0.7425
Fertilized–unfertilized	-2.44	4.18	4	-0.58	0.5908
<b>(c) Relative fruit set</b>					
Intercept	2.01	0.38	-	5.26	< 0.0001
Log( <i>N</i> open flowers)	-0.22	0.04	-	-5.49	< 0.0001
PI 40–10	-0.31	0.15	-	-2.01	0.0442
PI 70–40	-0.13	0.09	-	-1.42	0.1551
PI 100–70	0.07	0.09	-	0.73	0.4613
Shaded–unshaded	0.06	0.07	-	0.80	0.4219
Fertilized–unfertilized	0.21	0.63	-	0.33	0.7442
<b>(d) Relative fruit abortion</b>					
Intercept	-0.48	0.36	-	-1.34	0.1794
Log( <i>N</i> open flowers)	0.01	0.06	-	0.13	0.8954
PI 40–10	0.05	0.17	-	0.31	0.7602
PI 70–40	-0.03	0.10	-	-0.34	0.7371
PI 100–70	0.35	0.11	-	3.15	0.0016
Shaded–unshaded	0.19	0.10	-	2.03	0.0425
Fertilized–unfertilized	-0.08	0.30	-	-0.25	0.8000
PI 40–10 × S/Us	-1.21	0.34	-	-3.59	0.0003
PI 70–40 × S/Us	1.25	0.20	-	6.25	< 0.0001
PI 100–70 × S/Us	-0.18	0.18	-	-1.03	0.3023
PI 40–10 × F/Uf	0.61	0.32	-	1.89	0.0582
PI 70–40 × F/Uf	-0.96	0.24	-	-4.02	0.0001
PI 100–70 × F/Uf	0.04	0.20	-	0.23	0.8193

In the shade and fertilizer experiment a total of 5527 cacao flowers were hand pollinated from 11,736 open flowers (OF) on 45 trees during a two-month period. A total of 1630 flowers wilted within 4 days after fertilization, 1236 wilted after 4 days, and 1561 resulted in wilted cherelles. Disease resulted in 164 dead pods, 38 pods died off due to unknown causes, and 370 pods were harvested. Number of open flowers did not differ between shade and fertilizer treatments ( $P > 0.20$ , results not shown).

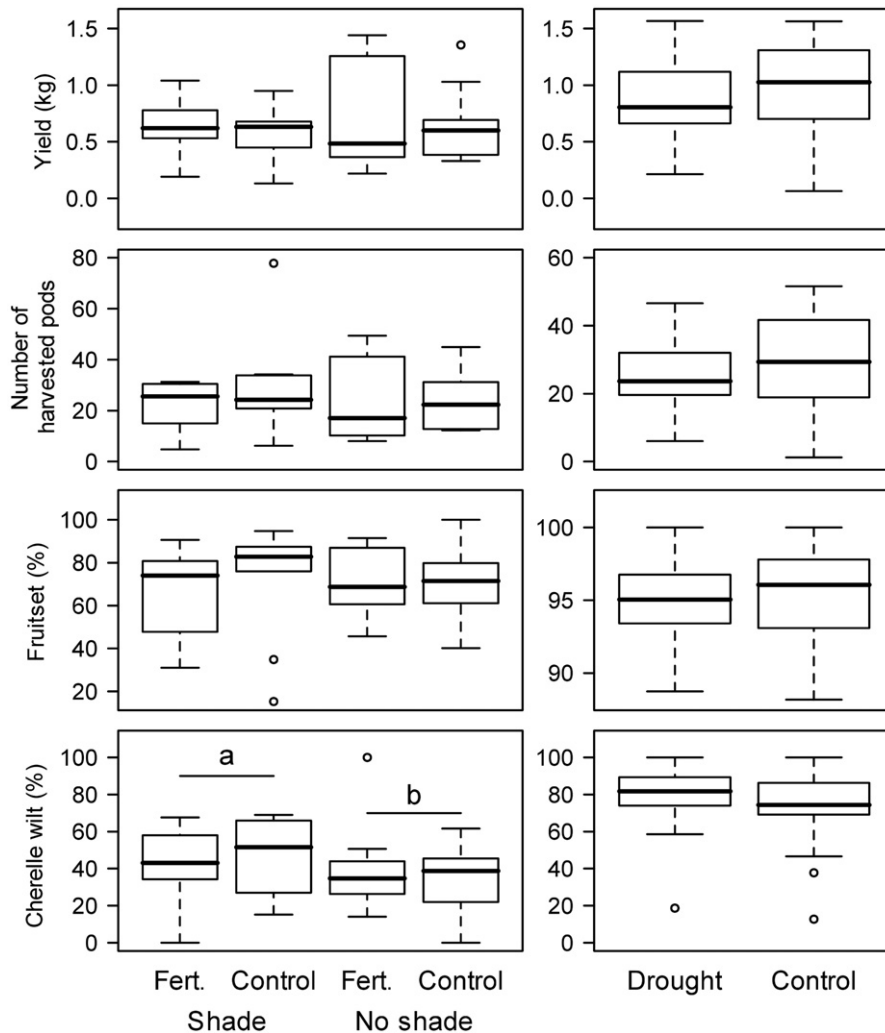
Dry yield increased significantly with number of open flowers ( $P=0.0001$ , Table 1) and was significantly higher in the 40% than the 10% pollination intensity treatments ( $P=0.0048$ ). The increase in pollination intensity from 40% through 70–100% did not markedly increase yield beyond values obtained with 40% pollination (all  $P > 0.1736$ ; Fig. 4, Table 1). Shade and fertilizer did not significantly affect yield ( $P=0.3185$  and  $0.6512$ , respectively; Fig. 5, Table 1). The same patterns were found for number of harvested pods (Fig. 5, Table 1). The analysis of relative fruit set (Table 1) shows that a decrease with number of open flowers on the tree ( $P < 0.0001$ ), but no differences between pollination intensity treatments (all  $P$  between 0.2041 and 0.2605) or shade and fertilizer treatments ( $P=0.4219$  and  $0.7442$ , respectively). Fruit set was somewhat higher in shaded than in unshaded trees at low pollination intensity (interaction:  $P=0.0442$ ).

