Early-season crop colonization by parasitoids is associated with native vegetation, but is spatially and temporally erratic

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1   **Early-season crop colonization by parasitoids is associated with native vegetation, but is**

2   **spatially and temporally erratic**

3

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26   **Abstract**

27   Semi-natural habitats in agricultural landscapes may support parasitoid populations that

28   provide biocontrol services by suppressing populations of crop pests, but little is known about

29   the spatial pattern and variability of these services at different levels of scale. Here we

30   investigate the rarely studied phenomenon of early-season crop colonization by parasitoids

31   and the relationship with the surrounding landscape. We assessed parasitism of whiteflies by

32   placing whitefly infested cotton seedlings in remnant vegetation, arable land 25-125 m from

33   remnant vegetation, and arable land further than 400 m from remnant vegetation. Twelve to

34   twenty sentinel plants were exposed in a 25 x 25 m grid pattern in plots in each habitat. The

35   experiment was conducted at 18 locations across two landscapes and repeated three times in a

36   2-week period in 2007 and 2008. Parasitism was observed during the first three days after the

37   introduction of the whitefly infested seedlings and was in all cases caused by *Encarsia* spp.

38   The mean number of parasitized whitefly per plant was 0.106 ± 0.025 and was highest on

39   cotton plants placed in remnant vegetation, declining with increasing distance from remnant

40   vegetation. A regression model with land use and meteorological variables received more

41   statistical support from the data than models with only landscape and time period as factors.

42   Parasitism levels were influenced by the proportion of remnant vegetation, grassland, as well

43   as wind, temperature, dew point temperature and year. Early-season colonization of whitefly

44   infested seedlings by parasitoids was erratic and characterized by large spatial (inter-plant and

45   inter-plot) and temporal variation. Our study confirms that remnant vegetation function as

46   reservoirs for parasitoids and that parasitoids can penetrate arable fields beyond 125 m within

47   3 days. However, variation in the occurrence of parasitism makes it difficult to predict

48   parasitoid colonisation at a specific place and time. Therefore, field-based scouting for pests

49   and parasitoids is necessary, even in landscapes with a high biocontrol potential.

50

51

52 **Highlights:**

53 • The rate of crop colonization from remnant vegetation exceeds 125 m in three days

54 • There is on average a clear effect of landscape variables on parasitism rates

55 • Land use and meteorological variables explain parasitism rates

56 • There is low predictability of parasitism at a specific place and time

57

58

Introduction

60 **Introduction**

61 With increasing concern that biocontrol services are declining at a world-wide scale

62 (Millennium Ecosystem Assessment 2005) there is growing interest in habitat management to

63 restore functional biodiversity in agricultural landscapes and strengthen natural biocontrol

64 potential (Gurr et al. 2012). Despite growing consensus that landscape heterogeneity can

65 support arthropod mediated biocontrol services in crops (Bianchi et al. 2006, Chaplin-Kramer

66 et al. 2011), there are many questions regarding the spatial pattern of these ecosystem services

67 around resource habitats of the natural enemies, and the distances over which these services

68 extend into crops (Kremen 2005, Schellhorn et al. 2008). Identifying the appropriate

69 management scales for conservation biological control requires understanding of how

70 landscape heterogeneity influences the processes underlying natural pest control, including

71 the frequency and spatial extent of dispersal of biological control agents from source habitats

72 to crops and the associated variability in time and space. While the importance of native

73 perennial vegetation for sustaining parasitoid populations and crop colonization has been

74 demonstrated (Landis and Haas 1992, Dyer and Landis 1997, Bianchi et al. 2008, Thomson

75 and Hoffmann 2009, Letourneau et al. 2012, Macfadyen and Muller 2013), the spatial and

76 temporal variation associated with the crop colonization process and how this is influenced by

77 the surrounding landscape has largely been overlooked. Information on the predictability of

78 the crop colonization process by natural enemies is essential to inform effective conservation

79 biological control programs that aim to strengthen biocontrol services by habitat management.

80   Despite growing concerns about the sustainability of pest management strategies

81 based on the use of synthetic insecticides, these chemicals provide short term pest control that

82 is highly predictable, which makes them attractive pest management option for conventional

83 farmers (Ekström and Ekbom 2011) even where there is a longer term risk of pest resistance.

84 The predictability of natural biocontrol differs fundamentally from that of synthetic

85 insecticides as it depends on the colonization of natural enemies from habitats outside the

86 field into the target crop (Wissinger 1997, Schellhorn et al. 2014). This process depends on

87 the frequency and timing of the colonization events and the number of colonizers, which in

88   turn can be influenced by the quality and spatial distribution of source habitats in the

89   surrounding landscape from which natural enemies are recruited and weather conditions

90   favouring or discouraging dispersal. The dispersal-driven colonization process is therefore a

91   variability generating process, and underlies the intrinsically stochastic nature of biocontrol as

92   compared to the use of synthetic insecticides.

93   The predictability of benefits from non-crop habitats could be improved when

94   biocontrol agents have large dispersal capacity and if different habitat patches have

95   overlapping areas of influence, providing spatial insurance in the case that a particular habitat

96   patch would not provide natural enemies (Loreau et al. 2003). While the dispersal capacity of

97   marked parasitoids are typically assessed at spatial scales less than 100 m (Schellhorn et al.

