Broad spectrum pesticide application alters natural enemy communities and may facilitate secondary pest outbreaks

Matthew P Hill [Corresp., 1], Sarina Macfadyen [1], Michael A Nash [2]

[1] Agriculture & Food, CSIRO, Canberra, Australian Capital Territory, Australia

[2] School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, South Australia, Australia

Corresponding Author: Matthew P Hill
Email address: matthill@protonmail.com

Background. Pesticide application is the dominant method for controlling arthropod pests in broad-acre arable systems. In Australia, organophosphate pesticides are often applied either prophylactically, or reactively, at a higher concentration, to control crop establishment pests such as false wireworms, earwigs and slaters. Organophosphates are reported to be disruptive to beneficial species, though this has not been widely assessed in Australian systems, nor has the risk that secondary outbreaks may occur if the beneficial community composition or function is changed.

Methods. We examined the community ecology of invertebrates in an arable field over successive seasons under rotation (barley, wheat then canola). Two organophosphates (chlorpyrifos and methidathion) were initially applied at recommended rates. After no discernible impact on target pests, the rate for chlorpyrifos was doubled to elicit a definitive response (a level used at establishment when crop damage is observed). Ground-dwelling invertebrates were sampled using pitfalls and refuge traps throughout the experiments. We applied measures of community diversity, principal response curves and multiple generalised linear modelling techniques to understand the changes in pest and beneficial communities. We recorded yield across the field during the experiments and attempted to relate this to the pesticide treatments.

Results. There was large variability due to seasonality and crop type. Nevertheless, both pest and beneficial communities were significantly affected by application of organophosphates. When the rate of chlorpyrifos was increased there was a reduction in the number of beetles that predate on slug populations. Slugs displayed opposite trends to many of the other target pests, and actually increased in numbers under the higher rates of chlorpyrifos in comparison to the other treatments. Slug numbers in the final rotation of canola resulted in significant yield loss regardless of pesticide application. Results indicate communities varied over the 36ha area of the experiment confounding interpretation of yield response.

Discussion. Organophosphates are a cost-effective tool to control emergent pests in broad-acre arable systems in Australia. We found risks associated with prophylactic application in fields under rotation between different crop types and significant changes to the community of pests and beneficials. Disrupting key predators reduced effective suppression of other pests, such as slugs, and may lead to secondary outbreaks when rotating with susceptible crops such as canola. Such non-target impacts are rarely documented when studies focus on single-species, rather than community assessments. This study represents a single demonstration of how pesticide application can lead to secondary outbreaks and reinforces the need for studies that include a longer temporal component to understand this process further.

1   **Broad spectrum pesticide application alters natural enemy communities and may facilitate**

2   **secondary pest outbreaks**

3   Organophosphates and invertebrates

4   Matthew P. Hill[1*], Sarina Macfadyen[1], Michael A. Nash[2]

5   [1]CSIRO, Clunies Ross St., Acton, ACT, Canberra 2601, Australia

6   [2] School of Agriculture, Food and Wine, Waite Campus, University of Adelaide, SA 5005

7   [*] matthill@protonmail.com

8   **Keywords:** organophosphate, pesticide, community ecology, pest suppression, secondary

9   outbreak

10   **Abstract**

11   **Background.**

12   Pesticide application is the dominant method for controlling arthropod pests in broad-acre arable

13   systems. In Australia, organophosphate pesticides are often applied either prophylactically, or

14   reactively, at a higher concentration, to control crop establishment pests such as false wireworms,

15   earwigs and slaters. Organophosphates are reported to be disruptive to beneficial species, though

16   this has not been widely assessed in Australian systems, nor has the risk that secondary outbreaks

17   may occur if the beneficial community composition or function is changed.

18   **Methods.**

19   We examined the community ecology of invertebrates in an arable field over successive seasons

20   under rotation (barley, wheat then canola). Two organophosphates (chlorpyrifos and

21   methidathion) were initially applied at recommended rates. After no discernible impact on target

22   pests, the rate for chlorpyrifos was doubled to elicit a definitive response (a level used at

23   establishment when crop damage is observed). Ground-dwelling invertebrates were sampled

24   using pitfalls and refuge traps throughout the experiments. We applied measures of community

25   diversity, principal response curves and multiple generalised linear modelling techniques to

26   understand the changes in pest and beneficial communities. We recorded yield across the field

27   during the experiments and attempted to relate this to the pesticide treatments.

28   **Results.**

29   There was large variability due to seasonality and crop type. Nevertheless, both pest and

30   beneficial communities were significantly affected by application of organophosphates. When the

31   rate of chlorpyrifos was increased there was a reduction in the number of beetles that predate on

32   slug populations. Slugs displayed opposite trends to many of the other target pests, and actually

33   increased in numbers under the higher rates of chlorpyrifos in comparison to the other treatments.

34   Slug numbers in the final rotation of canola resulted in significant yield loss regardless of

35   pesticide application. Results indicate communities varied over the 36ha area of the experiment

36   confounding interpretation of yield response.

37   **Discussion.**

38   Organophosphates are a cost-effective tool to control emergent pests in broad-acre arable systems

39   in Australia. We found risks associated with prophylactic application in fields under rotation

40   between different crop types and significant changes to the community of pests and beneficials.

41   Disrupting key predators reduced effective suppression of other pests, such as slugs, and may

42   lead to secondary outbreaks when rotating with susceptible crops such as canola. Such non-target

43   impacts are rarely documented when studies focus on single-species, rather than community

44   assessments. This study represents a single demonstration of how pesticide application can lead

45   to secondary outbreaks and reinforces the need for studies that include a longer temporal

46   component to understand this process further.

47 **Introduction**

48 Pesticides predominate management options for control of invertebrate pests in many parts of the

49 world (Thomson & Hoffmann, 2006; Guedes et al., 2016). The most widely used pesticide class

50 in Australia is organophosphates, with ~5,000 tonnes applied annually across agricultural systems

51 in 2002 (Radcliffe, 2002). Despite an increase in use of pesticides, crop losses due to pests have

52 remained largely unchanged for 30-40 years (Altieri & Nicholls, 2004). Beyond the target pests,

53 broad-spectrum pesticides (that kill insects and mites indiscriminately) may affect non-target

54 invertebrate species (Readshaw, 1975), including causing reductions in natural enemy population

55 abundance and activity (e.g. Wilson et al., 1998; 1999), and competition between pest species

56 (known as competitive release, Zeilinger et al., 2016). Assays of invertebrates against weathered

57 residues have shown the persistence of pesticides might play an important part in their negative

58 impacts on natural enemies in the field (Grundy et al., 2000).

59 A potential outcome of frequent broad-spectrum pesticide use is the emergence of pests not

60 controlled by the pesticides and but benefiting from reduced mortality from beneficials and/or

61 competitive release, commonly known as secondary pests (Dutcher, 2007; Gross & Rosenheim

62 2011; Steinmann et al, 2011). Reporting secondary pest outbreaks is challenging as they may also

63 be caused by other mechanisms, which inherently makes it difficult to determine how frequently

64 pesticide-use results in this outcome (Gross & Rosenheim 2011). In cotton fields, it was

65 estimated that 20% of late-season pesticide costs were attributable to secondary pest outbreaks

66 caused by early-season pesticide applications for *Lygus* pests (Gross & Rosenheim 2011). Higher

67 numbers of cotton aphids, *Aphis gossypii* Glover and spider mites, *Tetranychus urticae* Koch

68 were found in cotton fields that received early-season applications of insecticides against

69 *Helicoverpa* spp. (Wilson et al., 1998, 1999). Understanding interactions between resident

70 invertebrate communities and pesticides will help us predict when secondary pest outbreaks are

71 likely to occur, and lead to more informed pest control decision-making.

72 One standardised approach for assessing non-target impacts of pesticides is the International

73 Organization for Biological and Integrated Control - Pesticides and Beneficial Organisms (IOBC)

74 ratings system (Hassan, 1985). This approach has identified a range of toxic and harmful effects

75 of broad-spectrum pesticides on a number of non-target invertebrate species, particularly natural

76 enemies. As this ratings system focuses on standardized sets of "representative" organisms, it

77   does not consider the specific context in which pesticides are being applied, the rate at which they

78   are applied nor the cumulative effects of multiple chemical applications across a season (Nash et

79   al., 2008a). This means that the diverse range of sub-lethal effects are not assessed (Stark et al.,

80   2004). Subsequently, more bioassays under field conditions are needed to incorporate the

81   dynamic interaction between pest populations and their natural enemy communities (Thomson &

82   Hoffmann, 2007) and the environmental context at the time of application. While such studies are

83   rare for examining repeated pesticide applications in arable systems, community-level analyses to

84   examine the effects of genetically modified crops (e.g. Bt cotton) on non-target cspecies are more

85   commonplace (Naranjo 2005; Whitehouse et al., 2007; Rose & Dively, 2007). This suggest that

86   such methods should be transferable to examine repeated pesticide applications on communities

87   of pests and natural enemies within Australian arable systems.

88   In Australian broad-acre grains the pest management practitioners are primarily concerned with

89   pesticide efficacy, crop phytotoxicity and cost; seldom are broader impacts of pesticides included

90   in decision-making (van der Werf, 1996; Umina et al., 2015). As such, more expensive selective

91   pesticides are not favoured. Two broad-spectrum organophosphate pesticides, methidathion and

92   chlorpyrifos, are commonly used to control invertebrate pests. methidathion is typically used to

93   control earth mites and lucerne flea in emerging canola crops, and chlorpyrifos is used to control

94   mite and wireworm larvae around sowing (Gu et al. 2007). Chlorpyrifos is thought to be

95   increasingly applied for the control of pests such as earwigs, isopods (Armadillidiidae) and

96   millipedes (Portuguese millipede, *Ommatoiulus moreleti* Lucas, 1860) (MA Nash *personal*

97   *observations*), despite not being registered specifically to control those pests. A reduced

98   application rate of broad-spectrum pesticides may lessen the impact on natural enemies, but still

99   remain efficacious against pests (e.g. Wiles & Jepson 1995; Wilson et al., 1998). However, when

100   growers fail to achieve what they consider to be adequate pest control they often respond by

101   applying higher rates of pesticides, especially for high yielding crops that are likely to still

102   generate a significant profit despite the added input costs (Edwards et al., 2008). Repeated

103   applications of broad-spectrum pesticides to control typical pest species is common in broad-acre

104   crops, in particular canola (Gu et al., 2007) and pulses (Murray et al., 2013). There are few

105   economic thresholds for many pest species common in Australian grain crops (but see Arthur et

106   al., 2015), therefore growers cannot often relate the pest numbers observed in a field to likely

107   yield losses and adjust pesticide application accordingly. (e.g. aphids; Valenzuela & Hoffmann

108   2015). The outcome is that pesticides are often applied prophylactically or in response to some

109   observed crop damage that may or may not result in yield loss.