Relative fruit abortion (cherelle wilt; Table 1) was significantly higher in shaded than in unshaded trees ( $P=0.0425$ ). Fruit abortion was higher at 100% than at 70% pollination intensity ( $P=0.0016$ ). Within the 40% pollination level, it was higher in shaded ( $P < 0.0001$ ) than in unshaded and lower in fertilized than in unfertilized trees ( $P=0.0001$ ), but the reversal of this tendency at higher pollination levels suggest this effect may be due to outliers, rather than to effects driven by pollination intensity.

In the throughfall displacement experiment a total of 5202 flowers were hand pollinated from 11,495 open flowers on 47 trees. A total of 381 flowers wilted within 4 days, 303 were recorded as wilted flowers older than 4 days, 3540 as wilted



**Fig. 4.** Dry cocoa yield and number of harvested pods in the shade and fertilizer experiment (A) and the drought experiment (B) as a function of pollination intensity, corrected for number of open flowers (set at 400 per tree) on the trees. The horizontal line in each box is the median, the boxes define the hinge (25–75% quartile), and the line is 1.5 times the hinge. Points outside this interval are represented as dots.



**Fig. 5.** Dry cocoa yield, number of harvested pods, relative fruit set and relative fruit abortion as a function of resource availability in the shade and fertilizer (left) and the drought experiment (right), corrected for number of open flowers (set at 400 per tree) on the trees. The horizontal line in each box is the median, the boxes define the hinge (25–75% quartile), and the line is 1.5 times the hinge. Points outside this interval are represented as dots.

cherelles, 347 as dead fruits due to disease, 27 as dead fruits due to unknown causes, and 496 were harvested healthy.

Dry yield increased significantly with number of open flowers ( $P < 0.0021$ ), and with pollination intensity, but as in the shade and fertilizer experiment, only the effect of the increase from 10% to 40% was significant ( $P = 0.0131$ ) (Table 2). There is little evidence for a drought-related reduction in yield ( $P < 0.4448$ ), and no evidence for an interaction between drought and pollination intensity, which was dropped from the final model. A similar pattern was found for number of harvested pods (Table 2). Relative fruit set increased with number of open flowers ( $P < 0.0001$ ), the opposite pattern to what was found in the shade and fertilizer experiment, and decreased with an increase in pollination intensity from 10% to 40% ( $P = 0.0181$ ), but did not differ between drought and control treatments ( $P = 0.6721$ ). Relative fruit abortion increased with number of open flowers ( $P < 0.0001$ ) and from 10% to 40% and 40% to 70% pollination intensity, respectively ( $P = 0.0001$ ). Values decreased again as pollination intensity was increased from 70% to 100%. In addition, relative fruit abortion was significantly higher in drought than in control plots ( $P = 0.0474$ ). The only significant interaction was a reversal of the drought effect, with a higher rate of fruit abortion in the control plots than in the drought plots in the 100% pollination trees when compared to the 70% pollination trees.