98   2014), one of the few studies that quantified parasitoid dispersal over larger distances

99   indicates that the emergent spatial pattern is erratic (Kristensen et al. 2013). Estimates of the

100 dispersal capacity of parasitoids can also be derived from spatially-indexed regression

101 approaches, which suggest that crop colonization from source habitats can take place over

102 distances of 1-1.5 km and further (Thies et al. 2003, Bianchi et al. 2008), but these studies do

103 not always differentiate between the colonization process and local reproduction at target sites

104 (e.g., Thies et al. 2003). There is a need for further studies to underpin our understanding of

105 the initial crop colonization by parasitoids that capture the appropriate spatial and temporal

106 scales and provide information on the biotic and abiotic factors influencing the colonization

107 process.

108   Australia hosts many indigenous whitefly species (Martin 1999, De Barro et al. 2000,

109 Sequeira et al. 2009), but these are typically not considered agricultural pests. In 1994, the

110 major pest *Bemisia* tabaci (Middle East - Asia Minor 1 (MEAM1)) arrived in Australia (De

111 Barro et al. 2011), which now causes economic damage to melons, cotton, and soybean.

112 Whiteflies are attacked by indigenous and exotic aphelinid parasitoids (De Barro et al. 2000).

113 In cotton, parasitism of *B. tabaci* MEAM1 nymphs by *Eretmocerus* and *Encarsia* spp. is

114 highly variable, and can be as high as 80% depending on time of year, pest management, and

115 host density (Sequeira et al. 2009).

116   The 2-year study reported here focuses on the colonization of whitefly parasitoids

117   early in the growing season as this period is considered critical for effective suppression of

118   pest populations that have potential for exponential increase (Schellhorn et al. 2014). The aim

119   of the study is twofold. Firstly, to assess the factors that best explain the colonization of

120   parasitoids for plots in, near to and further than 400 m from remnant vegetation. Secondly, to

121   assess the variability of biocontrol services associated with remnant vegetation by comparing

122   the colonization in plots of remnant vegetation and plots in arable fields near and further away

123   from remnant vegetation. Variability is assessed at two spatial scales (plant and plot level)

124   and between time periods.

125

126   **Materials and Methods**

127

128   *Study sites*

129   The study was conducted at 18 locations spread among two 5 km radius landscape sectors in

130   an arable production area west of the Great Dividing Range, near Dalby, Queensland,

131   Australia. The areas were 50 km apart, with the centres located at 151 6' 2.28" E; 26 51'

132   31.52" S (North landscape) and 151 5' 47.83" E; 27 17' 43.43" S (South landscape). The

133   landscapes consisted of agricultural fields, including sorghum (*Sorghum bicolor* L. Moench),

134   barley (*Hordeum vulgare* L.), canary (*Phalaris canariensis* L.), chick pea (*Cicer arietinum*

135   L.), mungbean (*Vigna radiata* L.), oat (*Avena sativa* L.), wheat (*Triticum aestivum* L.) and

136   cotton (*Gossypium hirsutum* L.), as well as grassland, and remnant native vegetation in

137   various forms (linear strips of trees, patches of remnant vegetation and remnant vegetation

138   along creeks). The plant species composition of the remnant vegetation was similar in both

139   landscapes. Remnant vegetation was dominated by *Eucalyptus populnea* (F. Muell.), *Acacia*

140   *salicina* (Lindl.) and *A. harpophylla* (F. Muell.) in the tree and shrub layer, and several

141   chenopodiaceous species in the understory (Bianchi et al. 2012).

142

143   *Experimental setup and data collection*

144   Experiments were conducted in the spring (October) of 2007 and 2008, around the time at

145   which the first cotton is sown and colonization by cotton herbivores and their natural enemies

146   begins. In each landscape, 3 plots (75 m x 50 m) were laid out in patches of remnant

147   vegetation, 3 plots (75 m x 100 m), within in arable fields at 25 m from the edge of the

148   remnant vegetation plots, and 3 plots (75 m x 100 m) in arable fields with the nearest edge at

149   least 400 m from remnant vegetation, for a total of 18 plots (2 landscapes x 9 plots) in each

150   year (Fig. 1). We refer to the location of the plots (i.e. within, near and far from remnant

151   vegetation) as "treatment". In 2007, all arable fields were fallow, containing sorghum or

152   cereal stubble, whereas in 2008, which was wetter than 2007, most fallow fields were planted

153   and therefore plots were established in newly planted sorghum fields. At the start of the

154   experiment sorghum plants were 13.6 ± 1.56 cm high (mean $\pm$ SEM). Twelve sentinel cotton

155   plants (3 x 4) were placed in a 25 x 25 m grid pattern in each remnant vegetation plot (plot

156   size 50 x 75 m), while twenty sentinels (5 x 4) were placed in each of the arable plots (near

157   and far; plot size 100 x 75 m) (Fig. 1). By placing these cotton seedlings in fallow (2007) or

158   newly sown fields (2008) we mimicked an emerging cotton crop, and avoided potential

159   confounding effects of parasitism from within the field versus the surrounding landscape. In

160   both years, seedlings were set out and recollected three times in each plot within a time

161   window of approximately two weeks coinciding with the time of emergence of the cotton. In

162   each landscape we took half-hourly records of temperature, precipitation, wind, and dewpoint

163   temperature, using a Davis Vantage Pro2 weather station (South Windsor, Australia). In

164   summary, the design included 108 plots with 1872 plants: 3 treatments (within + near + far =

165   12 + 20 + 20 = 52 plants) $\times$ 3 spatial replicates $\times$ 2 landscapes $\times$ 3 periods $\times$ 2 years.

