

# *Insect pollination reduces yield loss following heat stress in faba bean (*Vicia faba* L.)*

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2 Insect pollination reduces yield loss following heat stress in faba bean (*Vicia faba* L.).

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20

21

## **Abstract**

22 Global food security, particularly crop fertilization and yield production, is threatened by heat waves  
23 that are projected to increase in frequency and magnitude with climate change. Effects of heat stress  
24 on the fertilization of insect-pollinated plants are not well understood, but experiments conducted  
25 primarily in self-pollinated crops, such as wheat, show that transfer of fertile pollen may recover yield  
26 following stress. We hypothesized that in the partially pollinator-dependent crop, faba bean (*Vicia*  
27 *faba* L.), insect pollination would elicit similar yield recovery following heat stress. We exposed  
28 potted faba bean plants to heat stress for 5 days during floral development and anthesis. Temperature  
29 treatments were representative of heat waves projected in the UK for the period 2021-2050 and  
30 onwards. Following temperature treatments, plants were distributed in flight cages and either  
31 pollinated by domesticated *Bombus terrestris* colonies or received no insect pollination. Yield loss  
32 due to heat stress at 30°C was greater in plants excluded from pollinators (15%) compared to those  
33 with bumblebee pollination (2.5%). Thus, the pollinator dependency of faba bean yield was 16% at  
34 control temperatures (18 to 26°C) and extreme stress (34°C), but was 53% following intermediate  
35 heat stress at 30°C. These findings provide the first evidence that the pollinator dependency of crops  
36 can be modified by heat stress, and suggest that insect pollination may become more important in  
37 crop production as the probability of heat waves increases.

38 Keywords: Faba bean; heat stress; pollination; climate change; yield stability; yield variability.

39

## **1 Introduction**

40 The Intergovernmental Panel on Climate Change projects that crop production and food security will  
41 be increasingly threatened this century due in part to increased climate variability, including the  
42 increased frequency and magnitude of heat waves (Kirtman et al., 2013; Porter et al., 2014;  
43 Seneviratne et al., 2012). Especially large yield losses can occur when high temperatures cause  
44 damage during crop floral development and anthesis (Hedhly, 2011; Luo, 2011), as many crop  
45 products (*e.g.* fruits, grains) are the direct result of successful fertilization. Insect pollinated crops  
46 constitute approximately a third of global food production (Klein et al., 2007), but there is no  
47 comprehensive evidence of how their fertilization may be affected by heat stress. Studies in these  
48 crops have typically measured the effect of heat stress in absence of insect pollinators (Peet et al.,  
49 1998; Young et al., 2004), potentially missing important changes in the interactions between plants  
50 and their pollinators following stress. Studies have shown that the yield of plants can be partially  
51 recovered following stress by hand provision of fertile pollen, in tomato (*Solanum lycopersicum*)  
52 (Peet et al., 1998), oilseed rape (*Brassica napus*) (Young et al., 2004), common bean (*Phaseolus*  
53 *vulgaris*) (Gross and Kigel, 1994; Monterroso and Wien, 1990) and wheat in (*Triticum aestivum*)  
54 (Briggs et al., 1999; Saini and Aspinall, 1982). Insect pollinators may promote similar yield resilience  
55 to heat stress in entomophilous crops, through their role as pollen vectors between flowers. Such a  
56 resilience mechanism is possibly an unexpected and unquantified benefit of insect pollination, which  
57 has already been estimated to be worth \$232-\$577 billion each year globally (Lautenbach et al., 2012)  
58 due to increases in total crop production of 3-8% (Aizen et al., 2009). This is pertinent at a time when

59 the threats of climate change to insect pollinator communities are becoming apparent (Carvalho et  
60 al., 2013).

61 This study investigates interactions between heat stress and insect pollination on the yield of faba  
62 bean (*Vicia faba* L.). In faba bean, vulnerability to heat stress varies between stages of floral  
63 development (Bennell et al., 2007). Therefore, heat stress at a given time point could damage some  
64 flowers while others remain undamaged through differences in the timing of their development,  
65 providing a source of fertile pollen. In a typical faba bean crop, a proportion of pollination is by  
66 spontaneous auto-fertilization, while the remainder requires an insect visit (e.g. Chen, 2009).  
67 Following heat stress however, all flowers with damaged pollen would effectively be male-sterile and  
68 unable to self-pollinate (Drayner, 1959). Yield in these flowers would therefore become more  
69 dependent upon the transfer of fertile pollen by insect pollinators (yield recovery by outcrossing). The  
70 pollinator dependency of faba bean can be influenced by many factors including cultivar and location  
71 (e.g. Suso et al., 2001), but under typical non-stress conditions approximately 25% of faba bean yield  
72 is dependent upon insect pollination (Ghamdi and Ghamdi, 2003; Somerville, 1999). Across the  
73 majority of Europe, the most common insect pollinators of faba bean are wild bumblebees (Carré et  
74 al., 2009; Free, 1993), populations of which are projected to undergo large distribution shifts due to  
75 climate change (Kerr et al., 2015; Rasmont et al., 2015). Faba bean is already a globally important  
76 grain legume (FAO, 2015) and demand for it is likely to increase with increasing recognition of the  
77 beneficial role of faba bean in sustainable cropping (Köpke and Nemecek, 2010), the rising  
78 requirements for plant protein for both human and animal nutrition (Tilman et al., 2011), and recent  
79 policy changes that encourage multiple cropping in Europe (European Parliament News, 2013).