110

111   Since the late 1990s, a number of exotic slug species have also emerged as pests of canola at the

112   crop establishment stage across the high rainfall (>500mm growing season rainfall) zones of

113   southern Australia (Nash et al., 2007). Two common species, *Deroceras reticulatum* Müller, 1774

114   and *Milax gagates* Draparnaud, 1801, can inflict significant damage to canola crops before the

115   four-leaf stage leading to plant death (Nash et al., 2007). The increased pest status of slugs is

116   often attributed to the retention of crop residues which serve as habitat and food (Glen, 1989). It

117   may also be due to a reduction in key predator numbers (e.g. predatory beetles Carabidae and

118   Staphylinidae) or a change in broader predator communities (including spiders, ladybeetles,

119   lacewings, predatory mites) as a result of widespread pesticide use (Nash et al., 2008a), including

120   insecticidal seed treatments (Douglas et al., 2015)

121   To predict the impact of pesticides on the interaction between pest species abundance, natural

122   enemy abundance, and crop yield we analyse the change in community composition under

123   application of organophosphates and across a rotation sequence in a commercial grain field. We

124   first investigate how the prophylactic use of pesticides to control earth mites at the establishment

125   stage of crops impacts both pest and natural enemy invertebrate communities. Secondly, we

126   investigate whether structural change to the natural enemy invertebrate community over a period

127   of three seasons led to the outbreak of secondary pest species, in this case slugs. We make an

128   assessment of the yield effects that may be attributed to the trade-offs involved in pesticide

129   applications and discuss both how our data support IOBC ratings, and how growers could use this

130   information in decision-making.

131 **Materials & Methods**

132 *Study site and experimental design*

133 The study site was situated near Mortlake, Victoria, Australia (38°00.5' S, 142°45.3' E) which has

134 a temperate climate with mean maximum annual temperature of 18-21°C and mean annual

135 precipitation is 625 mm. The soils are predominantly of grey sodosols (Isbell, 1996) based on

136 quaternary basalt. The experimental area was located in a 36 ha field managed according to standard

137 district agricultural practices using 2m wide raised (20cm) beds, constructed to alleviate water

138 logging, Seed treatments and fungicides were not applied during this long term experiment, only

139 herbicides to limit confounding non target impacts from other pesticides. The field was divided

140 into 72m wide strips and each was allocated to one of three treatments; methidathion **(**Supracide

141 ®, Syngenta**)** representing conventional district practice, chlorpyrifos (Lorsban ®,

142 DowAgroScience) representing a supposedly more disruptive treatment, and 80 l / ha of water as

143 a control (Fig. 1). The dates of sowing and treatment applications, and the seasonal weather

144 conditions for each year are shown in Table 1. In 2004 and 2005 the pesticides were applied at

145 recommended field rates of 40g active ingredient (a.i.) /ha for methidathion and 250g a.i./ha for

146 chlorpyrifos. Field observations indicated that treatments did not control pests in 2005-2006, so

147 the rate of chlorpyrifos was increased to 500 g a.i. /ha for 2006 and 2007. This double rate was

148 selected as it reflected grower practice when responding to a multitude of establishment pests and

149 was expected to increase disruption to beneficial communities (Table 2). This provided an

150 assessment of the effect of increased application rates on the pest and natural enemy community

151 and is similar to real-world practices where pesticide spray rates are increased in response to

152 visible signs of high pest abundance, or damage. The dates of sowing and treatment applications,

153 and the seasonal weather conditions for each year are shown in Table 1.

154 *Invertebrate sampling*

155 Since 2005, three transects (from now on referred to as blocks) were laid out perpendicular to the

156 treatment strips, so that each bisected the three treatments, achieved maximum interspersion of

157 treatments and achieved spatial independence (Fig. 1). The edges of the strips were avoided by

158 sampling towards the middle of the 72m wide strip. In each block, five census points (12m apart)

159 were selected per replicate (total of 5x3x3 census points). Each census point consisted of four

160 pitfall traps (7 cm deep by 11 cm in diameter filled 190 ml ethylene glycol) to capture macro-

161   invertebrates (> 2mm) communities, and four surface refuge traps to capture slugs (300mm by

162   300mm terracotta paving tile placed on the soil surface as per Nash et al.,2007).

163   Sampling was conducted three times a year to coincide with crop establishment (Zadok's Growth

164   Stage 1)(here in referred to as winter), stem elongation (Zadok's Growth Stage 3) here in referred

165   to as spring) and post-harvest (here in referred to as summer) (Table 1). Traps at each census

166   point were established after sowing and opened for one week, before being closed until later

167   sampling when reopened for one week. The pitfall trap catch was returned to the laboratory and

168   sieved (200 μm mesh) prior to sorting under a dissection microscope. The refuge traps were

169   turned over in the field and the number and identity of the slugs on the underside recorded in the

170   field, then all individuals were removed with a subset retained in 70% ethanol as vouchers.

171   From the invertebrate data collected in the pitfall traps we defined two functional communities,

172   *pests* and *beneficials* (natural enemies), and examined changes within these two broad groups as

173   well as targeted analysis on individual species or taxa (Table 2). As some taxa such as millipedes

174   and earwigs may sometimes act as either pest or beneficial organisms it can be difficult to

175   broadly classify them at this level. However, here we assigned them as pests as they may inflict

176   damage, but may also act as natural enemies at certain times. Taxa such as ants (Formicidae) are

177   also hard to assign to one of these two groups in this system, as they perform roles outside of

178   pests and beneficials, and so were omitted from subsequent analysis. For some common pest and

179   natural enemy species, their identification is straightforward. However, for many taxa we lack

180   species descriptions (especially for immature stages) and in these cases family-level identification

181   was conducted.

182   *Yield*

183   Yield of the crops grown during this study were recorded using Advanced Farming Systems

184   (AFS) features available for Case IH combine harvester, with different machines being used over

185   the course of the study. The software recorded clean grain flow and moisture whilst harvesting,

186   along with geographic co-ordinates (WGS 84). The data was calibrated in accordance with

187   individual user manuals, but to ensure accuracy actual weights obtained from point of sale where

188   used to correct data to tonnes/ha for each season. Initial data handling and maps were developed

189   using the SMS™ Advanced Software Ver. 8.0 (Ag Leader Technology®, Ames, Iowa). Krigged

190   data of yield was used to create contour maps to compare with the invertebrate samples collected

191   in the transects. Because of differences in collection of spatial data, tolerances were set at 10m^2

192 for yield data, and 6m² for invertebrate census points, not all points overlayed exactly so

193 corresponding data was matched and extracted manually. Geographic referenced information was

194 converted to Cartesian coordinates using the software GEOD Ver. 3.42 (Graham Samuel &

195 Associates Pty Ltd, Charlestown, NSW). Spatial analysis was conducted in the software Surfer©

196 Ver. 8.05 (Golden Software, Inc. Colorado).

197 *Statistical Analyses*

198 We first calculated species richness as total number of taxa present, for each sample across

199 treatments and sampling times to examine overall effect of pesticide application across the study

200 period. We assumed that each taxa represented one species (even if sorted to family-level),

201 although this is likely to underestimate species diversity. We calculated species turnover within

202 each treatment through time, using the "codyn" package in R (Hallett *et al.,* 2016). This analysis

203 allows for the total turnover to be calculated per time point (i.e. the proportion of species that

204 differ between time points either by appearing or disappearing), but also the proportion of species

205 that appear and disappear at each time point. We then examined the mean rank shifts for each

206 treatment, which give an indication of the degree of species reordering between two time points,

207 again using the codyn package (Hallett *et al.,* 2016).

208 To examine the effects of pesticides on the pest and natural enemy communities, we employed

209 two different methods that have recently been evaluated for use in ecotoxicology studies (Szöcs

210 et al., 2015). The first of these are principle response curves (PRC; Van den Brink & Ter Braak,

211 1999), which are widely used, from freshwater mesocosm studies through to non-target effects of

212 pest-resistant crops such as *Bt* cotton, to examine community level response to pesticides (or

213 control strategies) over time (e.g. Naranjo 2005; Whitehouse et al., 2007; Rose & Dively, 2007;

214 Pascual et al., 2010). The second method fits separate generalised linear models (GLMs) to each

215 species, to give an overall community analysis (Wang et al., 2012) and has recently been applied

216 to investigate pesticide effects on communities (Szöcs et al., 2015). A principal response curve

217 (PRC) is a time-dependent multivariate technique based on RDA (redundancy analysis). By

218 incorporating treatment, time and the interaction thereof, a PRC allows for visualizations of a

219 treatment effect through time on community structure, by highlighting variance in overall

220 response. We used the function "prc" in the R package "vegan" to conduct these analyses. To

221 evaluate the significance of treatment at each sampling point, we conducted single RDAs and

222 used a permutation structure to account for the blocking design of the study.