166   Sentinel cotton seedlings were grown in pots (8 cm diameter, 7 cm high) and brought

167   into the field when they were five weeks old (height 6-10 cm, 2-4 leaf stage). The seedlings

168   were infested with silverleaf whitefly eggs two weeks prior to exposure to ensure that nymphs

169   were in the second/third instar during exposure, which is the preferred stage for parasitism by

170   *Eretmocerus* and *Encarsia* spp. Whitefly density typically ranged between 50 and 150

171   nymphs per plant. The seedlings were put in trays which were secured to the ground. The

172   cotton seedlings were collected after approximately three days exposure in the field, covered

173   with polypropylene nets to prevent further parasitism, and returned to the laboratory to score

174   parasitism rate and rear out and identify parasitoids. Once the nymphs reached the 'redeye'

175   stage the seedlings were cut and put individually in polypropylene bags with an emergence

176   cup in one corner of the bag. The bags were placed in cardboard boxes with cups protruding

177   out the top, such that whiteflies and parasitoids were reared out and emerged individuals were

178   self-collecting in the cups due to positive phototaxis and negative geotaxis responses. After

179   six weeks the whiteflies and parasitoids were counted and identified.

180   Land use around each plot was assessed by quantifying the areas of woody remnant

181   vegetation, grassland, sorghum, barley, canary, chick pea, mungbean, oat, wheat and cotton

182   (in ha) at scales of 0-100, 100-500, 500-1000, 1000-1500 and 1500-2000 m using ground

183   survey and ArcGIS.

184

185   *Data analysis*

186   The final dataset contained observations of parasitism rates from 1698 plants. Data were

187   missing from 9 plants due to vertebrate grazing, while no whiteflies or parasitoids were found

188   in the rearing bags from 165 plants, indicating a problem during rearing out (e.g. inability to

189   move through the bag towards the light). These missing data were randomly distributed across

190   the plots and were therefore discarded from the analysis.

191   Parasitism was analysed as count data (i.e. the number of parasitoids emerged per

192   whitefly infested seedling) because the proportion parasitized whiteflies was very low for the

193   great majority of samples (mean parasitism rate was 0.39%, and 98.3% of the parasitism rates

194   were below 5%), indicating a practically unlimited host resource. Four discrete stochastic

195   distributions were considered for the error distribution of the data: Poisson, negative

196   binominal, zero-inflated Poisson and zero-inflated negative binominal. A log-link was used

197   for all models such that the response variable is related to the linear regression via:

$$Y \sim Poisson\left(\lambda = e^{(\beta_0 + \beta_1 \cdot X_1 + \beta_2 \cdot X_2 + \cdots)}\right)$$

$$Y \sim NegBin\left(\mu = e^{(\beta_0 + \beta_1 \cdot X_1 + \beta_2 \cdot X_2 + \cdots)}, k\right)$$

198   The expectation (λ and μ) was explained by the experimental variables, whereas the error

199   term accounts for the variation in the observations as compared to the expected value. The

200   zero-inflation factor of the zero-inflated distributions and the overdispersion parameter k of

201   the negative binominal distribution were assumed equal across treatments to avoid

202   overparameterization. The models were fitted using glm (for Poisson distribution), glm.nb

203   (for negative binominal distribution) and zeroinfl functions (for zero inflates Poisson and

204   negative binominal distributions) using the R packages *MASS* (Venables and Ripley 2002)

205   and *PSCL* (http://cran.r-project.org/web/packages/pscl/pscl.pdf).

206   Akaike's Information Criterion, corrected for finite sample sizes (AIC$_c$), was used to

207   rank and select models (Burnham and Anderson 2002). As the negative binominal distribution

208   gave a better fit than the other distributions, this distribution was used for all subsequent

209   analyses. The negative binomial distribution is characterized by two parameters: the mean μ

210   and overdispersion parameter k, whereby low values of k (e.g. $k < \mu$) reflect overdispersion

211   and high values of k low or no overdispersion. For values of $k > 10\mu$ the negative binomial

212   distribution approaches the Poisson distribution (Bolker 2008). Model selection of

213   explanatory variables was conducted using the dredge procedure in R package *MuMIN*

214   (http://cran.r-project.org/web/packages/MuMIn/MuMIn.pdf). This procedure generates a

215   complete set of sub-models with combinations of the terms of the full model, and sorts the

216   sub-models on the basis of AIC$_c$ values and associated Akaike weights. This procedure

217   typically resulted in the same optimal model as the automated model selection process

218   *stepAIC* of the R package *MASS*, but has the advantage that a set of models receiving support

219   from the data are identified, rather than a single "best" model.

220   We conducted three different analyses with our data. In the first analysis we

221   considered plant level data and experimental variables, which were used in the experimental

222   design: treatment, landscape, year and period. These factorial explanatory variables account

223   for variation in the biophysical system that drive the colonization process of parasitoids, but

224 do not capture information on the nature of the underlying biophysical variables. Two-way

225 interactions between treatment and landscape, and year and period were also considered. To

226 account for differences in the exposure time of sentinels in the field we included the log-

227 transformed exposure hours as an offset variable in the full model (Zuur et al. 2009). In

228 biological terms this means that the response variable is now expressed as the number of

229 parasitoids per unit of time.