80 Using a novel experimental approach replicated over three years, we exposed potted winter faba bean  
81 plants (cultivar Wizard) to five-day temperature treatments before moving them to flight cages to be  
82 either pollinated by domesticated bumblebee colonies, or to receive no insect pollination, in order to  
83 evaluate the following hypotheses: 1) pollination by *Bombus terrestris* reduces yield mass losses  
84 following heat stress in faba bean; 2) pollination by *Bombus terrestris* reduces losses in faba bean  
85 quality (e.g. mass per bean, protein content) following heat stress; 3) observed changes in yield can be  
86 attributed to changes in fertilization (e.g. bean number) following insect pollination.

## 87 **2 Methods**

### 88 **2.1 Experimental design and growing conditions**

89 Experiments were conducted over three growing seasons from 2012 to 2014 at the Plant Environment  
90 Laboratory (now succeeded by the Crop and Environment Laboratory), University of Reading, UK.  
91 All experimentation (Table 1) was designed to test whether insect pollination modifies the response of  
92 potted winter faba bean (*Vicia faba* L.) to heat stress during floral development and anthesis. Plants  
93 were exposed to temperature treatments for five days during early flowering (Table S1,  
94 Supplementary Material) and subsequently moved to flight cages where they were either exposed to a  
95 colony of domesticated bumblebees or received no insect pollination.

96 We used the synthetic cultivar, Wizard (Wherry & Sons Ltd), a UK recommended list commercial  
97 cultivar since 2003 (PGRO, 2015). Plants were randomly assigned to temperature treatments and  
98 flight cages in all experiments. All experimental plants were grown in plastic pots (180mm diameter;  
99 4l volume) containing vermiculite, sand, gravel and compost at a ratio of 4:2:4:1, mixed with 2kg m<sup>-3</sup>  
100 Osmocote slow-release granules (LBS Horticulture Ltd). Three seeds were sown per pot, allowing  
101 thinning to one plant per pot when 3 leaf pairs had unfolded on the majority of plants. Plants were  
102 maintained in a fully enclosed polytunnel until on average 4 leaf pairs had unfolded on each plant,  
103 when they were moved and randomly distributed either in the open (2012) or within flight cages  
104 (2013 and 2014) until temperature treatments. Plants were watered to maintain field capacity  
105 throughout experiments including during temperature treatments, at least daily by hand watering in  
106 2012, and drip-irrigation in 2013 and 2014. Three consecutive replicate experiments were conducted  
107 in 2013 over a period of 18 days (Table 1), and plants were manually assigned to replicates to  
108 standardise developmental stage.

## 109 **2.2 Temperature Treatment**

110

111 Five temperature treatments (18/10, 22/14, 26/18, 30/22, 34/26°C day/night temperature) were chosen  
112 to measure responses over a wide range of potential temperature anomalies, and because there was no  
113 prior information about heat stress vulnerability of faba bean. Temperature treatments 26, 30 and  
114 34°C were intended to represent heat wave scenarios that are projected to be common during the  
115 period 2021-2050 in the UK and Western Europe (Fischer and Schär, 2010), with 30 and 34°C in  
116 particular representing levels of stress that may occur through combinations of high temperatures and  
117 reduced soil moisture (Alghabari et al., 2014; Lobell et al., 2011). All treatments comprised  
118 transferring plants from flight cages at midday to five 1.37 x 1.47 m<sup>2</sup> Saxcil growth cabinets for a  
119 duration of five days during early flowering (Table S1, Supplementary Material). The photoperiod  
120 lasted 16 h and the transition between night and day temperatures took approximately 15 minutes.  
121 Conditions were monitored throughout temperature treatments; light levels were maintained at 650  
122  $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ ; relative humidity was 87±13 % in 2012, 80±20 % in 2013 and 85±15 % in  
123 2014; and CO<sub>2</sub> was 385 mg L<sup>-1</sup>. Temperature was measured by a thermocouple at pot height. Growth  
124 cabinet temperatures were randomly reassigned between years and during 24 h between replicate  
125 experiments in 2013.