We used the data of the large, homogeneous drought experiment plantation to plot number of fruit set, number of fruits harvested, and dry yield against number of pollinated flowers with simple linear model fits superposed (Fig. 6). This shows both the relative effects of fruit set (Fig. 6A) and losses during fruit development (Fig. 6B, C), as well as the reduction in incremental increase in number of mature fruits and yield with additional pollinated flowers.

## Discussion

Proximate reasons for low fruit set and fruit abortion mainly fall into two categories: pollen limitation and resource limitation (Ayres and Whelan, 1989; Brown and McNeil, 2006; Coupland et al., 2006; Bos et al., 2007b). Haig and Westoby (1988) predict that pollen limitation should be higher if resources are not limiting, as was found by Galen et al. (1985) in the lily *Clintonia borealis* by means of a resource addition experiment. Fruit and seed set by the masting tree *Sorbus aucuparia* was not found to be increased by manual cross-pollination, which was assumed to be due to resource limitation (Pías and Guitián, 2006). A recent study showed contrasting results of pollen supplementation and fertilization between two alpine plant species, suggesting that

resource and pollinator limitation may depend on specific life-history characteristics (Burkle and Irwin, 2009).

Our experimental investigation of the relationship between fertilization, shading, water stress, pollination intensity and yield in the tropical tree crop *T. cacao* revealed that (1) pollination intensity and the intensity of flowering are the main determinants of number of mature pods and (2) resource treatments did not interact with pollination intensity effects. An increase in pollination from 10% to 40% flowers results in a yield increase of around 350 g dry cacao per tree, which at densities of 1000 trees per

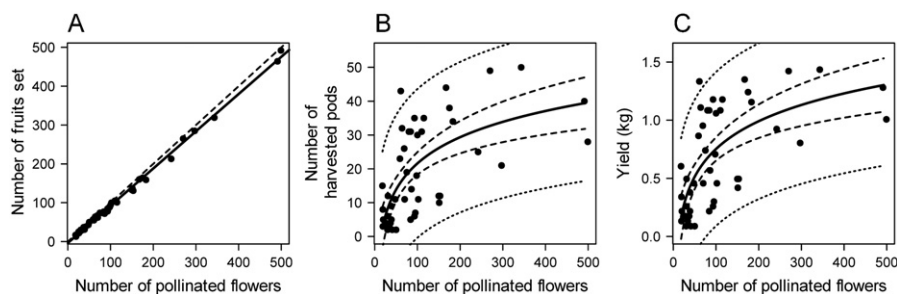
**Table 2**

Experiment manipulating pollination intensity (10%, 40%, 70% and 100% of open flowers) and water availability in *Theobroma cacao* stands: fixed-effects of mixed models explaining dry yield (a), number of harvested pods (b), relative fruit set (c) and relative fruit abortion (d) per tree. Error distribution was normal (a) and binomial (b, c). For pollination intensity, successive difference contrasts are used. Abbreviations are PI: pollination intensity; D: drought; C: control.

	Coefficient	SE	DF	t	P
<b>(a) Yield</b>					
Intercept	−922.34	477.45	37	−1.97	0.0611
Log(N open flowers)	303.65	91.60	37	3.36	0.0021
PI 40–10	392.90	150.67	37	2.61	0.0131
PI 70–40	122.89	140.48	37	0.88	0.3873
PI 100–70	138.98	135.29	37	0.99	0.3110
Drought–control	−127.44	150.51	4	−0.85	0.4448
<b>(b) Harvested pods</b>					
Intercept	−32.62	15.65	37	−2.08	0.0441
Log(N open flowers)	9.83	3.00	37	3.27	0.0023
PI 40–10	12.48	5.02	37	2.49	0.0176
PI 70–40	3.96	4.68	37	0.84	0.4038
PI 100–70	4.39	4.52	37	0.97	0.3376
Drought–control	−4.37	4.81	4	−0.91	0.4153
<b>(c) Relative fruit set</b>					
Intercept	−0.80	0.53	−	−1.51	0.1304
Log(N open flowers)	0.63	0.10	−	6.44	< 0.0001
PI 40–10	−0.67	0.29	−	−2.36	0.0181
PI 70–40	0.34	0.16	−	2.07	0.0382
PI 100–70	−0.25	0.14	−	−1.86	0.0624
Drought–control	−0.07	0.17	−	−0.42	0.6721
<b>(d) Relative fruit abortion</b>					
Intercept	−2.53	0.40	−	−6.30	< 0.0001
Log(N open flowers)	0.63	0.07	−	8.58	< 0.0001
PI 40–10	0.53	0.14	−	3.86	0.0001
PI 70–40	0.65	0.12	−	5.58	< 0.0001
PI 100–70	−0.51	0.10	−	−5.12	< 0.0001
Drought–control	0.35	0.18	−	1.98	0.0474
PI 40–10 × D–C	0.02	0.26	−	0.09	0.9254
PI 70–40 × D–C	0.29	0.23	−	−1.29	0.1958
PI 100–70 × D–C	−1.05	0.20	−	−5.35	< 0.0001