230   In a second analysis at the plant level a set of biologically meaningful variables were

231 used to acquire further insight in the biophysical factors driving whitefly parasitism. Land use

232 variables at five spatial scales were used to replace the factors "treatment" and "landscape".

233 In addition, meteorological variables were used to replace the factors "year" and "period".

234 The variable "rain" was discarded from the analysis because there was no precipitation during

235 the exposures. The variable "year" was also included as this accounts for any effects of year,

236 e.g. meteorological conditions in the period *before* exposure and the different vegetation

237 background in plots in arable fields (fallow vs. sorghum seedlings). Hours of exposure (log-

238 transformed) was included as an offset variable in the global model, and no interactions

239 between variables were included.

240   Finally, in a third analysis we tested the association between parasitism in plots in

241 remnant vegetation and plots near or far in arable fields using contingency tables and χ^2 test of

242 independence. For this analysis we used presence/absence data at the plot level (Appendix 1).

243 As there were 3 spatial replicates × 2 landscapes × 3 periods × 2 years, we considered a total

244 of 36 cases for each treatment, and expected frequencies were compared to a χ^2 test statistic

245 with one degree of freedom using the *chisq.test* procedure with Yates' continuity correction in

246 R (Crawley 2007). Separate analyses were conducted for the association of parasitism in and

247 near remnant vegetation, and in and far from remnant vegetation.

248

249 **Results**

250 In 2007 and 2008, 31,724 and 14,803 whiteflies were recovered and 147 and 33 parasitoids,

251 respectively. The average parasitism rates in the 3-day window of exposure were thus 0.46%

252 and 0.22%. All parasitoids were *Encarsia* spp. The overall mean number of whiteflies

253 emerging per cotton plant was 27.4 ± 0.64 (range 0-177), whereas the overall mean number of

254 emerging parasitoids was 0.106 ± 0.025 per plant (range 0-23). Parasitism was observed on

255 45 out of 1698 plants. The total number of parasitoids from plots in, near and far from

256 remnant vegetation was 119, 59 and 2, respectively (Appendix 2) and was influenced by the

257 variables of the experimental design (Fig. 2). At the within-plot level for plots near remnant

258 vegetation the total number of parasitoids encountered showed a declining trend with

259 increasing distance from remnant vegetation with values of 15 at 25 m, 25 at 50 m, 4 at 75 m,

260 8 at 100 m, and 7 at 125 m.

261 　　　　The model selection procedure of the first analysis considered variables of the

262 experimental design. The factors "treatment" and "year" were included in all six models that

263 received substantial support from the data ($\Delta AIC_c=6$; Table 1). The most parsimonious model

264 received an Akaike weight of 0.385 and contained main effects "treatment", "period", "year",

265 and the "period:year" interaction, and had a negative binomial error distribution with an

266 overdispersion factor k of 0.0217 (Table 2). The model indicated that the number of

267 parasitized whiteflies decreased with increasing distance from natural vegetation, and that the

268 number of parasitized whiteflies in the first period in 2008 were higher than in 2007, but

269 lower in the second and third period.

270 　　　　In the second analysis, the variables "treatment", "landscape", and "period" were

271 replaced by land use and meteorological data. This resulted in models with more support from

272 the data than the first analysis, reflected by a substantial drop in AIC_c values (Table 3 vs.

273 Table 1). The best models all included the variables area of remnant vegetation, area of

274 grassland, wind, dew point and year ($\Delta AIC_c = 6$; Table 3). The most parsimonious model

275 (Akaike weight 0.328) included all these factors and had a negative binomial error

276 distribution with a k of 0.0499 (Table 4). The k value of 0.0499 indicates that this model

277 explains the data better (i.e. the data becomes more Poisson distributed) than the model based

278 on the experimental design resulting from the first analysis ($k = 0.0217$), with a greater part of

279 the variability captured by explanatory variables and with lower residual variation. The

280   model indicates that remnant vegetation at 0-100 m, 100-500 and 500-1000 m had a positive

281   effect on the number of parasitoids per plant, whereas remnant vegetation at 1000-1500 m and

282   grassland at 100-500 m had a negative effect. The model also indicated that parasitism

283   increased as wind speed and dew point increased. Furthermore, parasitism decreased with

284   temperature, and was higher in 2007 than in 2008.

285   The third analysis focussed on presence or absence of parasitism at the plot scale. It

286   showed that parasitism was detected in, near, and far from remnant vegetation in 13 (36.1%),

287   5 (13.9%) and 2 times (5.6%) out of the 36 cases, respectively. There were 3 cases where

288   parasitism was observed in plots in remnant vegetation and nearby arable fields in the same

289   period (Appendix 1). The null-hypothesis that presence of parasitism in plots in and near

290   remnant vegetation is independent was not rejected (χ^2=0.486, df=1, P=0.486), indicating a

291   lack of association between incidence of parasitism in remnant vegetation and in nearby plots

292   in arable fields. There was one case when parasitism in remnant vegetation coincided with

293   parasitism in plants 400 m from remnant vegetation, but also in this case there was no support

294   for the hypothesis that the incidence of parasitism in plots in and far from remnant vegetation

295   are dependent (χ^2=0, df=1, P=1).