126

## 127 **2.3 Pollination Treatment**

128

129 Following temperature treatments, plants were moved to flight cages (Table 1) which were used to  
130 either retain single domesticated colonies of *Bombus terrestris audax* L. (a common wild visitor of  
131 faba bean in the field; Garratt et al., 2014) that were applied following temperature treatments, or to  
132 completely prevent visits from insect pollinators. While this method does not represent a typical  
133 pollinator community visiting faba bean in the field, it enables a controlled comparison between  
134 pollination treatments without confounding effects of bagging that could otherwise modify plant  
135 growth and yield accumulation in excluded plants (Free, 1993). All cages were custom-made

136 (Lancashire Sports Repair) from 1.33 mm<sup>2</sup> aperture polyethylene mesh (WM16, Wondermesh). In  
137 each year, all treatment cages were within an area of 12.5 x 5 m. Following common practise in  
138 reciprocal outcrossing experiments (e.g. Saini and Aspinall, 1982), experimental plants were housed  
139 with non-stressed pollen donor individuals to ensure provision of fertile pollen. The ratio of pollen  
140 donor to experimental plants was 3:1 in 2012, but was later reduced to 1:1 following an additional  
141 experiment which demonstrated this was a sufficient ratio to achieve good pollination (data not  
142 shown). Experimental plants that had been exposed to different temperatures were housed together in  
143 the same flight cage; thus maintaining the validity of temperature treatment comparisons. In 2013,  
144 flight cages were repeatedly allocated to the same pollination treatment across the three replicate  
145 experiments, but were analysed as independent replicates because a new *B. terrestris* colony was used  
146 each time. To standardise timing of pollinator exposure across all experiments, in 2013 the pollination  
147 treatment plants assigned to the third replicate experiment were held in the exclusion cage, while  
148 replicate two plants were exposed to stress, and replicate one plants received insect pollination.

#### 149 **2.4 Data collection**

150

151 Yield parameters were assessed when plants had reached senescence. Pods on all experimental plants  
152 were individually harvested with node and raceme position recorded, to allow changes in within-stem  
153 yield allocation to be investigated. Pods were oven dried at 80°C until dry mass was constant before  
154 recording bean mass. Bean size and number were measured using WINDIAS image analysis software  
155 (version 3, Delta T Devices), recorded to whole plant level in 2012 and pod-level in 2013 and 2014. A  
156 conservative threshold was applied to exclude beans with area <50 mm<sup>2</sup> (assumed to be non-fertilized  
157 ovules).

158 Yield mass per plant was calculated for all years, by summing the mass of beans produced by pods on  
159 each plant. The yield mass benefit due to insect pollination was calculated for each temperature  
160 treatment level, by dividing the average per-plant yield of an insect pollination cage by that of the  
161 exclusion cage, in each year, or replicate experiment in 2013. The 10 cages used in 2014 were  
162 randomly allocated to treatments and therefore not paired, so for 2014 the combined means of all  
163 cages containing bees and those excluding pollinators were compared, the statistical analysis was  
164 weighted accordingly. Mass per bean, and the number of beans per pod, were calculated by averaging  
165 across pods within each plant. Changes in yield allocation on the primary stem were tested using the  
166 first node to set pods on each plant. The yield ratio was measured by dividing yield mass by the mass  
167 of stems (with leaf and raceme branches removed) and pod casings for each plant in 2014. Seed  
168 nitrogen content per plant, as a proxy for protein content, was measured on a subset of plants in 2013  
169 (150 plants) and 2014 (100 plants) using a LECO FP-328 analyser.

#### 170 **2.5 Statistical analysis**

171 Plant level yield parameters (yield mass, bean number, pod number (data from all years); bean  
172 number per pod, mass per bean, first node with pod, nitrogen content (2013 and 2014), yield ratio,  
173 non-yield biomass (2014 only)) were analysed with linear mixed effects models (Table S3,

174 Supplementary Material) via the lme4 package (Bates et al., 2014) in R statistical software (version  
175 3.2.0, R Core Team 2015). Repeated measures of multiple plants within each cage, and differences in  
176 the number of replicate plants between years, were addressed by the random effect (1|cage).  
177 Temperature treatments were analysed as a categorical factor, to allow for simpler analysis and  
178 interpretation of complex non-linear relationships between temperature and pollination treatments.  
179 Plants within each cabinet were treated as independent replicates of a temperature treatment; the  
180 temperature treatment was the dominant factor affecting plants within each cabinet, and cabinets were  
181 randomly allocated to different temperature treatments between replicated experiments in 2013, and  
182 across years. Yield parameters that were calculated on a larger than plant level (yield benefit of  
183 pollination; yield variability), were analysed with ANOVA using the means of plants from each  
184 combination of flight cage and cabinet (Table S3, Supplementary Material). Analysis of yield benefit  
185 due to pollination included a weighting term (5 times higher weighting for 2014), as the single figure  
186 for 2014 was derived from 5 comparisons of cages containing and excluding insect pollinators. Year  
187 was considered a fixed effect in all models to assess the between-year variability.

188 To establish the effect of treatments on yield parameters (Table S3, Supplementary Material),  
189 maximal models, containing parameters: temperature, pollination, interaction of temperature and  
190 pollination, and year, were simplified by single term deletions tested with likelihood ratio tests  
191 (Shmueli, 2010). Single terms were dropped if  $p > 0.05$ . After all single term deletion tests had been  
192 performed, temperature treatment levels with similar model predicted estimates were grouped for  
193 simplicity of interpretation (Crawley, 2013), provided model explanatory power was not reduced  
194 ( $p > 0.1$ ). Model residuals were checked for normality and heteroscedasticity, yield ratio was  
195 exponential-transformed and yield variability was square-root transformed to improve model fit.  
196 Effect sizes provided in the text are model parameter estimates, raw data values are provided in the  
197 figures and table 2.