hectares, translates to 350 kg per hectare, which is between 30% and 100% of current smallholder yields in the highlands of Central Sulawesi (Clough, unpublished). Even though an increase in pollination beyond 40% did not result in any significant increases, these differences clearly indicate that natural pollination levels in cacao (around 10% of all flowers on a tree; Falque et al., 1995; Bos et al., 2007b; de Almeida and Valle, 2007) are likely to fall very short of the pollination optimum. Our data suggest the pollination optimum is beyond 40% of the flowers. A caveat of the study is that a whole-tree pollination study was not possible, given the size of the trees, and that possible differences in pollination success and cherelle wilt between flowers above and below 2 m make upscaling difficult. But despite not having covered the whole range of pollination intensity at the tree-scale, the increases in harvested pods, and consequently yield, due to hand pollination are very large even at the tree- or plantation-scale, as mentioned above, which contrasts with results obtained in other studies. Valle et al. (1990) and Bos et al. (2007b), in a whole and partial tree hand pollination study, respectively, found that pollinating all flowers on cacao trees did not result in increased yield due to cherelle wilt, i.e. the abortion of young cacao pods. We chose a somewhat different approach by varying the intensity of pollination along a gradient from 10% to 100%. In combination with the naturally high variability of flowering intensity in cacao, we obtained a gradient in the number of pollinated flowers, which is more appropriate to study processes expected to be non-linear (Fig. 2). A large percentage of the pollinated flowers set fruit (Figs. 5, 6A) and while fruit set decreased with pollination intensity, the overall percentage of wilted flowers as well as the impact on final number of fruits was minimal. Cherelle wilt on the other hand is known as the major physiological yield-reducing “disorder” (McKelvie, 1956, 1960; Valle et al., 1990; Bos et al., 2007b), and is seen as the mechanism with which trees adapt the number of maturing pods to resource availability (Valle et al., 1990). Cherelle wilt, relative to the number of fruit set, increased with high pollination intensity, thereby offsetting part of the benefits of enhanced pollination as assessed at the fruit-setting stage (Fig. 6). In contrast to the studies mentioned above, we found the offset to be only partial: the benefits of successive increases in hand pollination intensity erode beyond 40%, but number of pods and yield remained much higher than when only 10% of the flowers were pollinated.

As highlighted by Burkle and Irwin (2009), studies on wild species show that there are species for which pollinator availability is more important than resource availability in determining reproductive success (Mattila and Kuitunen, 2000) as well as vice versa (Ne’eman et al., 2006; Burkle and Irwin, 2009). In our study, drought and nitrogen fertilizer treatments did not have any measurable effects on fruit set, cherelle wilt or number of harvestable pods of cacao, and there was little



**Fig. 6.** Fruit set before cherelle wilt (A), number of harvested pods (B) and yield (C) as a function of number of pollinated flowers in *Theobroma cacao* (data from drought experiment site). Fitted models are: (A) bold line is model fit:  $y = -3.52 + 0.96 \times x$  ( $R^2 = 0.99$ ,  $P < 0.0001$ ), broken line indicates slope=1; (B)  $y = -29.84 + 11.17 \times \log(x)$  ( $R^2 = 0.45$ ,  $P < 0.0001$ ); (C)  $y = -0.83 + 0.34 \times \log(x)$  ( $R^2 = 0.46$ ,  $P < 0.0001$ ),  $N = 47$  for all three models. Outer broken line is tolerance interval in which 95% of the population is expected to fall, inner broken line 95% confidence interval for model predictions.