296

297   **Discussion**

298   The main finding of this study is that early-season crop colonization by *Encarsia* parasitoids

299   is associated with remnant vegetation, but that early-season crop colonization is an infrequent

300   event, resulting in an erratic spatio-temporal pattern that is difficult to predict. Hence, while

301   *on average* there is a clear effect of landscape variables on parasitism, we also show that there

302   is major variability and hence low predictability of the response at a specific place and time.

303   At the plant level, the variation in the probability of colonization is reflected in the low values

304   of the overdispersion factor *k* of the negative binomial distribution (0.0217 and 0.0499,

305   respectively). As these *k* values are well below the mean value of the number of parasitized

306   whiteflies per plant of 0.106, the probability density functions have a high probability of zeros

307   and a long tail (i.e. a relatively high probability of having a high number of parasitized

308   whiteflies per plant). In addition, the variable nature of the crop colonization process by

309   parasitoids at the plot level is illustrated by the limited number of occasions that parasitism in

310   remnant vegetation coincided with parasitism in the adjacent plots, and the fact that parasitism

311   in plots near and far from remnant vegetation were never observed in two sequential periods.

312   Our study thus shows that the spatial pattern of crop colonization by parasitoids from source

313   habitats early in the growing season is heterogeneous, which contrasts with the more even

314   colonization patterns of arable fields by ground-dwelling predators from field edges (e.g.,

315   Thomas et al. 1991). The high level of variability makes predicting the spatial distribution of

316   biocontrol services by parasitoids around source habitats early in the growing season

317   challenging. The difficulty of predicting parasitism events in this context is intrinsically

318   related to the low frequency of parasitism; it is impossible to predict a stochastic event that

319   has a low frequency of occurrence at small temporal or spatial scales.

320   While crop colonization by parasitoids is intermittent at a temporal scale of several

321   days, there are examples of effective pest suppression mediated by natural enemies that

322   sporadically visit pest-infested plants early in the growing season (Costamagna and Landis

323   2007). In particular ladybeetles are considered beneficial because they increase their aphid

324   intake at higher aphid densities (Costamagna and Landis 2007). However, the role of

325   parasitoids in early-season pest suppression is less well documented. Our data indicate that

326   *Encarsia* parasitoids can penetrate arable fields and parasitize whiteflies at distances beyond

327   125 m from their source habitats in approximately three days, and occasionally reaching up to

328   400 m. This is a substantially higher dispersal capacity than found for *Encarsia inaron* in an

329   orchard, which only moved up to 30 m in a 2-week period (Pickett and Pitcairn 1999).

330   Although the rate of parasitism detected was low in our experiment, it is important to consider

331   that we focused only on the colonization stage, so the significance for pest suppression could

332   still be great. Early-season parasitism in crops is likely to result in effective pest suppression

333   because there is more time available for the parasitoid population to build-up before pests

334   reach economic damage thresholds (Rauwald and Ives 2001).

335   The colonization rates of crops by parasitoids were quantified at the plant and plot

336   scale (100 x 75 m) and appeared scale dependent. For arable fields near remnant vegetation,

337   the probability of parasitism was 4.8% at the plant scale and 13.9% at the plot scale, while for

338   fields far from remnant vegetation this was 0.04 and 5.6%, respectively. These contrasting

339   findings between plant and plot scale prompt the question "what is the most relevant spatial

340   scale of crop colonization for biocontrol?" For *Encarsia* parasitoids, which can have multiple

341   generations within the growing season of a cotton crop, the plot scale is likely more relevant

342   provided that the progeny of the colonizing parasitoid population remains in the crop and

343   continue parasitizing hosts. Under favourable conditions even a few founding female

344   parasitoids could establish an effective population of parasitoids and influence the biocontrol

345   outcome in a growing season. In addition, because the trials lasted approximately 3 days, it

346   could well be the case that we have documented a series of snapshots of a continuous

347   colonization process taking place over longer time scales. This would suggest that fields that

348   were not colonized by parasitoids during the exposure periods, might nevertheless have been

349   colonized at a later stage.

350   Despite intensive sampling we observed parasitism in remnant vegetation plots in

351   only 13 out of 36 cases, indicating that the spatial distribution of parasitoid populations within

352   remnant vegetation is scattered. Assessment of the arthropod community on the dominant

353   plant species in these plots (*Eucalyptus*, *Acacia* and *Chenopodium* spp.) showed no adult

354   whiteflies, which suggests that these species are not a source of alternative hosts for

355   parasitoids of whiteflies (Bianchi et al. 2012). However, less prevalent plant species that were

356   not included in the vegetation assessment could have supported whiteflies. Although our

357   experiment does not irrefutably demonstrate that remnant vegetation was the source of the

358   parasitoids found in the arable fields, it is very likely that the parasitoids that colonized the

359   sentinel plants in arable fields originated from locations outside these fields because fallow

360   and sorghum seedlings (the only other local habitats) do not support whiteflies and their

361   parasitoids. The relatively high levels of parasitism in remnant vegetation in combination with

362   observations that parasitoids move from remnant vegetation into crops early in the season

14

363 (Dyer and Landis 1997, Macfadyen and Muller 2013) provide circumstantial support that

364 remnant vegetation is a source habitat for parasitoids. Ironically, based on the χ^2 test of

365 independence there was no statistical support for the hypothesis that presence of parasitism in

366 remnant vegetation plots was associated with parasitism in nearby arable fields. Further

367 research on the spatial distribution of parasitoids in remnant vegetation patches and the crop

368 colonization process is needed to resolve this apparent paradox.