223   Whilst PRCs are useful for examining the overall community response to different treatments,

224   methods that incorporate GLMs appear more robust for indication of responding taxa within the

225   groups, particularly for identify responding taxa that would be missing on the first axis in the

226   PRC (Szöcs et al., 2015). Further to this, PRC (and RDA) are also based on Euclidean distance,

227   so that double zeroes can affect interpretation of the results. An additional strength of using

228   GLMs here is that they are better able to handle count data, through using poisson or negative

229   binomial distributions, to provide a non-normal data parametric framework (O'Hara et al., 2010)

230   and avoid the need for data transformation. However, PRCs are better for describing the direction

231   of the effect on the community and thus using PRC and GLMs together allows for complimentary

232   analysis on community and treatment data through time. Using the R package "mvabund" and

233   function "manyglm" we ran separate GLM per species, using negative binomial distributions, for

234   the two communities (pests and beneficials), with the three treatments and time points, and the

235   interaction thereof, as the dependent variables. We ran null models to investigate the overall

236   effect of treatment, and to investigate the interaction of treatment and time. Finally, we performed

237   separate analyses at each sampling time point to examine differences between communities for

238   each treatment, using Likelihood Ratio tests on the univariate responses (species) and 1000

239   bootstrap repeats. All of these analyses used a permutation structure incorporating the blocked

240   design of the study. To visualise changes in the pest and beneficial communities in response to

241   the treatments, described through the GLM analyses, we plotted the combined deviance (effect

242   size) for the members of each community across each of the sampling points.

243   Assessing the impact of the ultimate outcome of the pesticides treatments on crop yield was

244   challenging due to the large seasonal fluctuations in conditions for crop growth and underlying

245   spatial patterns in crop productivity across large fields. However, ideally every pesticide input

246   should provide some yield benefit for the grower each year, regardless of seasonal conditions,

247   usually through protecting the crop from damage due to pests. In our study this should manifest

248   as a significant increase in crop yield in the treated parts of the field in comparison to the control

249   area. Prior to analysis of yield we removed outliers (high values for yield) that corresponded to

250   instances where the header stops during harvest and extra ingrain is collected. These outliers were

251   identified as being further than two standard deviations from the mean, per block, per year. We

252   then performed separate season GLMs with the yield as response, and Treatment and Block as

253   fixed effects, including the interaction between Treatment and Block. As there was almost always

254   a significant interaction, pairwise contrasts using the "lsmeans" package in R, were used to

255   determine where treatments differed within blocks.

256   **Results**

257   115 different species or taxa were identified in total from our samples. Prior to the increased rate

258   of chlorpyrifos application, the initial winter samples at crop emergence in July 2005 were the

259   lowest in species richness, but the October 2005 spring sample contains the highest species

260   richness. This large amount of seasonal variation in community composition is further

261   highlighted by the first 2006 sample yielding low species richness again (Figure 2a). Importantly

262   richness through time suggests there was no difference between treatments for the first three

263   sampling points, supporting the rationale behind increasing the chlorpyrifos application rate.

264   Species turnover was high and similar across treatments (65-80%) for the samples prior to the

265   rate increase of chlorpyrifos, again reflecting the seasonal nature of the species examined (Figure

266   2b). After the chlorpyrifos concentration was increased in 2006, species richness starts to change

267   between the treatments (Figure 2a), with chlorpyrifos having the highest richness in the spring

268   2006 sampling, before the lowest in the winter 2007 sample. The methidathion treatment had a

269   higher richness than the control and chlorpyrifos in the last two sampling time points. Species

270   turnover began to differ between treatments following the increase in chlorpyrifos, and is much

271   more variable in the chlorpyrifos treatment than the control or methidathion. Over the course of

272   the entire experiment, the mean rank shift pattern reflects richness and turnover, and suggests that

273   variability between treatments for the abundance of different species becomes increased through

274   time, compared to the control (Figure 2c).

275   To display how key species from the PRC and multiple GLM analyses (see below) changed in

276   abundance patterns through the trial, we plotted the temporal abundance per treatment for slugs

277   and their potential predators, carabids and staphylinids, as well as other species displaying large

278   responses: earth mites and earwigs (Figure 3). Outside the summer samples in 2006 and 2007

279   (reflecting their seasonality and lack of suitable resources for them post crop harvest), slugs were

280   consistently more abundant in the chlorpyrifos treatment than the control and methidathion. This

281   is contrast to other pest species such as earth mites and earwigs, which display lower abundance

282   in the organophosphate treatments, especially towards the end of the trial (Figure 3). While some

283   of the slug abundance patterns may be explained by less mortality from predators, the response of

284   the main predator group (in this dataset) is complex. The Carabidae (most belonging to the tribes

285   Broscinnae and Pteristninnae) initially show quite high abundance, but then for July 2006 and

286   June 2007, carabid numbers in the chlorpyrifos treatment are well below the control and

287   methidathion treatments. After the 2006 pesticide application (including the higher rate) carabids

288   are absent in the chlorpyrifos treatment, with only a few individuals in the methidathion and

289   control treatments. Carabidae numbers recover and increase from this point, possibly responding

290   to the high abundance of slugs, until the 2007 pesticide application: following this event the

291   Carabids are reduced again to zero in the chlorpyrifos treatment, whilst persisting in the

292   methidathion and the control treatments. The staphylinids were heavily reduced in numbers in the

293   chlorpyrifos treatment (and methidathion but not to as great extent) following the 2006 pesticide

294   application.

295   The natural enemy community initially displayed an increase over the control as shown by the

296   principal response curve (Figure 4a). In 2006 the effect switches to become negative, and for

297   methidathion it stays negative. Chlorpyrifos, however, goes back to a positive effect at the final

298   time point. The carabids are strongly weighted against the community trend, indicating that they

299   likely had fewer numbers in the chlorpyrifos treatment by the end of the study period. Predatory

300   bugs also do not follow the treatment effects on the community patterns. For the pests PRC

301   (Figure 4b), there was no differences between the control and chlorpyrifos or methidathion for

302   the initial applications. Over time, the target pests (earwigs, earth mites and millipedes) all

303   exhibit strong positive weightings to the negative effect of the pesticide applications, in particular

304   chlorpyrifos (Figure 4b). Unlike the target pests, slugs show an opposite trend towards the

305   temporal pest community response (Figure 4b).

306   The multiple GLM approach broadly agrees with the results from the PRC, as reflected in

307   significant ($P < 0.05$) and non-significant ($P > 0.05$) community differences at most of the same

308   sampling periods. The exception is that for the pestcommunity, the GLM approach determined

309   the June 2005 and September 2006 samplings as significantly different from the control, with the

310   PRC only marginally significant (0.062 and 0.094, respectively). For both the beneficials and

311   pests, the community was significantly affected following the increase in the rate of chlorpyrifos

312   (Table 3). The carabids and staphylinids showed the largest contributions to the overall

313   community trends, with 23.7 and 13.4%, respectively (Figure 5c). The carabids were significantly

314   affected in October 2006 and June 2007 (Table 3), whereas the Staphylinids in July 2006 and

315   marginally in March 2006 (Table 3). Other species, such as the predatory bugs (June 2007) and

316   wasps (July 2006) also display significant effect sizes following the spray events (Figure 5c;

317   Table 3). The multiple GLMs on the pest community indicates that effect sizes were also greatest

318   following the spray events (Fig 6a), especially for chlorpyrifos (Fig 6b). Overall, the pest

319   community in all but two samples (both prior to increased rate of chlorpyrifos) was significantly

320   affected by pesticide application (Table 3). The effect sizes also appear to increase with time, but

321   this may also be due to the rotation into canola, where isopods, mites, aphids and earwigs were

322   all significantly affected by the organophosphate treatments. The earwigs and isopods had the

323   largest contributions across the trial (16.5 and 12.2%, respectively; Figure 6c), but this appears to

324   be driven mostly by the last sample taken in the canola crop. The weevils, millipedes and

325   orthopterans also provided contributions between 10-11% each (Figure 6c). As opposed to

326   following a spray event, the effect size for the slugs is greatest in September 2006.

327   There was a large amount of spatial variation in the yield in the areas of the field corresponding

328   to the difference treatments, and this was further complicated due to an interaction with the block

329   (Figure 7). For the barley (2004 crop) yield, harvested in 2005, there was an overall treatment

330   effect ($\chi^2_2 = 7.154$, $p < 0.028$), but this appears to be driven by block 3 (yield in the Control was

331   significantly lower than yield in both treatments). Following this, there was a significant block

332   effect ($\chi^2_2 = 10.4381$, $p < 0.001$) and significant interaction between the treatments and the block

333   ($\chi^2_4 = 14.3202$, $p < 0.001$) (Fig. 7). Following an increase in the rate of chlorpyrifos, for the wheat

334   (2005 crop) yield harvested in 2006 the overall treatment effect was significant ($\chi^2_2 = 43.381$, $p <$

335   0.001) with yield significantly higher in the chlorpyrifos treatment than the control in blocks 1

336   and 3, but lower in block 2 (all significant, $p < 0.001$). Methidathion yielded significantly lower

337   than the control and chlorpyrifos in blocks 1 and 3 ($p < 0.05$), and significantly higher than

338   chlorpyrifos in block 2 ($p < 0.001$). These differences gave an overall significant block effect (χ^2_2

339    $= 96.990$, $p < 0.001$) and a significant overall interaction effect ($\chi^2_4 = 162.896$, $p < 0.001$) (Fig. 7).

340   For the wheat (2006 crop) harvested in 2007, the chlorpyrifos gave consistently higher yields

341   than the control and methidathion in blocks 1 and 3 ($p < 0.01$), and there was a significant overall

342   Treatment effect ($\chi^2_2 = 65.186$, $p < 0.001$). There was no significant difference between all

343   treatments in block 2. The control and methidathion were only significantly different from one

344   another in block 1. Again, there was a significant block effect ($\chi^2_2 = 37.103$, $p < 0.001$) and a

345   significant overall interaction ($\chi^2_4 = 40.995$, $p < 0.001$) (Fig. 7). Finally, for the canola yield

346   harvested in 2008, there was no data in blocks 1 and 2 as seedlings were completely lost due to

347   slug predation at establishment (July 2007), regardless of the treatments (i.e. the treated areas still

348   suffered the same damage). In the remaining block (3), Chlorpyrifos (at the higher application

349   rate) was significantly lower in yield than the control and methidathion treatment ($p < 0.001$) and

350   there was an overall significant treatment effect ($\chi^2_2 = 56.911$, $p < 0.001$)(Fig. 7).