evidence for interaction between pollination and resource treatments. Several authors showed an increase in yield under N-fertilization (Murray, 1958; Cunningham and Arnold, 1962; Ahenkorah et al., 1987; Edwin and Masters, 2005) in this species. On poor soils in Ghana, fertilization (however with P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O<sub>2</sub>) increased yield by 62% (FAO 2005). Furthermore, cherele wilt is probably triggered by nutrient deficiency (Valle et al., 1990), according to Bos et al. (2007a) by a lack of nitrogen. Thus, we expected higher yields from nitrogen fertilized plots than from unfertilized plots, especially in interaction with pollination intensity. That this was not the case may be due to the fact that soils in the study region are very fertile. In a study conducted at comparable sites in Central Sulawesi the measured nitrogen soil stock was 9900 kg ha<sup>-1</sup>; losses due to leaching accounted for 16.2 kg ha<sup>-1</sup> a<sup>-1</sup> and those due to harvest for 57 kg ha<sup>-1</sup> a<sup>-1</sup> (Dechert et al., 2005). The authors suggested that biological nitrogen fixation lead to an input of ~71 kg N ha<sup>-1</sup> a<sup>-1</sup>. Thus, even without N-fixating shade trees, as on our plots, an additional input of nitrogen may at this point in time have no effect, because N availability is not yet limiting. The differences in nutrient content of the soils and other variables such as climate, cacao variety and number of replicates may explain the contrast to the results of Valle et al. (1990) and Bos et al. (2007a), and thus the differences in outcome may indicate the existence of condition-dependent pollination optima beyond the range of resource availability included in this study. In addition, it is known from wild plant species that respective importance of pollination and resource availability may vary between populations (Eppley, 2005), which suggests that the identity of cacao variety, which could not be investigated further in this study, may also play a role in this system.

In the drought experiment it was hypothesized that those trees under water stress would undergo nutrient shortage due to inhibited uptake and, thus, produce less pods. Drought-related cacao yield reductions as high as 38% of their normal level have been reported by farmers in the area for the years 1997 and 2002 (Keil et al., 2008). Despite a successful reduction of the water availability for cacao in the soil (Schwendenmann et al., 2010) we found only a slight trend towards a reduction of number of harvested pods in drought plots when compared to controls (Fig. 5, Table 1). Whole-plot data (Schwendenmann et al., 2010) show that drought reduces yield, but the effect may not have been strong enough to be detectable on the 47 focal trees used in this analysis.

The response to resource availability may differ between perennial plants, including trees, and annuals. Woody perennials may be strongly dependent on nutrients, light and water but may store these resources instead of directly allocating these resources to reproduction. Also, only long-term effects of increased pollination may be sufficient to make interactions between pollination availability and resources apparent. Ehrlén and Eriksson (1995) found that pollen supplementation increased seed production of the perennial herb *Lathyrus verna* but plants were smaller and produced fewer flowers in the next year relative to control plants. A central question that remains to be answered is thus for how long increased cacao yield can be sustained under supplemented pollination, as our study only covered one growing season.

While manipulation of water, nitrogen and light availability had no or only minor effects on number of cacao pods harvested and yield, we found that enhancing pollination intensity from 10% to 40% may result in yield increases up to 100%, independently of resource availability. The results of our study suggest that for this tree crop species (i) pollination is a very important factor in determining yield, contrary to what has been found previously; (ii) the pollination optimum may depend on site and cacao variety, but is not reached in nutrient-rich cacao growing regions

under natural pollination levels; and (iii) even small improvements in the pollination service by cacao pollinators, i.e. mainly ceratopogonid midges (Soetardi, 1950; Groeneveld, unpublished) whose ecology is poorly known due to their very small size and low densities (e.g. Glendenning, 1972), could result in major increases in cacao yield. These results are interesting, firstly because experimental manipulations of both plant resources and pollination levels have never been reported for crop species before, and secondly because they suggest that pollination ecosystem services have been underestimated. This highlights the need for more joint studies on pollen and resource limitation, as well as on the effect of changes in management intensity and landscape configuration in human-dominated landscapes on pollination (Klein et al., 2007; Kremen et al., 2007).

## Acknowledgements

We thank Mardi, Baswan, Aco, Gayus, Sahrim, Sadryk, Nining and Team Kakao for assistance during field work and acknowledge S. Andirahman, M. Grosse, W. Lorenz, S. Tarigan at the coordination offices of STORMA in Germany and Indonesia and counterparts D. Buchori and A. Anshary for their kind support, and A.-M. Klein, J. Nason and an anonymous reviewer for helpful comments on a previous version of the manuscript. The study was conducted within the DFG-funded project STORMA ("Stability of Rainforest Margins in Indonesia"; SFB-552, subproject C3). JG was funded by DAAD with a scholarship during field work.

