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

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1 **Title:**

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

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20

21 **<u>Abstract</u>**

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 **<u>1 Introduction</u>**

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 (*Phaselous*

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 (Carvalheiro 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.

2 Methods

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^{-3}

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^2 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   µmol photon m^{-2} s^{-1}; relative humidity was 87±13 % in 2012, 80±20 % in 2013 and 85±15 % in

123   2014; and CO_2 was 385 mg L^{-1}. 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^2 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^2 (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.

3 Results

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.

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.

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 (≥6 consecutive days with peak temperature ~26°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 [CO_2] will not increase, or affect yield resilience, ii) that soil moisture will not limit

305 plant evapotranspiration. Future [CO2] emissions greatly depend upon human actions, and impacts of

306 increased [CO2] 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.,

9

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.

6 References

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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

542

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; χ^2 tests for mixed models or *F* tests for

545 linear models, between candidate models following single-term deletions.

Treatments		Parameters (mean ± 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±5.2	17.2±1.8	2.9±0.1	0.510±0.014	0.378±0.080	4.295±0.098	35.848±1.953
	Exclusion	42.8±5.8	15.5±6.7	2.5±0.1	0.534±0.018	0.425±0.062	4.542±0.066	37.566±2.164
22/14	Pollinated	46.0±4.5	15.4±2.5	2.6±0.2	0.521±0.014	0.414±0.047	4.398±0.110	34.162±0.927
	Exclusion	40.3±4.7	15.6±7.6	2.5±0.1	0.528±0.019	0.430±0.076	4.469±0.143	34.018±1.912
26/18	Pollinated	48.3±4.6	17.0±3.8	2.7±0.0	0.494±0.013	0.322±0.054	4.560±0.087	32.993±1.299
	Exclusion	42.0±5.1	16.8±8.8	2.4±0.1	0.565±0.021	0.403±0.075	4.624±0.115	36.232±1.419
30/22	Pollinated	43.7±4.8	17.5±4.1	2.5±0.1	0.591±0.017	0.307±0.049	4.512±0.130	30.723±1.988
	Exclusion	31.8±3.0	15.0±9.7	2.0±0.0	0.556±0.020	0.496±0.084	4.559±0.090	35.238±1.064
34/26	Pollinated	32.8±5.2	14.5±5.2	2.1±0.1	0.608±0.023	0.432±0.094	4.539±0.089	27.445±0.654
	Exclusion	31.5±5.4	15.0±10.6	2.1±0.0	0.552±0.022	0.570±0.136	4.517±0.078	33.135±0.760
Treatment effects								
Interaction Pollination : Temperature		χ^2=5.671; p=0.117	χ^2=3.441; p=0.487	χ^2=26.91; **p<0.001**	χ^2=7.873; **p=0.005**	F=0.703; p=0.593	χ^2=6.7102; p=0.152	χ^2=4.126; p=0.389
Pollination		χ^2=5.178; **p=0.023**	χ^2=0.753; p=0.386	-	-	F=5.508; **p=0.021**	χ^2=0.6945; p=0.405	χ^2=4.725; **p=0.030**
Temperature		χ^2=118.84; **p<0.001**	χ^2=33.175; **p<0.001**	-	-	F=0.865; p=0.488	χ^2=10.100; **p=0.039**	χ^2=16.181; **p=0.003**
Year		χ^2=25.002; **p<0.001**	χ^2=33.680; **p<0.001**	χ^2=28.625; **p<0.001**	χ^2=13.845; **p<0.001**	F=21.489; **p<0.001**	χ^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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547

Figure 1: Responses of yield parameters to heat stress and pollination treatments. Point styles

represent pollination treatment and year, filled points = insect pollination; open points = exclusion;

triangles = 2012; squares = 2013; circles = 2014. Points are jittered to aid viewing. **A**: Yield mass per

plant. Lines represent model estimated means for each temperature category, for insect pollinated

plants (solid line) or plants excluded from pollination (dashed line); **B**: Proportion of yield attributable

to insect pollination (yield mass of insect pollinated plants/excluded plants). Line represents model

estimated mean for each temperature category, dashed line indicates level at which yield mass of

insect pollinated and excluded plants are equal.
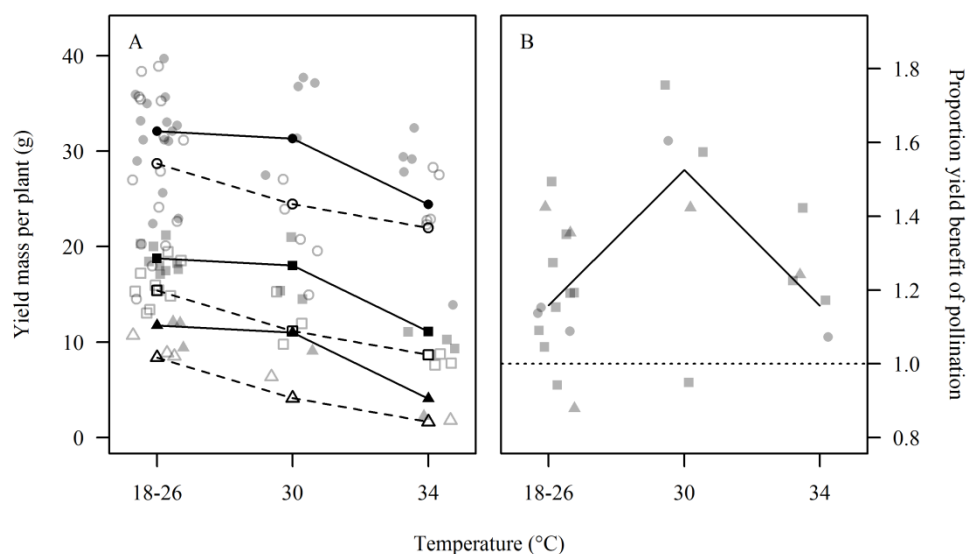
558 **Figure 2:** Point styles represent pollination treatment and year, open points = exclusion (panel A1);

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.

566