351   **Discussion**

352   Although the overall interactions of season, pesticide application and crop type on both pest and

353   natural enemy communities are complex (e.g. Brust et al., 1985; Holland & Luff, 2000), this

354   study provides some indication of how rotational systems may change in response to

355   conventional pesticide use. Importantly, the community composition with standard rates of

356   pesticides was very similar to the control treatments, however after higher rates were used, the

357   change in community composition was marked. We demonstrated that such pesticide applications

358   are likely to come with trade-offs associated with the reduction in important predatory species,

359   and that the timing of these reductions may have profound effects on pest suppression and crop

360   production. While the absence of key predators may not always be a problem for one crop type, it

361   may be critical for a rotation into another, where key establishment pests are not adequately

362   suppressed. There was no apparent benefit to yield by applying the organophosphates, or any

363   benefit was confounded by spatial variation in crop productivity.

364   Not all invertebrates will be directly affected by organophosphates in the field, but the disruption

365   of important predators at critical times (e.g. a certain crop type) may be more consequential to

366   pest suppression than overall community effects. The final rotation into canola here demonstrates

367   how the reduction of carabids at this point was more detrimental to the grower than the

368   reductions of either carabids and staphylinids in the prior wheat rotations, and the subsequent

369   outbreak of slugs in the increased chlorpyrifos treatment appears indicative of a secondary

370   outbreak. Whilst it is difficult to draw a causal link between absence of predators and the

371   outbreak of slugs in this study, the reduction of slugs by carabids has been demonstrated in

372   similar systems (Nash 2008b) . Our results draw an interesting parallel to a recent study which

373   found that imidacloprid applications also increased slug issues due to disruption of adequate

374   biological control through non-target effects (Douglas et al., 2015). Such field response data are

375   important, as there are few studies that use field evaluation of non-target effects of pesticides (e.g.

376   Staübli et al., 1984; Jenkins et al., 2013), most studies typically use laboratory bioassays

377   (Thomson & Hoffmann, 2006), or short-term small-plot trials (e.g. Macfadyen et al., 2012;

378   Macfadyen et al., 2014). Further testing of acute and sub-lethal effects under semi-field

379 conditions is required to test our findings, and like Jenkins et al., (2013), we suggest that

380 laboratory assessments of toxicity should be extrapolated with caution to the field setting. More

381 longer term studies to examine the cumulative effects of repeated pesticide applications are

382 required.

383 In Australia, short-term semi-field studies (Jenkins et al., 2013) have suggested that the impacts

384 of chlorpyrifos are not as disruptive to natural enemies as previously thought (Curtis and Horne,

385 1995), however cumulative impacts over longer time periods are considered disruptive in

386 viticulture (Nash 2010) and arable systems (Nash 2008a). The strong negative response of

387 carabids (Pteristcninnae) to chlorpyrifos is concordant with overseas data on the closely related

388 *Pterostichus melinarius*, with slightly higher rates (720g a.i.) being considered harmful (IOBC

389 rating 3) (Hassan et al., 1988). Lower rates (480 a.i.) have been shown to be less harmful (IOBC

390 2) to the carabid *Bembidion* sp, in field trials when compared to lab assays (Floate et al., 1989),

391 however toxicity responses vary between studies and methodologies, ranging from IOBC 2-4

392 (Cockfield & Potter, 1983; Bale et al., 1992; Turner et al., 1990). There is limited data on

393 methidathion impacts on beneficial species, however it has been considered as very harmful

394 (IOBC 4) to green lacewings in semi-field trials (Hassan et al, 1985) and harmful (IOBC 3) to

395 spiders, predatory bugs and green lacewings in the field (). We did not find methidathion to cause

396 significant reductions on those populations here, however this may be due to the lower rate

397 applied (40 g a.i.) compared to a previous field study (120 g a.i.) (Staübli et al., 1984). Outside of

398 acute toxicity, behavioral aspects can change exposure to pesticides. As important as the results

399 are for the species that did show strong effects, the same can be said for those species groups that

400 did not appear to be heavily impacted by the pesticides. Although spiders, ladybirds and

401 hoverflies were not targeted using the pitfall methods herein, these species groups may have also

402 avoided the winter pesticide application due to their activity at later crop stages, or they may have

403 a greater tolerance to organophosphates.

404 Target pests, earth mites, earwigs and millipedes, were all controlled by the organophosphates

405 (particularly chlorpyrifos at the increased rate). Varying susceptibilities to organophosphates,

406 including methidathion, are found within the common earth mite speces: *Penthaleus falcatus* has

407 a higher tolerance to methidathion than either of *P. major, P. tectus* or *Halotydeus destructor*

408 (Umina & Hoffmann, 1999). Chemical control is the dominant control option for earth mites,

409 despite this study indicating no discernible yield response warranting application targeting these

410　pests. Earwigs (including *Forficula auricula*) are widespread in southern Australian grain

411　systems, and although they are typically considered as sporadic pests (e.g. Murray et al., 2013),

412　their role as pest or beneficial species is presently unclear. In addition to acute toxicity and high

413　rates of mortality, chlorpyrifos-ethyl has been shown to reduce the predatory behaviour of the

414　earwig *F. auricula* in orchards (Malagnoux et al., 2015), where they are considered effective

415　biological control agents. The strong response of earwigs to organophosphates suggests that any

416　form of pest suppression service in grains crops could be hampered by the application of harmful

417　organophosphates such as chlorpyrifos. Despite some limited data (unpublished report, Hart Field

418　Site Group SA 2015), there is little known about the ability of organophosphates to control

419　millipedes, and there are no currently registered chemicals for control in Australian grains.

420　To better understand if secondary outbreaks in the canola crop followed previous suppression of

421　natural enemies by organophosphates, this study should have ideally continued through two

422　complete rotations (6-7 years). Despites this, undertaking this study over four seasons and

423　different crop rotations gives some indication of the trade-offs and long-term effects of pesticides

424　on the pest species response, and the response of the natural enemy communities that co-occur

425　with them. Most of the key invertebrate species here would have undergone multiple generations

426　during the experiment, which implies that some sublethal effects (e.g. reduced fecundity,

427　survivorship of immature stages, short-term toxicity effects) should have been captured during

428　this experiment. While changing the application rate of chlorpyrifos during the experiment is not

429　ideal from an analysis point of view, it provides a realistic scenario of how growers adjust rates

430　and frequency of application in response to perceived pest threats. The continued use of

431　organophosphates as a default for control of insect pests in Australian grains is perhaps best

432　exemplified by an emergency permit for use of chlorpyrifos and Pirimicarb (APVMA 82792) to

433　control Russian Wheat Aphid (*Diuraphis noxia* Kurdjumov 1913) in response to its incursion in

434　2016.

435　We did not observe spatially consistent yield benefits from applying pesticides, and in canola the

436　application of pesticides did not prevent widespread seedling loss from slugs. Furthermore, there

437　was only one instance where the control had significantly lower yield than both the chlorpyrifos

438　and methidathion treatments, and this benefit was not seen across the whole study area (barley

439　04/05 block 3, Fig. 7). Large spatial variation within the field in the yield response may be related

440　to spatial variation in the abundance of pests and beneficials. However, given the significant

441   interactions observed between the effect of pesticide treatments and the blocks used to control for

442   spatial variation, it is likely that other invertebrate species that we did not record may be

443   involved. The detrimental effects of chlorpyrifos on the key slug predators may be evident from

444   the lower yield seen in the last remaining block of canola during the final year of the study.

445   Unfortunately, having only one block surviving makes it hard to test this pattern further.

446   The lack of a spatially consistent yield benefit from the application of organophosphates suggests

447   that growers could limit broad-spectrum pesticide applications without risking any crop losses

448   due to invertebrate pests. This could be achieved through either applying a threshold-based

449   approach to spray decisions, or selectively targeting areas of the field that may be at risk. For *H.*

450   *destructor* a recently published study examined thresholds associated with economic crop losses

451   (Arthur et al., 2015), and recommendations for control have called for rotation in the use of

452   chemicals, non-chemical management options and crop rotations (National Insecticide Resistance

453   Management Working Group, 2016). Managing for control failures due to resistance in pests is an

454   important component of grains pest management in Australia, and the risk of secondary outbreaks

455   appears to require similar attention. As much of Australian grains production includes rotation

456   with other crops, understanding and responding to the risk of secondary pest outbreaks will

457   require growers to manage their pesticide-use across an entire rotation. More ecologically-based

458   management approaches are being developed, including promoting conditions to favour higher

459   abundances of carabids and other slug predators (Le Gall & Tooker, 2017). This may involve

460   managing the field-margins to provide more suitable habitat for these predators, or planting

461   cover-crops that are more attractive to the slugs, before the cash crop. This earlier planting of a

462   cover crop allows for predator numbers to build up, before terminating the cover crops with

463   herbicides, perhaps allowing for more effective control during the growth of the cash crop (Le

464   Gall & Tooker, 2017). Despite some Australian growers investigating the use of cover crops, to

465   our knowledge this approach to pest slug management is not yet explored adequately in

466   Australian grains.

467   **Conclusions**

468   Demonstrating the long-term effects of organophosphates on the ecology of invertebrate species

469   within Australian grains systems is complex, due to the scale of production, diverse rotation

470   practices, and inter-annual variation of species diversity and abundance. Despite this, there are

471 important points that arise from this experiment conducted across a standard crop rotation.

472 Firstly, the prophylactic use of organophosphates as a management strategy requires

473 understanding of the risks of secondary outbreak in both the current crop and subsequent crops in

474 the rotation. Secondly, quantifying the impact of reactive management strategies (such as

475 increasing pesticide rates) on pest and natural enemy communities will allow growers to make

476 more informed judgements on the risk of disrupting biological control services.

477 **Acknowledgements**

481   **References**

482   Altieri MA, Nichols CI. 2004. Biodiversity and Pest Management in Agrosystems, CRC Press,

483   2nd edition.