## References

- Ackerman, J.D., Montalvo, A.M., 1990. Short- and long-term limitations to fruit production in a tropical orchid. *Ecology* 71, 263–272.
- Ahenkorah, Y., Halm, B., Appiah, M., Akrofi, G., Yirenykyi, J., 1987. Twenty years' results from a shade and fertilizer trial on amazon cocoa (*Theobroma cacao*) in Ghana. *Exp. Agric.* 23, 31–39.
- Aizen, M.A., Harder, L.D., 2007. Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. *Ecology* 88, 271–281.
- Ashman, T.L., Knight, T.M., Steets, J.A., Amarasekare, P., Burd, M., Campbell, D.R., Dudash, M.R., Johnston, M.O., Mazer, S.J., Mitchell, R.J., Morgan, M.T., Wilson, W.G., 2004. *Ecology* 85, 2408–2421.
- Ayre, D.J., Whelan, R.J., 1989. Factors controlling fruit set in hermaphroditic plants: studies with the Australian Proteaceae. *Trends Ecol. Evol.* 4, 267–272.
- Bates, D., Maechler, M., Bin Dai, 2008. lme4: Linear mixed-effects models using Eigen and R. R package version 0.999375-28 <<http://lme4.r-forge.r-project.org/>>.
- Beer, J., 1988. Litter production and nutrient cycling in coffee (*Coffea arabica*) or cacao (*Theobroma cacao*) plantations with shade trees. *Agroforest. Syst.* 7, 103–114.
- Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.-S.S., 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* 24, 127–135.
- Bookman, S., 1983. Costs and benefits of flower abscission and fruit abortion in *Asclepias speciosa*. *Ecology* 64, 264–273.
- Bos, M.M., Steffan-Dewenter, I., Tschardtke, T., 2007a. Shade tree management affects fruit abortion, insect pests and pathogens of cacao. *Agric. Ecosyst. Environ.* 120, 201–205.
- Bos, M.M., Veddeler, D., Bogdanski, A.K., Klein, A.-M., Tschardtke, T., Steffan-Dewenter, I., Tylianakis, J.M., 2007b. Caveats to quantifying ecosystem services: fruit abortion blurs benefits from crop pollination. *Ecol. Appl.* 17, 1841–1849.
- Brookes, R.M., Jesson, L.K., Burd, M., 2008. A test of simultaneous resource and pollen limitation in *Styloidium armeria*. *New Phytol.* 179, 557–565.
- Brown, A.O., McNeil, J.N., 2006. Fruit production in Cranberry (*Vaccinium macrocarpon*): a bet-hedging strategy to optimize reproductive effort. *Am. J. Bot.* 93, 910–916.
- Burd, M., 1988. "Excess" flower production and selective fruit abortion: a model of potential benefits. *Ecology* 79, 2123–2132.
- Burkle, L.A., Irwin, R.E., 2009. The effects of nutrient addition on floral characters and pollination in two subalpine plants, *Ipomopsis aggregata* and *Linum lewisii*. *Plant Ecol.* 203, 83–98.
- Campbell, D.R., Halama, K.J., 1993. Resource and pollen limitations to lifetime seed production in a natural plant population. *Ecology* 74, 1043–1051.
- Charlesworth, D., 1989. Evolution of low female fertility in plants: pollen limitation, resource allocation and genetic load. *Trends Ecol. Evol.* 4, 289–292.