## 198 **3 Results**

### 199 **3.1 Yield parameters**

200 Whole-plant yield and the yield benefit attributable to insect pollination were analysed to understand  
201 the response of faba bean plants to insect pollination following heat stress.

#### 202 *3.1.1 Per plant yield*

203 The response of whole-plant yield to heat stress (Fig. 1A) was significantly modified by pollination  
204 ( $p = 0.036$ ). Following the 30°C temperature treatment the yield of plants grown in cages without bees  
205 was reduced by 4.2g per plant (at least 15 %), while the yield of insect-pollinated plants was reduced  
206 by 0.8g (at least 2.5 %) compared to control temperatures. Yields of both insect-pollinated and  
207 excluded plants were reduced following the 34°C temperature treatment, with reductions of 7.6g and  
208 6.7g compared to the respective control treatments. The heat wave scenario treatment of 26°C did not  
209 significantly differ from control temperatures 18 and 22°C, so these temperatures were grouped as  
210 one control level ( $p = 0.539$ ) after significance of the treatments had been established.



211 *3.1.2 Yield benefit from pollination*

212 In addition to modifying the relationship of yield and heat stress in terms of absolute yield values, the  
213 proportional yield benefit attributable to insect pollination (Fig. 1B) increased from 15.8% under  
214 control temperatures (18, 22 and 26°C; grouping  $p=0.591$ ) to 52.5% following the 30°C heat stress  
215 treatment ( $p=0.004$ ). Following exposure to 34°C, however, the benefit of pollination (15.8%) was  
216 identical to control temperatures.

217 **3.2 Fertilization and yield quality parameters**

218 The number of beans per pod and per plant were analysed to assess changes in fertilization success.  
219 To explore the mechanisms by which pollinators modified yield and their impact on yield quality,  
220 yield allocation; yield ratio; yield variability; and mass of individual beans were analysed.

221 *3.2.1 Bean and pod number*

222 Bean number per plant (Table 2) was not affected by an interaction between temperature and  
223 pollination treatments ( $p=0.117$ ), however, temperature treatments of 30 and 34°C (18 to 26°C were  
224 grouped,  $p=0.101$ ) reduced bean number by 6.6 and 14.7 respectively ( $p<0.001$ ), and plants excluded  
225 from insect pollinators produced on average 6.9 (at least 12 %) fewer beans. Bean number per pod  
226 (Table 2) was affected by an interaction between heat stress and pollination ( $p<0.001$ ), each level of  
227 temperature was significantly different. Pod number per plant (Table 2) was not affected by insect  
228 pollination ( $p=0.386$ ), but was reduced following the 30 and 34°C treatments ( $p<0.001$ ).

229 *3.2.2 Yield ratio and within-plant yield allocation*

230 The first node to set pods moved away from those flowers present prior to stress with temperature  
231 (Fig. 2A) and was 5.5 and 7.8 nodal positions higher following 30 and 34°C temperature treatments in  
232 plants excluded from insect pollination, while smaller changes of 1.9 and 3.8 nodes were measured in  
233 pollinated plants ( $p=0.005$ ), each level of temperature was significantly different. Insect pollinated  
234 plants produced around 3g less non-yield biomass (table 2) per plant ( $p=0.030$ ) and non-yield biomass  
235 was also reduced by an average of 3.5g per plant across both pollination treatments following the 30  
236 and 34°C temperature treatments ( $p=0.001$ ). There was no interaction between temperature and  
237 pollination ( $p=0.389$ ) and no significant difference between the two hottest treatments ( $p=0.126$ ).  
238 Yield ratio (Fig. 2B) of insect pollinated plants was approximately 20% higher following the 30°C  
239 temperature treatment (interaction term;  $p=0.001$ ).

240 *3.2.3 Yield variability*

241 The yield of plants within a combination of temperature treatment and flight cage was approximately  
242 18% less variable in cages that contained bees, than in cages without bees (Table 2;  $p=0.021$ ). The  
243 coefficient of variation (standard deviation/mean) was unaffected by temperature treatments  
244 ( $p=0.488$ ) but changed between years of experimentation ( $p<0.001$ ). Other yield parameters changed  
245 between years; total yield mass per plant ( $p<0.001$ ), bean number per pod ( $p<0.001$ ) and per plant

246 ( $p < 0.001$ ) all differed between years, while the proportional benefit of pollination remained stable  
247 between years ( $p = 0.784$ ).

#### 248 3.2.4 Mass per bean and nitrogen content.

249 Thousand grain weight (*i.e.* individual bean mass \* 1000) of insect pollinated plants increased by 45  
250 and 55 g following the 30 and 34°C temperature treatments from 460.15g at control temperatures,  
251 compared to an increase of 31 g and a decrease of 52 g measured in plants excluded from pollinators  
252 (interaction term;  $p = 0.020$ ). Percentage nitrogen content was 0.18 higher following the 26, 30 and  
253 34°C temperature treatments ( $p = 0.039$ ) and differed with year ( $p = 0.032$ ), though these differences are  
254 small and equate to around a one percent change in protein content.