484   Arthur AL, Hoffmann AA, Umina PA. 2015. Challenges in devising economic spray thresholds

485   for a major pest of Australian canola, the redlegged earth mite (*Halotydeus destructor*).

486   *Pest Management Science* **71(10):**1462-1470

487   Bale JS, Ekebuisi M, Wright C. 1992. Effect of seed bed preparation, soil structure and release

488   time on the toxicity of a range of grassland pesticides to the carabid beetle *Pterostichus*

489   *melanarius* (Ill.) (Col., Carabidae) using a microplot technique. *Journal of Applied*

490   *Entomology* **113(1-5):**175-182

491   Brust GE, Stinner BR, McCartney DA. 1985. Tillage and soil insecticide effects on predator-

492   black cutworm (Lepidoptera: Noctuidae) interactions in corn agroecosystems. *Journal of*

493   *Economic Entomology* **78:** 389–1392

494   Cockfield SD, Potter, DA. 1983. Short-term effects of insecticidal applications on predaceous

495   arthropods and oribatid mites in Kentucky bluegrass turf. *Environmental Entomology*

496   **12(4):** 1260-1264

497   Curtis JE, Horne, PA. 1995. Effect of chlorpyrifos and cypermethrin applications on non-target

498   invertebrates in a conservation-tillage crop. *Australian Journal of Entomology* **34:**229–

499   231

500   Douglas MR, Rohr, JR, Tooker JF. 2015. Neonicotinoid insecticide travels through a soil food

501   chain, disrupting biological control of non   target pests and decreasing soya bean yield.

502   *Journal of Applied Ecology* **52(1):**250-260

503   Dutcher JD. 2007. A review of resurgence and replacement causing pest outbreaks in IPM. In

504   *General concepts in integrated pest and disease management* (pp. 27-43). Springer

505   Netherlands.

506   Edwards OR, Franzmann B, Thackray D, Micic S. 2008. Insecticide resistance and implications

507   for future aphid management in Australian grains and pastures: a review. *Australian*

508   *Journal of Experimental Agriculture* **48(12):** 1523-1530

509   Floate KD, Elliott RH, Doane JF, Gillott C. 1989. Field bioassay to evaluate contact and residual

510   toxicities of insecticides to carabid beetles (Coleoptera: Carabidae). *Journal of Economic*

511   *Entomology* **82(6):**1543-1547Glen DM. 1989. Understanding and predicting slug

512   problems in cereals. In: Henderson IF. (ed.) Slugs and Snails in World Agriculture.

513   Thorton Heath: British Crop Protection Council.

514 Gu H, Fitt GP, Baker GH. 2007. Invertebrate pests of canola and their management in Australia: a

515   review. *Australian Journal of Entomology* **46:**231-243

516 Guedes RNC, Smagghe G, Stark JD, Desneux N. 2016. Pesticide-induced stress in arthropod

517   pests for optimized integrated pest management programs. *Annual Review of Entomology*

518   **61:**43-62

519 Gross K, Rosenheim JA. 2011. Quantifying secondary pest outbreaks in cotton and their

520   monetary cost with causal   inference statistics. *Ecological Applications* **21(7):**2770-2780

521 Grundy PR, Maelzer D, Collins PJ, Hassan E. 2000. Potential for integrating eleven agricultural

522   insecticides with the predatory bug *Pristhesancus plagipennis* (Hemiptera: Reduviidae).

523   *Journal of Economic Entomology* **93:**584-589

524 Hallett LM, Jones SK, MacDonald AAM, Jones MB, Flynn DF, Ripplinger J, Slaughter P, Gries

525   C, Collins SL. 2016. codyn: An R package of community dynamics metrics. *Methods in*

526   *Ecology and Evolution* **7(10):**1146-1151

527 Hassan SA. 1985. Standard methods to test the side-effects of pesticides on natural enemies of

528   insects and mites developed by the IOBC/WPRS Working Group 'Pesticides and

529   beneficial organisms'. Bulletin OEPP/EPPO **15:**214-255

530 Hassan SA, Bigler F, Bogenschütz H, Boller E, Brun J, Chiverton P, Edwards P, Mansour F,

531   Naton E, Oomen PA, Overmeer WPJ, Polgar L, Rieckmann W, Samsøe-petersen L,

532   Stäubli A, Sterk G, Tavares K, Tuset JJ, Viggiani G, Vivas AG. 1988. Results of the fourth

533   joint pesticide testing programme by the IOBC/WPRS-Working Group "Pesticides and

534   Beneficial Arthropods". *Journal of Applied Entomology* **105:**321-329

535 Holland J, Luff M. 2000. The effects of agricultural practices on Carabidae in temperate

536   agroecosystems. *Integrated Pest Management Reviews* **5:**109-129

537 Isbell RF. 1996. The Australian Soil Classification, Melbourne, CSIRO Publishing.

538 Jenkins S, Hoffmann AA, McColl S, Tsitsilas A, Umina PA. 2013. Synthetic pesticides in agro-

539   ecosystems: are they as detrimental to nontarget invertebrate fauna as we suspect?

540   *Journal of Economic Entomology* **106(2):**756-75

541 Le Gall M, Tooker JF. 2017. Developing ecologically based pest management programs for

542   terrestrial molluscs in field and forage crops. *Journal of Pest Science* **3(90):** 825-838

543 Macfadyen S, Zalucki MP. 2012. Assessing the short-term impact of an insecticide
544   (Deltamethrin) on predator and herbivore abundance in soybean glycine max using a
545   replicated small-plot field experiment. *Insect Science* **19(1):**112–20

546 Macfadyen S, Hardie DC, Fagan L, Stefanova K, Perry KD, DeGraaf HE, Holloway J, Spafford
547   H, Umina PA. 2014. Reducing insecticide use in broad-acre grains production: an
548   Australian study. *PloS one* **9(2):** e89119

549 Malagnoux L, Capowiez Y, Rault M. 2015. Impact of insecticide exposure on the predation
550   activity of the European earwig *Forficula auricularia*. *Environmental Science and*
551   *Pollution Research* **22(18):** 14116-14126

552 Murray DAH, Clarke MB, Ronning DA. 2013. Estimating invertebrate pest losses in six major
553   Australian grain crops. *Australian Journal of Entomology* **52:**227-241

554 Naranjo SE. 2005. Long-term assessment of the effects of transgenic Bt cotton on the abundance
555   of nontarget arthropod natural enemies. *Environmental Entomology* **34(5):** 1193-1210

556 Nash MA, Thomson LJ, Hoffmann AA. 2007. Slug control in Australian canola: monitoring,
557   molluscicidal baits and economic thresholds. *Pest Management Science* **63(9):** 851-859

558 Nash MA, Thomson LJ, Hoffmann AA. 2008a. Effect of remnant vegetation, pesticides and farm
559   management on abundance of the beneficial predator *Notonomus gravis* (Chaudoir)
560   (Coleoptera: Carabidae). *Biological Control* **46:**83-93

561 Nash, MA, Thomson, LJ, Horne, PA, Hoffmann, AA (2008b) *Notonomus gravis* (Chaudoir)
562   (Coleoptera: Carabidae) predation of *Deroceras reticulatum* Muller (Gastropoda:
563   Agriolimacidae), an example of fortuitous biological control. Biological Control 47, 328-
564   334.Nash MA, Hoffmann AA, Thomson LJ. 2010. Identifying signature of chemical
565   applications on indigenous and invasive nontarget arthropod communities in vineyards.
566   *Ecological Applications* **20(6):**1693-1703

567 O'Hara RB, Kotze DJ. 2010. Do not log-transform count data. *Methods in Ecology & Evolution*
568   **1:**118–122

569 Radcliffe JC. 2002. 'Pesticide Use in Australia.' (Australian Academy of Technological Sciences
570   and Engineering: Melbourne.)

571 Readshaw JL. 1975. Biological control of orchard mites in Australia with an insecticide-resistant
572   predator. *Journal of the Australian Institute of Agricultural Science* **41(3):** 213-214

573 Rose R, Dively GP. 2007. Effects of insecticide-treated and lepidopteran-active Bt transgenic
574   sweet corn on the abundance and diversity of arthropods. *Environmental Entomology*
575   **36(5):** 1254-1268

576 Stark JD, Banks JE, Acheampong S. 2004. Estimating susceptibility of biological control agents
577   to pesticides: influence of life history strategies and population structure. *Biological*
578   *Control* **29(3):**392-398

579 Staübli A, Hächler M, Antonin P, Mittaz C. 1984. Tests de nocivité de divers pesticides envers les
580   ennemis naturels des principaux ravageurs des vergers de poiriers en Suisse romande.
581   *Revue Suisse Viticulture Arboriculture Horticulture* **16(5):**279-286

582 Steinmann KP, Zhang M, Grant JA. 2011. Does use of pesticides known to harm natural enemies
583   of spider mites (Acari: Tetranychidae) result in increased number of miticide
584   applications? An examination of California walnut orchards. *Journal of Economic*
585   *Entomology* **104(5):** 1496-1501

586 Szöcs E, Van den Brink PJ, Lagadic L, Caquet T, Roucaute M, Auber A, Bayona Y, Liess M,
587   Ebke P, Ippolito A, ter Braak CJ, Brock TCM, Schäfer RB. 2015. Analysing chemical-
588   induced changes in macroinvertebrate communities in aquatic mesocosm experiments: a
589   comparison of methods. *Ecotoxicology* **24(4):**760-769

590 Thomson LJ, Hoffmann AA. 2006. Field validation of laboratory-derived IOBC toxicity ratings
591   for natural enemies in commercial vineyards. *Biological Control* **39(3):**507-515

592 Thomson LJ, Hoffmann AA. 2007. Ecologically sustainable chemical recommendations for
593   agricultural pest control? *Journal of Economic Entomology* **100(6):**1741-1750

594 Turner AS, Bale JS, Clements RO. 1990. Effects of a range of pesticides on the carabid
595   beetle *Pterostichus melanarius* (Ill.) using a microplot technique. J*ournal of Applied*
596   *Entomology* **109(1-5):**463-469