- Coupland, G.T., Paling, E.I., McGuinness, K.A., 2006. Floral abortion and pollination in four species of tropical mangroves from northern Australia. *Aquat. Bot.* 84, 151–157.
- Crawley, M.J., 2002. *Statistical Computing. An Introduction to Data Analysis using S-Plus*. Wiley, Chichester, UK.
- Cunningham, R., Arnold, P., 1962. The shade and fertiliser requirements of cocoa (*Theobroma cacao*) in Ghana. *J. Sci. Food Agric.* 13, 213–221.
- Cunningham, R., Lamb, J., 1958. Cocoa shade and manurial experiment in Ghana. *Nature* 182, 119.
- de Almeida, A.-A., Valle, R.R., 2007. Ecophysiology of the cacao tree. *Braz. J. Plant Physiol.* 19, 25–448.
- Dechert, G., Veldkamp, E., Brumme, R., 2005. Are partial nutrient balances suitable to evaluate nutrient sustainability of land use systems? Results from a case study in Central Sulawesi, Indonesia. *Nutr. Cycl. Agroecosyst.* 72, 201–212.
- Edwin, J., Masters, W., 2005. Genetic improvement and cocoa yields in Ghana. *Exp. Agric.* 41, 491–503.
- Egbe, N., Adenikinju, S., 1990. Effect of intercropping on potential yield of cacao in South Western Nigeria. *Café, Cacao, Thé* 34, 281–284.
- Ehrlén, J., 1992. Proximate limits to seed production in a herbaceous perennial legume, *Lathyrus vernus*. *Ecology* 73, 1820–1831.
- Ehrlén, J., Eriksson, O., 1995. Pollen limitation and population growth in a herbaceous perennial legume. *Ecology* 76, 652–656.
- Eppley, S.M., 2005. Spatial segregation of the sexes and nutrients affect reproductive success in a dioecious windpollinated grass. *Plant Ecol.* 18, 1179–1190.
- Falque, M., Vincent, A., Vaissière, B.E., Eskes, A.B., 1995. Effect of pollination intensity on fruit and seed set in cacao (*Theobroma cacao* L.). *Sex. Plant Reprod.* 8, 354–360.
- FAO, 2005. *Fertilizer use by crop in Ghana. Technical Report, Food and Agricultural Organization of the United Nations*, Rome, Italy.
- Galen, C., Plowright, R.C., Thomson, J.D., 1985. Floral biology and regulation of seed set and seed size in the lily, *Clintonia borealis*. *Am. J. Bot.* 10, 1544–1552.
- Ghazoul, J., 2005. Buzziness as usual: questioning the global pollination crisis. *Trends Ecol. Evol.* 20, 367–373.
- Glendinning, D.R., 1972. Natural pollination of cocoa. *New Phytol.* 71, 719–729.
- Haig, D., Westoby, M., 1988. On limits to seed production. *Am. Nat.* 131, 757–759.
- Horvitz, C.C., Schemske, D.W., 1988. A test of the pollinator limitation hypothesis for a neotropical herb. *Ecology* 69, 200–206.
- Johns, N.D., 1999. Conservation in Brazil's chocolate forest: the unlikely persistence of the traditional cocoa agroecosystem. *Environ. Manage.* 23, 31–47.
- Keil, A., Zeller, M., Wida, A., Sanim, B., Birner, R., 2008. What determines farmers' resilience towards ENSO-related drought? An empirical assessment in Central Sulawesi, Indonesia. *Climatic Change* 86, 291–307.
- Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. *Proc. R. Soc. London B: Biol.* 274, 303–313.
- Kremen, C., Williams, N.M., Aizen, M.A., Gemmill-Herren, B., Lebuhn, G., Minckley, R., Packer, L., Potts, S.G., Roulston, T., Steffan-Dewenter, I., Vazquez, D.P., Winfree, R., Adams, L., Crone, E.E., Greenleaf, S.S., Keitt, T.H., Klein, A., Regetz, J., Ricketts, T., 2007. Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land use change. *Ecol. Lett.* 10, 299–314.
- Maciel Sousa, R., de Sousa Aguiar, O., Magalhães Freitas, B., Abreu da Silveira Neto, A., Fladner Costa Pereira, T., 2009. Requerimentos de Polonização do Meloeiro (*Cucumis melo*). *Rev. Caatinga* 22, 238–242.
- Mattila, E., Kuitunen, M.T., 2000. Nutrient versus pollination limitation in *Platanthera bifolia* and *Dactylorhiza incarnata* (Orchidaceae). *Oikos* 89, 360–366.
- McKelvie, A., 1956. Cherrille wilt of cacao: I. Pod development and its relation to wilt. *J. Exp. Bot.* 7, 252–263.
- McKelvie, A., 1960. Cherrille wilt of cacao: II. Wilt in relation to yield. *J. Exp. Bot.* 11, 413–424.
- Murray, D., 1958. Response of cacao to fertilizers. *Nature* 182, 1613.