369   The second analysis of whitefly parasitism by *Encarsia* at the plant level showed the

370 importance of remnant vegetation at different scales. The effects of woody remnant

371 vegetation at 0-100, 100-500 and 500-1000 m on parasitism were all positive. However, the

372 effect of woody remnant vegetation at 1000-1500 m was negative. As the woody remnant

373 vegetation at 1000-1500 m was correlated with the factor landscape (Pearson-correlation

374 coefficient of 0.60), this result may have accounted for the fact that parasitism in the

375 landscape with 13% remnant vegetation (at a scale of 5 km radius) was lower than in the

376 landscape with 6% remnant vegetation. In addition, the area of grassland (at two spatial

377 scales), wind, temperature, dew point and year were also selected as explanatory variables in

378 the most parsimonious model (Table 4). The role of grassland for whitefly-*Encarsia*

379 interactions is unknown, but possibly these habitats provided host plants for whitefly and

380 *Encarsia*. Nevertheless, since this variable had opposing signs for 100-500 and 500-1000 m,

381 we cannot be certain of the ecological function of grassland for whitefly parasitoids. Wind

382 had a positive effect on the parasitism rates. Although parasitoids typically do not fly under

383 windy conditions as it may hinder directed flight (Fink and Volkl 1995), strong winds may

384 also result in occasional undirected redistribution of parasitoids over larger distances. This

385 conjecture could explain the few cases that parasitism was observed in plots further than 400

386 m from remnant vegetation. Temperature had a negative effect on parasitism. Given the

387 relative high day temperatures with some recorded maxima above 30 ºC, this aligns well with

388 the observation that parasitoid wasps avoid flying and have a reduced survival at high

389 temperatures (Dyer and Landis 1996). The positive effect of dewpoint on parasitism indicates

390 that *Encarsia* parasitoids are more active under humid conditions, which limits the risk of

391 dehydration (Sorribas et al. 2012). The variable "year" may have accounted for the different

392 vegetation background in arable fields (fallow in 2007 vs. seedling sorghum in 2008), the

393 weather conditions in 2007 and 2008 prior to the experiment or differences in cropping

394 history in the arable fields. The year of 2007 was characterised by drought, whereas 2008 had

395 more rain. This analysis highlights that both land use and meteorological variables are needed

396 to understand the colonization dynamics of parasitoids in agro-landscapes.

397   In conclusion, our study suggests that remnant vegetation provides important habitats

398 for the conservation of parasitoids that contribute to pest suppression in crops, and that

399 biocontrol services extend beyond 125 m from remnant vegetation into crops. We also show

400 that this colonization is fast (within three days after the introduction of pests) and recurrent

401 (parasitism occurred at all time periods), but the spatial pattern of crop colonization is

402 scattered across the landscape. Therefore, in practice, it may be necessary to monitor whether

403 or not colonization has occurred before a grower should put his trust in natural enemies for his

404 whitefly control. The likelihood of encountering parasitoids is higher near remnant vegetation

405 than in the field centre, hence for the assessment of presence-absence of parasitoids,

406 monitoring should be concentrated near remnant vegetation-field interfaces. The reliability of

407 biocontrol services in an agricultural landscape is expected to increase as more remnant

408 vegetation is available because the frequency of crop colonization by parasitoids from

409 remnant vegetation is increased.