597 Umina PA, Jenkins S, McColl S, Arthur A, Hoffmann AA. 2015. A framework for identifying
598   selective chemical applications for IPM in dryland agriculture. *Insects* **6(4):** 988-1012

599 Umina PA, Hoffmann AA. 1999. Tolerance of cryptic species of blue oat mites (*Penthaleus* spp.)
600   and the redlegged earth mite (*Halotydeus destructor*) to pesticides. *Australian Journal of*
601   *Experimental Agriculture* **39(5):**621-628

602 Valenzuela I, Hoffmann AA. 2015. Effects of aphid feeding and associated virus injury on grain
603   crops in Australia. *Austral Entomology* **54:**292-305

604 van der Werf HM. 1996. Assessing the impact of pesticides on the environment. *Agriculture,*
605   *Ecosystems & Environment* **60(2-3):**81-96.Van den Brink PJ, Ter Braak CJF. 1999.
606   Principal response curves: Analysis of time dependent multivariate responses of
607   biological community to stress. *Environmental Toxicology and Chemistry* **18(2):**138-148

608   Wang Y, Naumann U, Wright ST, Warton DI. 2012. mvabund–an R package for model   based
609   analysis of multivariate abundance data. *Methods in Ecology and Evolution* **3(3):**471-474
610   Whitehouse MA, Wilson LJ, Fitt GP. 2005. A comparison of arthropod communities in transgenic
611   Bt and conventional cotton in Australia. *Environmental Entomology* **34(5):**1224-1241
612   Wiles JA, Jepson PC. 1995. Dosage reduction to improve the selectivity of deltamethrin between
613   aphids and coccinellids in cereals. *Entomologia Experimentalis et Applicata* **76(1):**83-96
614   Wilson LJ, Bauer LR, Lally DA. 1998. Effect of early season insecticide use on predators and
615   outbreaks of spider mites (Acari: Tetranychidae) in cotton. *Bulletin of Entomological*
616   *Research* **88(4):**477-488
617   Wilson LJ, Bauer LR, Lally DA. 1999. Insecticide☐induced increases in aphid abundance in
618   cotton. *Austral Entomology* **38(3):**242-243
619   Zeilinger AR, Olson DM, Andow DA. 2016. Competitive release and outbreaks of non   target
620   pests associated with transgenic Bt cotton. *Ecological Applications* **26(4):**1047-1054

Table 1(on next page)

Sowing dates, seasonal and experimental conditions throughout the study period at the experimental site near Mortlake in Victoria.

*Very delayed sowing this season due to seasonal conditions. The yield for Kellalac from

Hamilton National Variety Trials for each season is included, as is the site mean for Triazene

Tolerant canola varieties for comparative purposes as obtained from relevant trial reports (

http://www.farmtrials.com.au/trial_report_library.php?action=search&query=Hamilton

accessed 13 Feb 2017).

	Crop sown	Planting date (treatments applied)	Growing season rainfall (mm)	Average yield in region t/ha
2004	Barley (Gardner)	* 15 Oct 2014 (19 Oct)	Sep-Feb 310	Wheat 2.06 Canola 1.05
2005	Wheat (Kellalac)	28 Jun 2015 (1 Jul)	Apr-Nov 348	Wheat 2.67 Canola 2.31
2006	Wheat (Kellalac)	17 Jun 2016 (21 Jun)	Apr-Nov 269	Wheat 2.75 Canola 1.7
2007	Canola (Thunder TT Pacific seeds)	5 June 2007 (6 Jun)	Apr-Nov 470	Wheat 5.37 Canola 2.65

Table 2(on next page)

Beneficial and pest communities and groups defined for this study. IOBC ratings for representative members of each beneficial group are included, as well as which target pests are registered for the respective chemical use in Australia.

IOBC (International Organization for Biological and Integrated Control Pesticides and Beneficial Organisms) ratings for the natural enemies are taken for a representative from that grouping. IOBC toxicity ratings are on a 4 point scale (1 "harmless" <25% mortality to 4 "very harmful" >75%). The registration for pests is taken from the chemical labels for the respective pesticides (Lorsban (Chlorpyrifos), Dow Chemicals, APVMA Approval No: 32887/56655; Supracide (Methidathion), Syngenta, APVMA Approval No: 33041/5). Registration is marked (Y)es if the target pest is included for grains crops, if there is registration for a target pest outside of grains, it is included in parentheses.

Beneficials

Group	Includes	IOBC – Chlorpyrifos	IOBC – Methidathion
lacewings	*Micromus tasmaniae*	4	4
carabids	Coleoptera: Carabidae	2-4	
staphylinids	Coleoptera: Staphylinidae	3-4	
ladybirds	Coleoptera: Coccinellidae	3-4	
wasps	all hymenopteran parasitoids	1&4	
predatory bugs	predatory bugs, e.g. assassin bugs	4	3
spider1	hunting Spiders (Lycosidae, Miturgidae, Lamponidae	3	
spider2	web-building spiders and harvestmen (Linyphiidae, Opiliones)		3
spider3	sac-spiders (Clubionidae, Gnaphosidae, Corinnidae)		
salticids	jumping spiders (Salticidae)		
predatory mites	snout mites, mesotig mites, trombididae mites	4	
syrphids	hoverflies		
centipedes	all centipedes		

Pests

Group	Includes	Registered – Chlorpyrifos	Registered – Methidathion
slaters	Isopoda: Armadillidiidae		
millipedes	*Ommatoiulus moreleti* (Portuguese millipedes)		
slugs	*Milax gagates, Deroceras reticulatum*		
earwigs	predominantly *Forficula auricula*, some natives	(Stonefruit)	
earth mites	*Halotydeus destructor, Penthaleus* spp., *Balaustium* spp.	Y	Y
aphids	*Rhopalosiphum padi, Myzus persicae*	Y	(Lucerne, Lupins etc.)
lucerne flea	*Sminthurus viridis* (Collembola: Sminthuridae)		(Lucerne)
scarab beetles	Coleoptera: Scarabidae	Y	(Pasture)
lepidopterans	pest catepillars	Y	
weevils	Coleoptera: Curculonidae	Y	(Lucerne)
orthopterans	crickets and grasshoppers	Y	(Ornamentals)

Table 3(on next page)

Significance of treatments from Principal Response Curve (PRC) and multiple GLM analyses at each sampling time.

The PRC analysis score is for the whole community and reflects the redundancy analysis (RDA) score for that single time point. The GLM scores are for significance of the community or species deviance related to the treatments at each sampling time. All bold values indicate significant score ($p < 0.05$)

Beneficial community

Year	Month	Community RDA (PRC)	GLM	lacewings	wasps	carabids	staphylinids	predatory mites	ladybirds	syrphids	centipedes	spider1	spider2	spider3	salticids	predatory bugs
2005	July	0.571	0.442	1.000	1.000	0.937	0.639	1.000	1.000	1.000	1.000	0.639	1.000	1.000	0.639	1.000
	October	0.697	0.515	0.930	0.866	0.975	0.991	0.991	0.796	0.929	0.983	0.991	0.974	0.983	0.991	0.783
2006	March	0.695	0.312	1.000	0.997	0.997	0.068	0.997	1.000	1.000	0.633	0.733	0.997	1.000	1.000	0.984
	July	**0.001**	**0.000**	1.000	**0.009**	0.088	**0.038**	**0.021**	1.000	1.000	0.547	0.547	1.000	1.000	1.000	1.000
	October	**0.001**	**0.002**	0.057	0.648	**0.042**	0.195	1.000	0.648	0.271	0.648	0.648	0.648	0.648	0.648	0.951
2007	March	0.272	0.374	1.000	0.818	0.865	0.818	0.721	1.000	1.000	1.000	0.865	0.818	0.721	1.000	0.865
	June	**0.001**	**0.000**	1.000	0.732	**0.001**	0.933	0.933	1.000	1.000	0.885	0.933	0.227	0.826	1.000	**0.019**