### 255 **4 Discussion**

256 The main aim of this study was to investigate interactions between heat stress and insect pollination  
257 on the yield of faba bean. Our results suggest that sufficiently pollinated faba bean crops could have  
258 less variable yields that are more resilient to heat stress. We measured an increase in the pollinator-  
259 dependency of experimental plants with heat stress, from 16 % dependency at control temperatures, to  
260 53 % dependency in plants exposed to 30°C treatment, before dropping back to 16 % dependency at  
261 34°C. This change in the benefit of insect pollination occurred because following heat stress at 30°C,  
262 yield losses of at least 15 % occurred in plants that were excluded from pollinators, while  
263 significantly lower yield losses occurred in plants that were pollinated by *Bombus terrestris*. At 34°C,  
264 female floral organs may have been damaged to the point that fertilization was not possible, or other  
265 processes such as plant vegetative growth may have been affected so that bee-dependent yield  
266 recovery could not be realised. Enhanced yield resilience to stress was a previously unknown benefit  
267 of insect pollination. Experiments to compare the vulnerability of male and female floral organs have  
268 however measured similar yield recovery following stress and the manual transfer (*e.g.* by hand) of  
269 fertile pollen in tomato (Peet et al., 1998), oilseed rape (Young et al., 2004), common bean (Gross and  
270 Kigel, 1994; Monterroso and Wien, 1990), and wheat (Briggs et al., 1999; Saini and Aspinall, 1982).  
271 This suggests that there is potential for pollination to mitigate the negative effects of heat stress on  
272 productivity of other insect-pollinated crops. It is interesting that yield increased during the three  
273 years of our experimentation, this was likely due to continuous optimisation of growth conditions of  
274 our potted plants. The benefit to yield or yield stability provided by insect pollination was conserved  
275 across the range of faba bean productivity.

276 It is not clear from our experiment whether insect pollinators actually improved yield resilience to  
277 heat stress by moving fertile pollen to pollen-deficient flowers (yield recovery by outcrossing). In  
278 faba bean, a floral visit can either lead to outcrossing, or can facilitate within-flower self-pollination  
279 by disrupting (tripping) a physical barrier between the stigma and anthers that otherwise prevents self-  
280 pollination in some flowers (Kambal et al., 1976). Insect pollination may have simply facilitated  
281 greater levels of self-pollination in flowers that were less damaged by the stress treatment. The  
282 number of beans per plant, arguably a more direct measure of fertilization, was not augmented by

283 insect pollination to the same extent as yield mass. However, yield allocation was retained on lower,  
284 more productive floral nodes following heat stress in insect pollinated plants (and was retained closer  
285 to flowers present prior to stress), while yield at these nodes was lost in excluded plants. This may  
286 have promoted yield resilience through changes in resource use efficiency, which increased  
287 dramatically following the 30°C treatment in insect pollinated plants, contrasting with a reduction in  
288 excluded plants. Confirming the mechanism by which resilience occurred is important to effectively  
289 target interventions. We studied a single cultivar to control differences in outcrossing, but resilience  
290 could be higher in certain faba bean cultivars that increase outcrossing rate through *e.g.* high floral  
291 attractiveness to pollinators (Suso et al., 2005). If resilience is due to the increased outcrossing  
292 following heat stress, this could be established using a genetic approach (*e.g.* Ritland and Jain, 1981).

293 To understand the importance of beneficial interactions that we observed, it is useful to quantify the  
294 likelihood of extreme temperatures occurring during crop floral development and anthesis. However,  
295 while there is consensus among projections that heat waves are likely to become hotter and more  
296 frequent in the future (Donat and Alexander, 2012; Hansen et al., 2012; Kirtman et al., 2013;  
297 Seneviratne et al., 2012), projecting the absolute temperatures and timing of extreme events remains  
298 problematic and susceptible to bias (Seneviratne et al., 2012). Available projections for the UK  
299 suggest that heat waves ( $\geq 6$  consecutive days with peak temperature  $\sim 26^\circ\text{C}$ ) will increase from  
300 approximately a 1 in 5 year to a 1 in  $<2$  year occurrence in summer months of the period 2021-2050  
301 (Fischer and Schär, 2010), occurrences of rarer, hotter, heat waves are more difficult to predict and  
302 were not provided. Furthermore, directly relating our experimental temperature treatments to climate  
303 change scenarios relies on at least two other assumptions, i) that atmospheric carbon dioxide  
304 concentrations [ $\text{CO}_2$ ] will not increase, or affect yield resilience, ii) that soil moisture will not limit  
305 plant evapotranspiration. Future [ $\text{CO}_2$ ] emissions greatly depend upon human actions, and impacts of  
306 increased [ $\text{CO}_2$ ] on crop production are variable (Ainsworth and Long, 2005). Drought is projected to  
307 increase in the future (Kirtman et al., 2013), so the temperature treatments of 30 and 34°C may  
308 represent stress levels that plants will experience at lower temperatures, if combined with low soil  
309 moisture (*e.g.* ‘compound events’; Seneviratne et al., 2012). Experimental plants were well watered  
310 and evaporative cooling undoubtedly increased the temperature at which yield reductions occurred  
311 (Alghabari et al., 2014; Lobell et al., 2011). Further work is required to quantify the relative  
312 likelihoods of stress levels represented by the 30 and 34°C treatments, to understand how frequently  
313 faba bean pollinator dependency will increase above typical levels.