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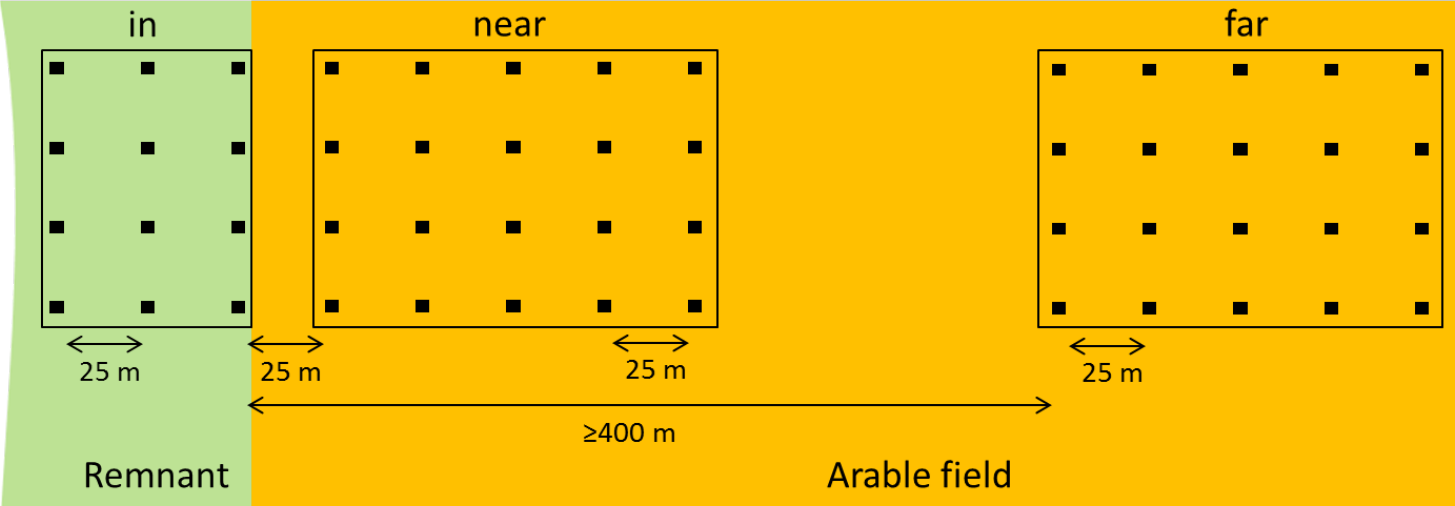
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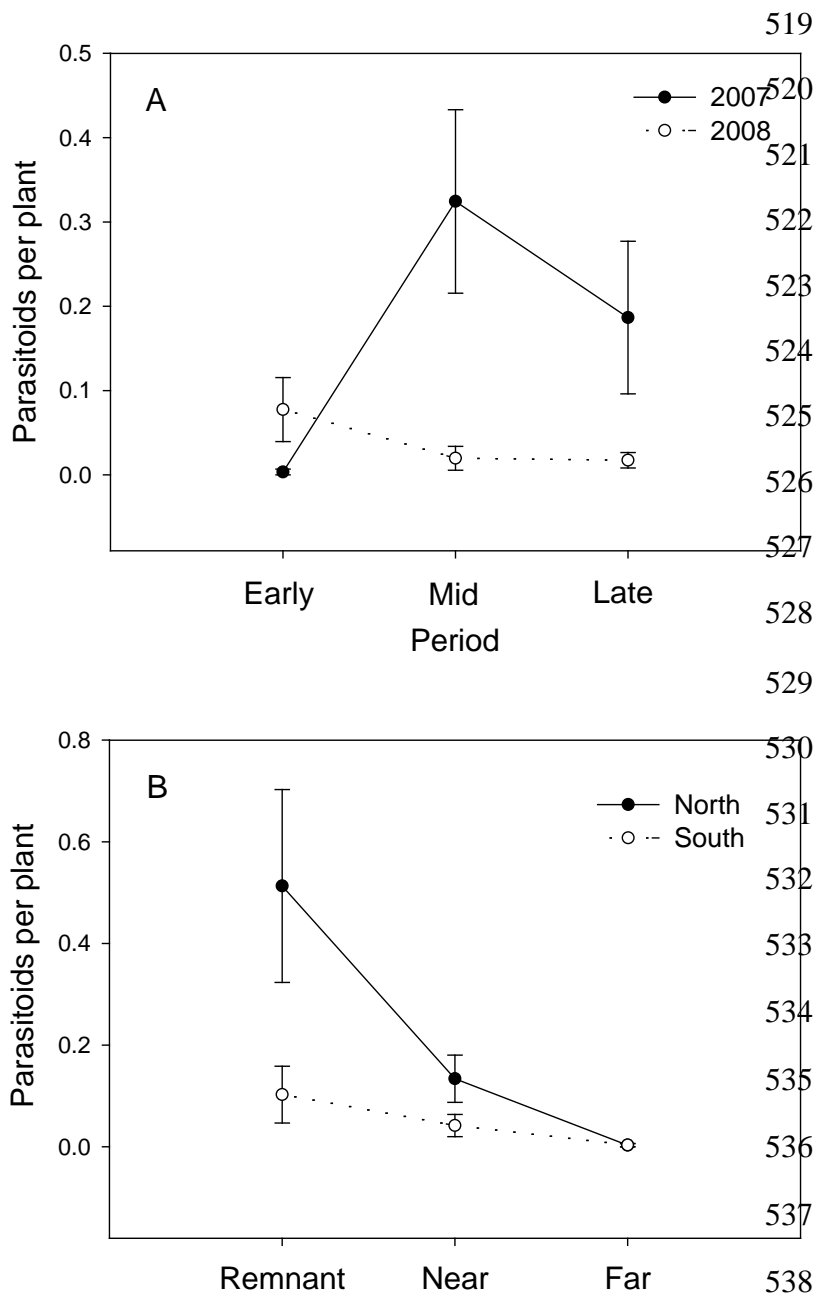
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518   Fig. 1

Fig. 2

Table 1: Overview of model selection statistics for models predicting the number of parasitized whitefly nymphs per plant using factorial variables (landscape, period, treatment, year, and the offset variable log-exposure hours). The number of parameters in the model is indicated by df, AIC_c is the corrected AIC for finite sample sizes, ΔAIC_c is the difference in AIC_c with the model with the lowest AIC_c value, and Akaike weights (Weight) represent the posterior probabilities or "degrees of belief" of the models in the set. Only models with substantial support from the data (i.e. within an AIC_c range of 6 points from the most parsimonious model) are presented.