Pest community

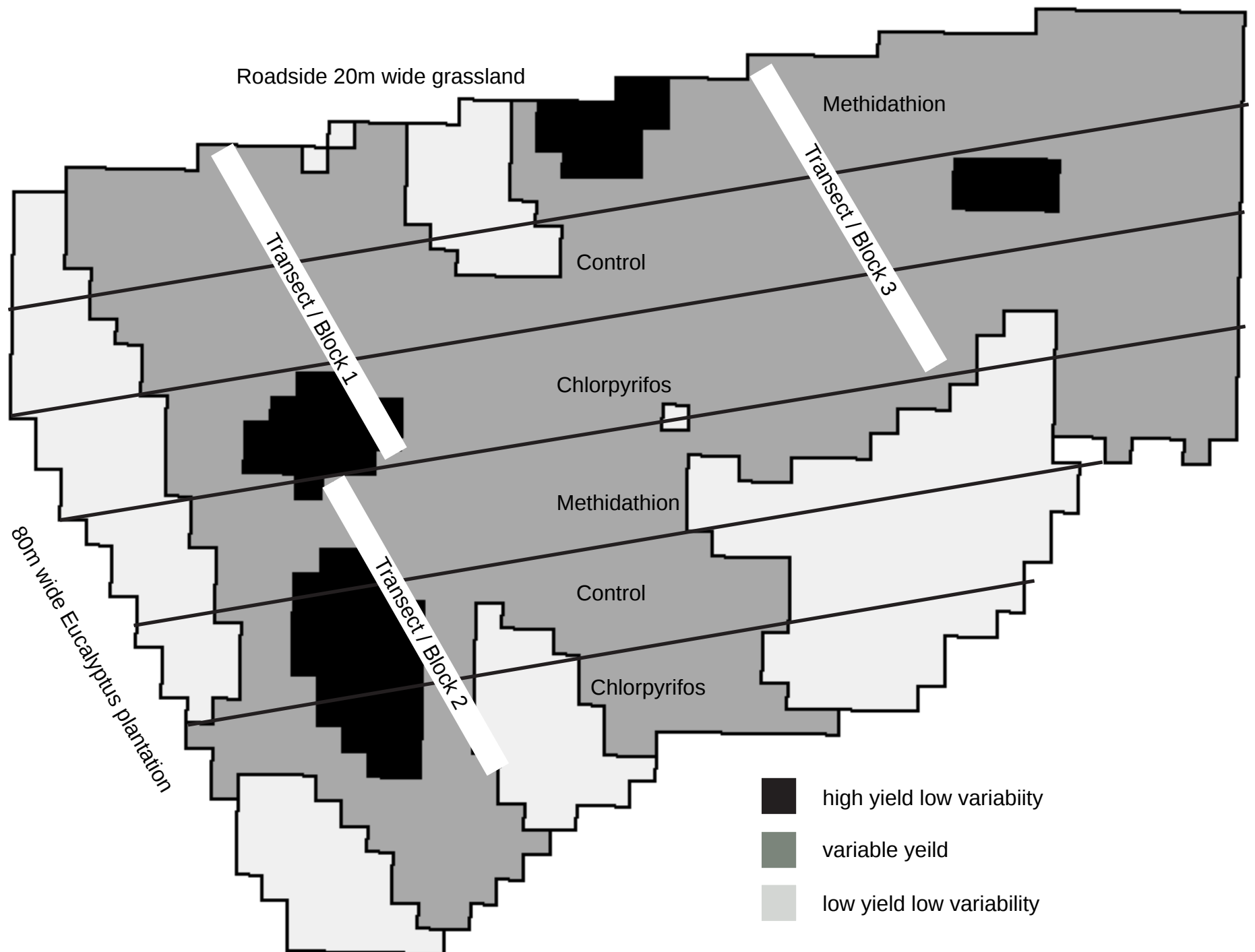| Year	Month	Community RDA (PRC)	GLM	slaters	millipedes	slugs	earth mites	lucerne flea	aphids	earwigs	scarab beetles	lepidopterans	weevils	orthopterans
2005	July	0.067	**0.028**	0.230	0.230	0.968	1.000	1.000	1.000	0.968	0.069	1.000	0.656	0.643
	October	0.593	0.116	0.733	0.707	0.579	1.000	0.274	0.948	0.974	0.961	0.809	0.961	0.529
2006	March	0.859	0.713	0.946	0.976	1.000	1.000	1.000	1.000	0.976	0.976	0.976	0.976	0.577
	July	**0.028**	**0.025**	0.446	0.500	0.446	0.579	1.000	1.000	0.108	0.749	1.000	0.500	0.617
	October	0.087	**0.011**	0.495	0.421	**0.038**	1.000	1.000	1.000	0.116	0.221	1.000	1.000	0.274
2007	March	**0.003**	**0.006**	0.346	0.346	1.000	1.000	1.000	1.000	0.555	0.555	0.656	**0.007**	0.285
	June	**0.004**	**0.000**	**0.017**	0.703	0.703	**0.018**	1.000	0.027	**0.005**	1.000	0.059	0.426	0.703

Figure 1(on next page)

Site layout

Site layout indicating insecticide treatments and invertebrate sampling transects (blocks) in relation to overall yield from the 36 ha field (pooled data from 2003-2007).
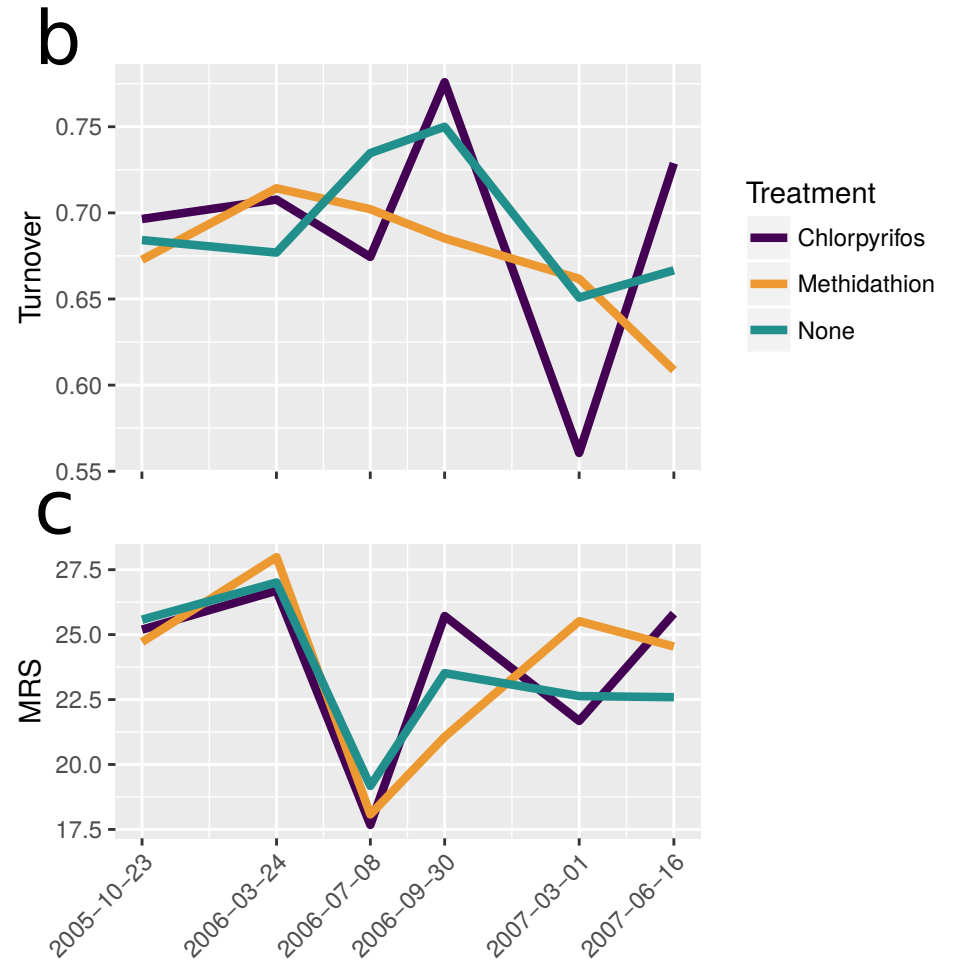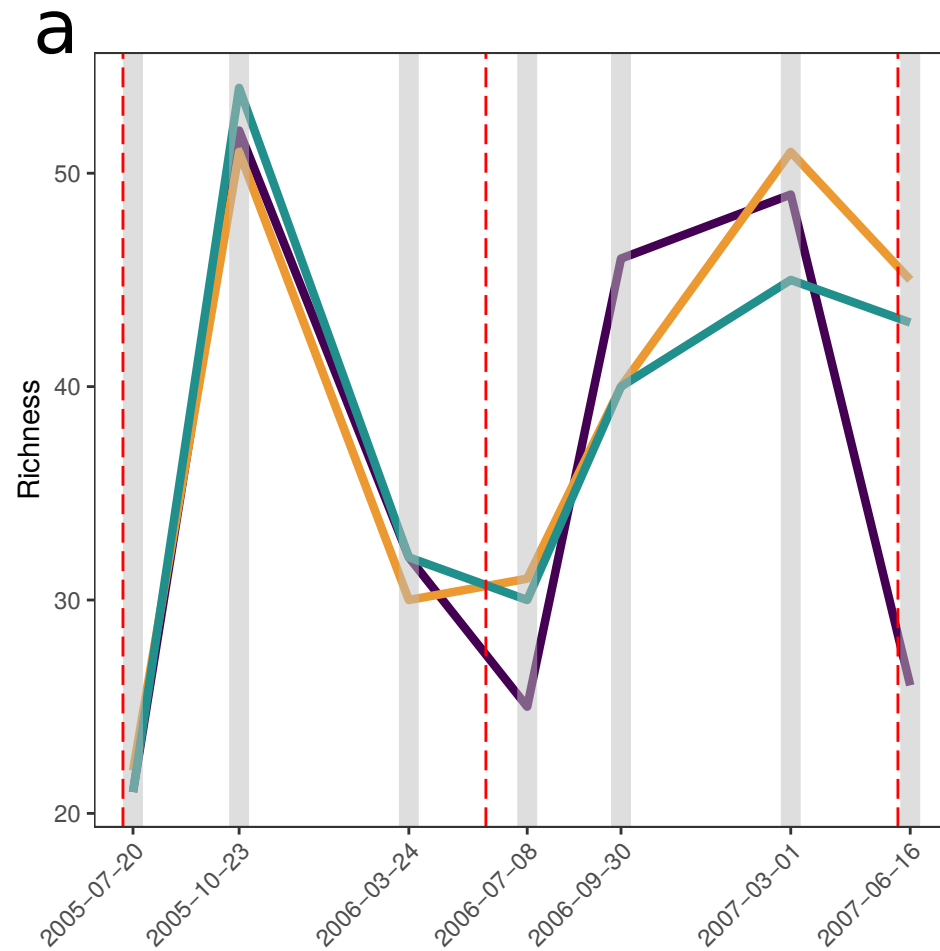
Figure 2(on next page)
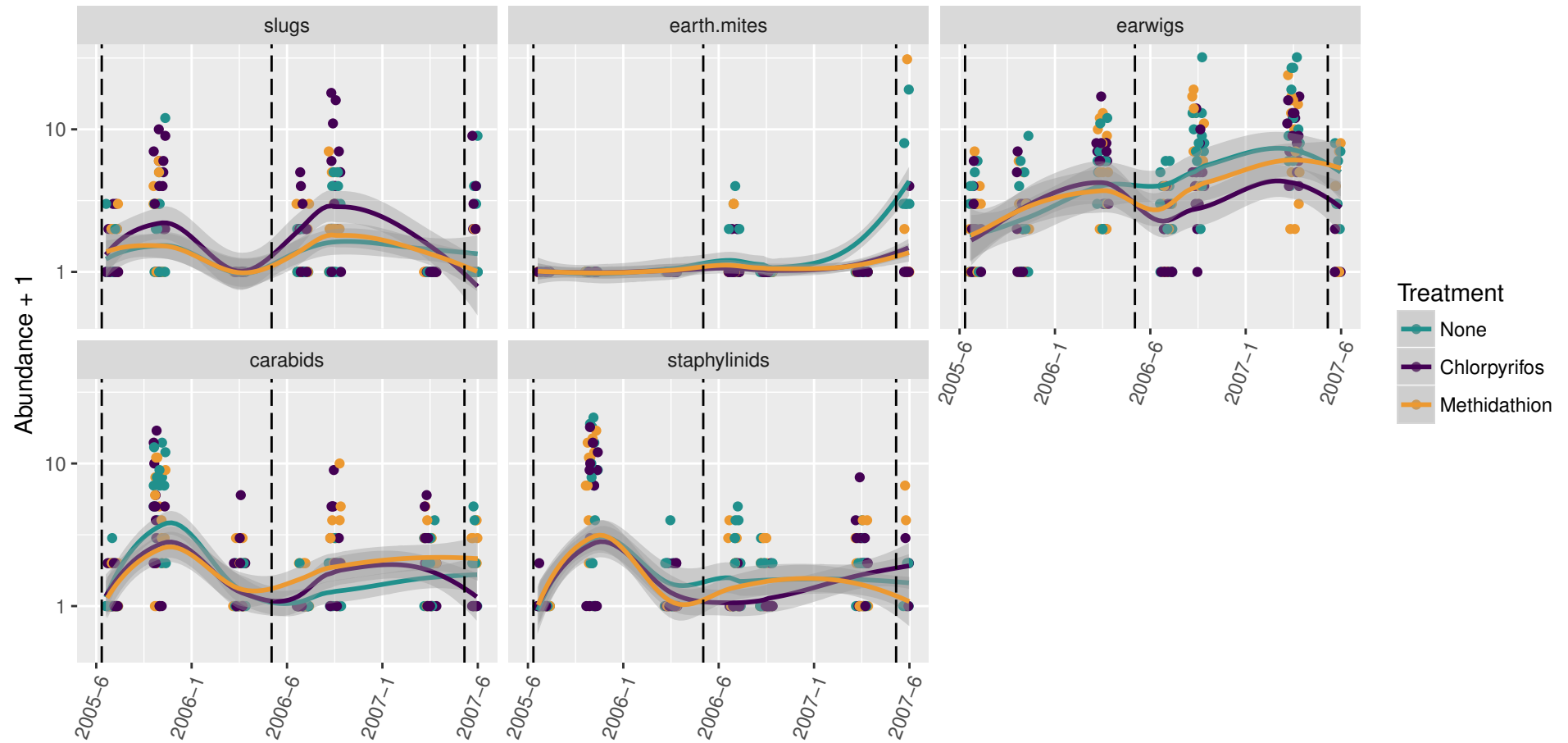
Species richness