314 The average yield benefit of insect pollination of approximately 16% that we measured at control  
315 temperatures falls within the range of other studies comparing faba bean plants in cages with and  
316 without insect pollinators *e.g.* 15% (Garratt et al., 2014); 26% (Ghamdi and Ghamdi, 2003) and 25%  
317 (Somerville, 1999). Higher reported benefits may be due to varietal differences, plant stress, or  
318 detrimental effects of bagging in experiments that compared yields of bagged plants with openly  
319 pollinated controls (Benachour et al., 2007; Free, 1993; Nayak et al., 2015). We found additional  
320 benefits of pollination across all tested temperatures, in agreement with existing literature, pollination  
321 increased the number of beans per plant (Ghamdi and Ghamdi, 2003) and per pod (Garratt et al.,

322 2014) indicating that improved fertilization enabled allocation of yield on lower nodes (Somerville,  
323 1999; Suso et al., 1996). This can reduce lodging risk and improve uniformity of ripening (Stoddard,  
324 1993), but did not affect seed nitrogen content (Bartomeus et al., 2014). Between-plant variability was  
325 high in all experiments but insect pollination reduced this variability in yield across all temperature  
326 treatments. This is of high importance as yield variability is a key concern for faba bean growers (*e.g.*  
327 Rubiales, 2010).

328 Our findings provide robust evidence that insect pollinators can elicit partial yield compensation  
329 following stress in faba bean, and therefore that pollinator dependency of faba bean and other self-  
330 compatible crops may increase with greater likelihood of heat stress during flowering. Our  
331 experimental methodology assumed that insect pollinators will be present, and able to provide this  
332 yield resilience benefit in the future. However, the current literature suggests that pollinator  
333 communities will be strongly affected by climate change (Kerr et al., 2015; Polce et al., 2014;  
334 Rasmont et al., 2015). More research is required to help understand (and mitigate) the threats of both  
335 gradual climate change on pollinator populations, and the effects of extreme weather on floral  
336 visitation by insect pollinators. With an eroded pollinator population in the future, methods to  
337 improve the interactions of crop plants and their pollinators (*e.g.* Garibaldi et al., 2014) will be further  
338 necessitated. In faba bean, evidence suggests that pollination services are higher and more stable  
339 when fields are closer to semi-natural habitats (Andersson et al., 2014; Garibaldi et al., 2011; Garratt  
340 et al., 2014; Nayak et al., 2015, but see Bartomeus et al., 2014). In landscapes where the natural  
341 pollinator community has been degraded, provision of managed pollinators to supplement wild  
342 pollinators may be the only feasible option to improve crop pollination. Supplementation with  
343 honeybees (*Apis mellifera*) can enhance yield (Stoddard, 1986) and has been shown to be  
344 economically viable in Australia (Cunningham and Le Feuvre, 2013). Further work is required to  
345 quantify the density and diversity of pollinators necessary to achieve optimal pollination in faba beans  
346 and also to determine whether the beneficial interactions that we measured occur in field conditions  
347 with a wild pollinator community. Beneficial interactions may be achieved with fairly low pollinator  
348 numbers; a study that controlled pollinator visits to individual flowers found no effect of visit number  
349 on pod set (Garratt et al., 2014).

350 This study was novel in exploring interactions between abiotic stress and insect pollination and their  
351 effects on crop yield production. In our experimental system, caged *Bombus terrestris* colonies  
352 contributed to a significant proportion of faba bean yield under all temperature treatments, and  
353 mitigated observed reductions in yield mass and some yield quality parameters (yield ratio, individual  
354 bean mass) following the 30°C heat stress treatment. Yield production became dramatically more  
355 dependent on insect pollination following the 30°C treatment, suggesting that insect pollination may  
356 become increasingly important with increasing incidence of heat stress. The potential impacts of this  
357 could be great in less developed countries where climate change is expected to have  
358 disproportionately large effects for food security (Porter et al., 2014) and where the cultivation of  
359 pollinator-dependent crops is higher (Aizen et al., 2009). Given that 75% of global crops benefit from  
360 insect pollination (Klein et al., 2007) it is important to understand how widespread this phenomenon

361 is for production stability. Our findings highlight the importance of understanding the threats to and  
362 conserving key pollinating species that may improve the resilience of crop production to projected  
363 climate change, in order to promote both current and future food security.

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## Figures and tables

540 **Table 1:** Summary of experimental designs, treatment combination refers to an individual  
541 combination of flight cage and controlled environment cabinet.