Landsc	Period	Treat	Year	Landsc:Treat	Period:Year	off(lexph)[†]	df	AIC_c	$\Delta AICc$	Weight
	×	×	×		×		9	554.9	0	0.385
	×	×	×		×	×	9	554.9	0	0.379
×	×	×	×	×	×		12	557.7	2.8	0.095
×	×	×	×	×	×	×	12	558.3	3.4	0.070
×		×	×			×	6	560.7	5.8	0.021
		×	×	×	×	×	5	560.8	5.9	0.020

[†] offset variable log-exposure hours

563   Table 2: Model estimates and 95% confidence intervals (on a natural logarithm scale) of the

564   most parsimonious model based on the factors treatment, year, period and the

565   period:year interaction. The model has an AIC of 554.77 ($\text{AIC}_c = 554.9$) and uses a

566   negative binominal error distribution with $k = 0.0217$ and has an overall mean of

567   0.106.

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	Estimate	lower 95% CI	upper 95% CI
Intercept	-3.981	-7.095	-1.671
Near	-1.735	-2.912	-0.664
Far	-4.716	-6.903	-2.984
Year 2008	2.302	-0.149	5.544
Period 2	4.357	2.001	7.592
Period 3	3.423	1.037	6.657
Year 2008:Period 2	-5.765	-9.418	-2.763
Year 2008:Period 3	-4.418	-8.027	-1.401

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Table 3: Overview of model selection statistics for models predicting the number of parasitized whitefly nymphs per plant using land use and meteorological variables, and the offset variable log-exposure hours. The number of parameters in the model is indicated by df, AIC_c is the corrected AIC for finite sample sizes, ΔAIC_c is the difference in AIC_c with the model with the lowest AIC_c value, and Akaike weights (Weight) represent the posterior probabilities or "degrees of belief" of the models in the set. Only models with substantial support from the data (i.e. within an AIC_c range of 6 points from the most parsimonious model) are presented.

Remnant 0-100 m	Remnant 100-500 m	Remnant 500-1000 m	Remnant 1000-1500 m	Grass 100-500 m	Grass 500-1000 m	Temp	Wind	Dewpoint	Year	off(lexph)[†]	df	AICc	ΔAICc	Weight
×	×	×	×	×	×	×	×	×	×		12	523.7	0	0.328
×	×	×	×	×	×	×	×	×	×	×	12	523.8	0.1	0.313
×	×	×	×	×		×	×	×	×	×	11	525.2	1.5	0.150
×	×	×	×	×		×	×	×	×		11	525.7	2.0	0.118
×	×	×		×		×	×	×	×	×	10	527.5	3.8	0.047
×	×	×		×	×	×	×	×	×	×	11	527.7	4.0	0.038
×	×	×		×		×	×	×	×		10	527.9	4.2	0.036
×	×	×		×	×	×	×	×	×		11	528.3	4.6	0.029
×	×	×		×	×		×	×	×	×	11	528.6	4.9	0.025

[†] offset variable log-exposure hours

Table 4: Model estimates and 95% confidence intervals (on a natural logarithm scale) of the most parsimonious model based on land use and meteorological variables. The model has an AIC of 523.49 (AIC$_c$ = 523.7) and a negative binominal error distribution with $k = 0.0499$.

	Estimate	lower 95% CI	upper 95% CI
Intercept	-5.249	-12.087	1.280
Remnant vegetation 0-100 m	2.412	1.562	3.517
Remnant vegetation 100-500 m	0.276	0.149	0.461
Remnant vegetation 500-1000 m	0.134	0.027	0.259
Remnant vegetation 1000-1500 m	-0.177	-0.362	-0.039
Grassland 100-500m	-0.482	-1.020	-0.162
Grassland 500-1000m	0.163	0.005	0.364
Wind	0.758	0.409	1.177
Temperature	-0.748	-1.304	-0.239
Dew point temperature	0.814	0.433	1.249
Year 2008	-1.288	-2.308	-0.273

Appendix 1

Presence/absence of parasitism at the plot level in plots in, near and far from remnant vegetation. There are 3 spatial replicates $\times$ 2 landscapes $\times$ 3 periods $\times$ 2 years = 36 cases for each distance treatment. A "+" indicates that parasitism was detected, while "-" indicates that no parasitism was observed. These contingency tables are used to test the association between parasitism in plots in remnant vegetation and plots near (A) or far (B) in arable fields using χ^2 test of independence with 1 degree of freedom. The null-hypothesis that presence of parasitism in plots in and near remnant vegetation is independent was not rejected (χ^2=0.486, df=1, P=0.486). Similarly, there was no support for the hypothesis that the incidence of parasitism in plots in and far from remnant vegetation are dependent (χ^2=0, df=1, P=1).

A

		Near +	-	Total
Remnant	+	3	10	13
	-	2	21	23
	Total	5	31	36

B

		Far +	-	Total
Remnant	+	1	12	13
	-	1	22	23
	Total	2	34	36

Appendix 2

Number of parasitized whiteflies per year, landscape, treatment and time period. The numbers are pooled across the 3 spatial replicate plots in each landscape. Treatments are described by the spatial relationship with remnant vegetation.

year	Landscape	Treatment	Period 1	2	3	**Total**
2007	North	In	0	53	37	**90**
		Near	0	41	0	**41**
		Far	0	1	0	**1**
	South	In	0	0	0	**0**
		Near	0	1	13	**14**
		Far	1	0	0	**1**
2008	North	In	4	3	2	**9**
		Near	2	0	2	**4**
		Far	0	0	0	**0**
	South	In	17	2	1	**20**
		Near	0	0	0	**0**
		Far	0	0	0	**0**