- Ne'eman, G., Ne'eman, R., Ellison, A.M., 2006. Limits to reproductive success of *Sarracenia purpurea* (Sarraceniaceae). *Am. J. Bot.* 93, 1660–1666.
- Nicodemo, D., Nogueira Couto, R.H., Braga Malheiros, E., De Jong, D., 2009. Honey bees as an effective pollinating agent of pumpkin. *Sci. Agric.* 66, 476–480.
- Pías, B., Guitián, P., 2006. Breeding system and pollen limitation in the masting tree *Sorbus aucuparia* L. (Rosaceae) in the NW Iberian Peninsula. *Acta Oecol.* 29, 97–109.
- Pinheiro, J., Bates, D., 2000. *Mixed-effects Models in S and S-Plus*. Statistics and Computin Series, Springer-Verlag, New York.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., the R Core Team, 2008. nlme: Linear and Nonlinear Mixed Effects Models. R package, version 3.1-90.
- R Development Core Team, 2008. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 3-900051-07-0, URL <http://www.R-project.org>.
- Ricketts, T.H., Daily, G.C., Ehrlich, P.R., Michener, C., 2004. Economic value of tropical forest to coffee production. *Proc. Natl. Acad. Sci. USA* 101, 12579–12582.
- Schwendenmann, L., Veldkamp, E., Moser, G., Hölscher, D., Anas, I., Clough, Y., Djajakirana, G., Erasmí, S., Hertel, D., Köhler, M., Leitner, D., Leuschner, C., Michalzik, B., Propastin, P., Tjoa, A., Tscharntke, T., van Straaten, O., 2010. Effects of an experimental drought on the functioning of a cacao agroforestry system, Sulawesi, Indonesia. *Global Change Biol.* Published Online: 21 July, 2009, doi:10.1111/j.1365-2486.2009.02034.x.
- Soetardi, R.G., 1950. The importance of insects in the pollination of *Theobroma cacao*. *Archief Koffiecultuur Indonesie* 17, 3–31.
- Steffan-Dewenter, I., Kessler, M., Barkmann, J., Bos, M., Buchori, D., Faust, H., Gerold, G., Glenk, K., Gradstein, S.R., Guhardja, E., Harteveld, M., Hertel, D., Hoehn, P., Kappas, M., Köhler, S., Leuschner, C., Maertens, M., Marggraf, R., Migge-Kleian, S., Mogeia, J., Pitopang, R., Schaefer, M., Schwarze, S., Sporn, S.G., Steingrebe, A., Tjitrosoedirdjoet, S.S., Tjitrosoemito, S., Twele, A., Weber, R., Woltmann, L., Zeller, M., Tscharntke, T., 2007. Tradeoffs between income, biodiversity, and ecosystem functioning during tropical rainforest conversion and agroforestry intensification. *Proc. Natl. Acad. Sci. USA* 104, 4973–4978.
- Stephenson, A.G., 1981. Flower and fruit abortion: proximate causes and ultimate functions. *Annu. Rev. Ecol. Syst.* 12, 253–279.
- Sutherland, S., 1986. Patterns of fruit-set: what controls fruit-flower ratios in plants? *Evolution* 40, 117–128.
- Sutherland, S., Delph, L.F., 1984. On the importance of male fitness in plants: patterns of fruits-set. *Ecology* 65, 1095–1104.
- Torres, C., Galetto, L., 1999. Factors constraining fruit set in *Mandevilla pentlandiana* (Apocynaceae). *Bot. J. Linn. Soc.* 129, 187–205.
- Udovic, D., 1981. Determinants of fruit set in *Yucca whipplei*: reproductive expenditure vs. pollinator availability. *Oecologia* 48, 389–399.
- Valle, R.R., Almeida, A.-A.F., de O. Leite, R.M., 1990. Energy costs of flowering, fruiting, and cherrille wilt in cacao. *Tree Physiol.* 6, 329–336.
- Vaughton, G., 1991. Variation between years in pollen and nutrient limitation of fruit-set in *Banksia spinulosa*. *J. Ecol.* 78, 389–400.
- Veddeler, D., Olschewski, R., Tscharntke, T., Klein, A.-M., 2008. The contribution of non-managed social bees to coffee production: new economic insights based on farm-scale yield data. *Agroforest. Syst.* 73, 109–114.
- Young, A.M., 1982. Effects of shade cover and availability of midge breeding sites on pollinating midge populations and fruit set in two cocoa farms. *J. Appl. Ecol.* 19, 47–63.
- Young, A.M., 1994. *The Chocolate Tree: A Natural History of Cacao*. Smithsonian Institution Press, Washington and London.
- Zuidema, P., Leffelaar, P., Gerritsma, W., Mommer, L., Anten, N., 2005. A physiological production model for cacao (*Theobroma cacao*): model presentation, validation and application. *Agric. Syst.* 84, 195–225.