Year	Sow date	Plant number		Replicate Experiments	Flight cage specifications			Donor: experimental plant ratio
		Total	Per treatment combination		Location (lat, long)	Number	Dimensions (m)	
2012	8 Dec 11	100	10	1	Sonning Farm (51 48' N, 00 89' W)	2	2.4 x 2.4 x 2.1	3:1
2013	11 Jan 13	190 (570)	19 (57)	3	Plant Environment Lab (51 27' N, 00 56' W)	2 (6)	12.5 x 2.5 x 2.5	1:1
2014	13 Jan 14	200	4	1	Plant Environment Lab (51 27' N, 00 56' W)	10	2.5 x 2.5 x 2.5	1:1

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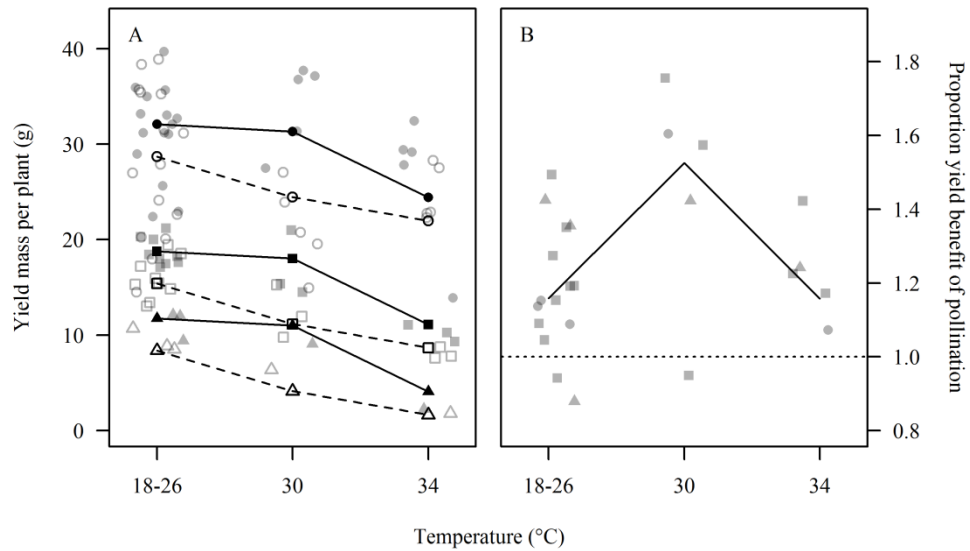
543 **Table 2:** Absolute yield parameter values aggregated across experimental years and cages. Test  
544 statistics and  $p$  values provided are from likelihood ratio tests;  $\chi^2$  tests for mixed models or  $F$  tests for  
545 linear models, between candidate models following single-term deletions.

Treatments		Parameters (mean $\pm$ SEM)						
Temperature (day/night; °C)	Pollination	Bean number	Pod number	Beans per pod	Mass per bean (g)	Yield mass variability	% Nitrogen	Non-yield biomass (g)
18/10	Pollinated	51.9 $\pm$ 5.2	17.2 $\pm$ 1.8	2.9 $\pm$ 0.1	0.510 $\pm$ 0.014	0.378 $\pm$ 0.080	4.295 $\pm$ 0.098	35.848 $\pm$ 1.953
	Exclusion	42.8 $\pm$ 5.8	15.5 $\pm$ 6.7	2.5 $\pm$ 0.1	0.534 $\pm$ 0.018	0.425 $\pm$ 0.062	4.542 $\pm$ 0.066	37.566 $\pm$ 2.164
22/14	Pollinated	46.0 $\pm$ 4.5	15.4 $\pm$ 2.5	2.6 $\pm$ 0.2	0.521 $\pm$ 0.014	0.414 $\pm$ 0.047	4.398 $\pm$ 0.110	34.162 $\pm$ 0.927
	Exclusion	40.3 $\pm$ 4.7	15.6 $\pm$ 7.6	2.5 $\pm$ 0.1	0.528 $\pm$ 0.019	0.430 $\pm$ 0.076	4.469 $\pm$ 0.143	34.018 $\pm$ 1.912
26/18	Pollinated	48.3 $\pm$ 4.6	17.0 $\pm$ 3.8	2.7 $\pm$ 0.0	0.494 $\pm$ 0.013	0.322 $\pm$ 0.054	4.560 $\pm$ 0.087	32.993 $\pm$ 1.299
	Exclusion	42.0 $\pm$ 5.1	16.8 $\pm$ 8.8	2.4 $\pm$ 0.1	0.565 $\pm$ 0.021	0.403 $\pm$ 0.075	4.624 $\pm$ 0.115	36.232 $\pm$ 1.419
30/22	Pollinated	43.7 $\pm$ 4.8	17.5 $\pm$ 4.1	2.5 $\pm$ 0.1	0.591 $\pm$ 0.017	0.307 $\pm$ 0.049	4.512 $\pm$ 0.130	30.723 $\pm$ 1.988
	Exclusion	31.8 $\pm$ 3.0	15.0 $\pm$ 9.7	2.0 $\pm$ 0.0	0.556 $\pm$ 0.020	0.496 $\pm$ 0.084	4.559 $\pm$ 0.090	35.238 $\pm$ 1.064
34/26	Pollinated	32.8 $\pm$ 5.2	14.5 $\pm$ 5.2	2.1 $\pm$ 0.1	0.608 $\pm$ 0.023	0.432 $\pm$ 0.094	4.539 $\pm$ 0.089	27.445 $\pm$ 0.654
	Exclusion	31.5 $\pm$ 5.4	15.0 $\pm$ 10.6	2.1 $\pm$ 0.0	0.552 $\pm$ 0.022	0.570 $\pm$ 0.136	4.517 $\pm$ 0.078	33.135 $\pm$ 0.760
<b>Treatment effects</b>								
Interaction Pollination : Temperature		$\chi^2=5.671$ ; $p=0.117$	$\chi^2=3.441$ ; $p=0.487$	$\chi^2=26.91$ ; $p<0.001$	$\chi^2=7.873$ ; $p=0.005$	$F=0.703$ ; $p=0.593$	$\chi^2=6.7102$ ; $p=0.152$	$\chi^2=4.126$ ; $p=0.389$
Pollination		$\chi^2=5.178$ ; $p=0.023$	$\chi^2=0.753$ ; $p=0.386$	-	-	$F=5.508$ ; $p=0.021$	$\chi^2=0.6945$ ; $p=0.405$	$\chi^2=4.725$ ; $p=0.030$
Temperature		$\chi^2=118.84$ ; $p<0.001$	$\chi^2=33.175$ ; $p<0.001$	-	-	$F=0.865$ ; $p=0.488$	$\chi^2=10.100$ ; $p=0.039$	$\chi^2=16.181$ ; $p=0.003$
Year		$\chi^2=25.002$ ; $p<0.001$	$\chi^2=33.680$ ; $p<0.001$	$\chi^2=28.625$ ; $p<0.001$	$\chi^2=13.845$ ; $p<0.001$	$F=21.489$ ; $p<0.001$	$\chi^2=4.612$ ; $p=0.032$	-
Simplified temperature categories		18-26, 30, 34	18-26, 30, 34	-	18-26, 30, 34	-	18-22; 26-34	18-26, 30-34

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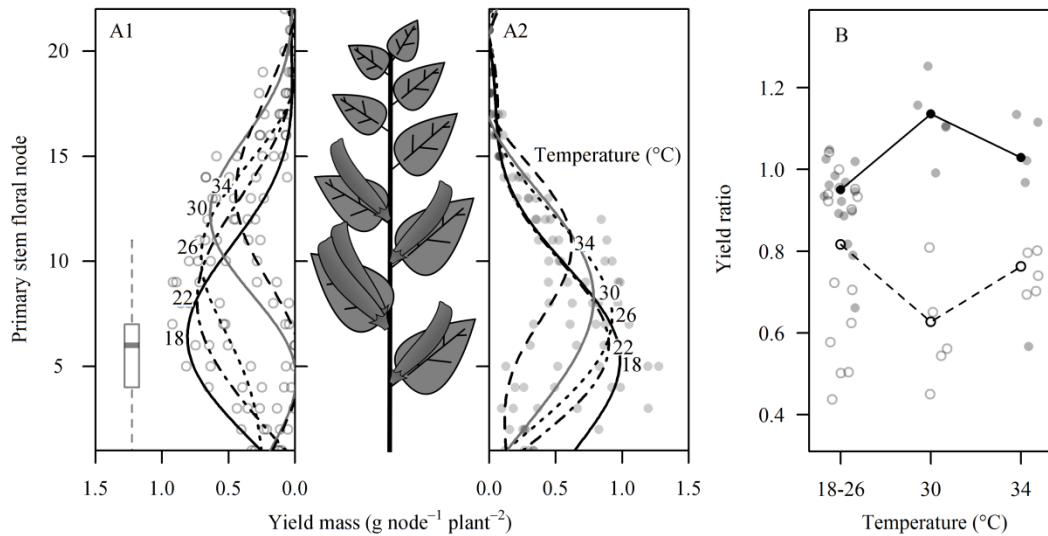
548 **Figure 1:** Responses of yield parameters to heat stress and pollination treatments. Point styles  
 549 represent pollination treatment and year, filled points = insect pollination; open points = exclusion;  
 550 triangles = 2012; squares = 2013; circles = 2014. Points are jittered to aid viewing. **A:** Yield mass per  
 551 plant. Lines represent model estimated means for each temperature category, for insect pollinated  
 552 plants (solid line) or plants excluded from pollination (dashed line); **B:** Proportion of yield attributable  
 553 to insect pollination (yield mass of insect pollinated plants/excluded plants). Line represents model  
 554 estimated mean for each temperature category, dashed line indicates level at which yield mass of  
 555 insect pollinated and excluded plants are equal.



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558 **Figure 2:** Point styles represent pollination treatment and year, open points = exclusion (panel A1);  
 559 filled points = insect pollination (panel A2). **A:** Distribution of yield on the primary stems of  
 560 experimental plants in 2013 and 2014; lines are model predictions from generalised additive models  
 561 restricted to 5 basis dimensions to produce readily comparable model fits, of the average yield mass  
 562 per node per plant for separate heat stress treatments. Line styles represent different temperature  
 563 treatments. Boxplot shows number of floral nodes on main stems with flowers present (counts  
 564 included un-opened flowers at green bud stage) prior to temperature treatments, across all treatments.  
 565 **B:** Yield ratio of plants in 2014. Lines represent model estimated mean for each temperature category.



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