(a) Change in total species richness over time. The grey bars represent the sampling times and the red dashed lines represent the application of pesticides associated with each treatment. (b) Total proportional species turnover for each time point through the study period. (c) Mean rank shifts. Note for b and c the initial sampling and spray event is not present, as each point represents the change from the previous sampling event. The first spray event is immediately before the beginning of these panels, however.

Figure 3(on next page)
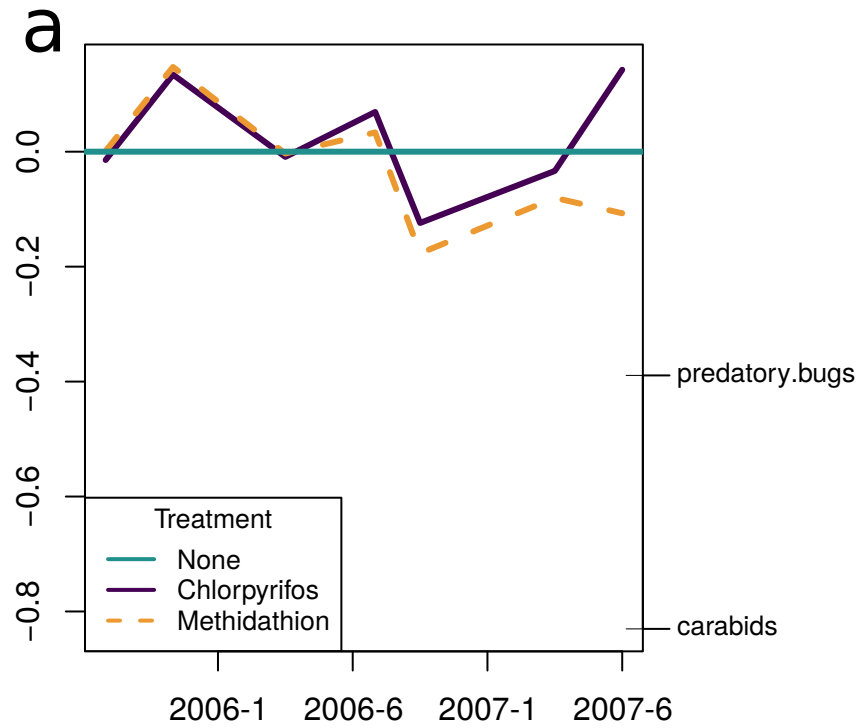
Selected species abundances

Abundances through time for selected pest and beneficial species displaying important responses to both PRC and GLMmv analyses (See Figures 4-6 and Table 3). Pest slugs, earthmites and earwigs, and the predatory beetles: carabids and staphylinids

PeerJ

Figure 4(on next page)

Principal response curves

(a) Principal response curve for the natural enemy community. The left y-axis dhows the Effect size. The position on the right y-axis reflects the weighting of the species to the overall respons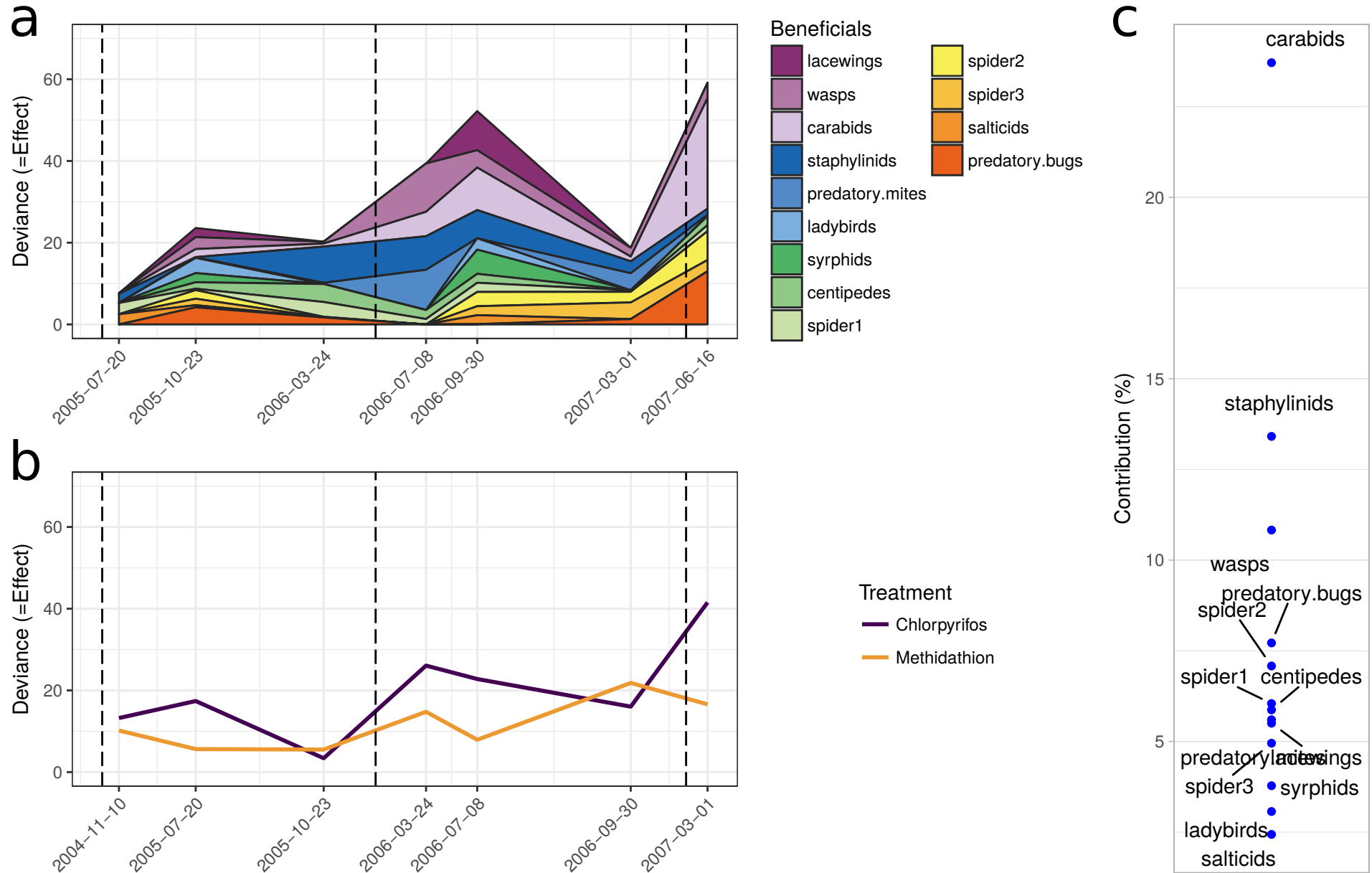e. The 0 line reflects the untreated control. **(b)** Principal response curve the for pest community. The left y-axis dhows the Effect size. The position on the right y-axis reflects the weighting of the species to the overall response. The 0 line reflects the untreated control.

Figure 5(on next page)

Beneficial community

Effect size through time on the beneficial community identified in this study. Each species grouping has had individual generalised linear models performed on abundance. The effect size is relative to the control, and the different colours represent the species contribution to that effect size, at that point in time. The dashed vertical lines represent the application of the pesticide treatments. (b) The overall community response to the application of the treatments through time. The three dashed vertical lines represent the application of the pesticide treatments. (c) The proportional contribution of each species to the overall deviance.
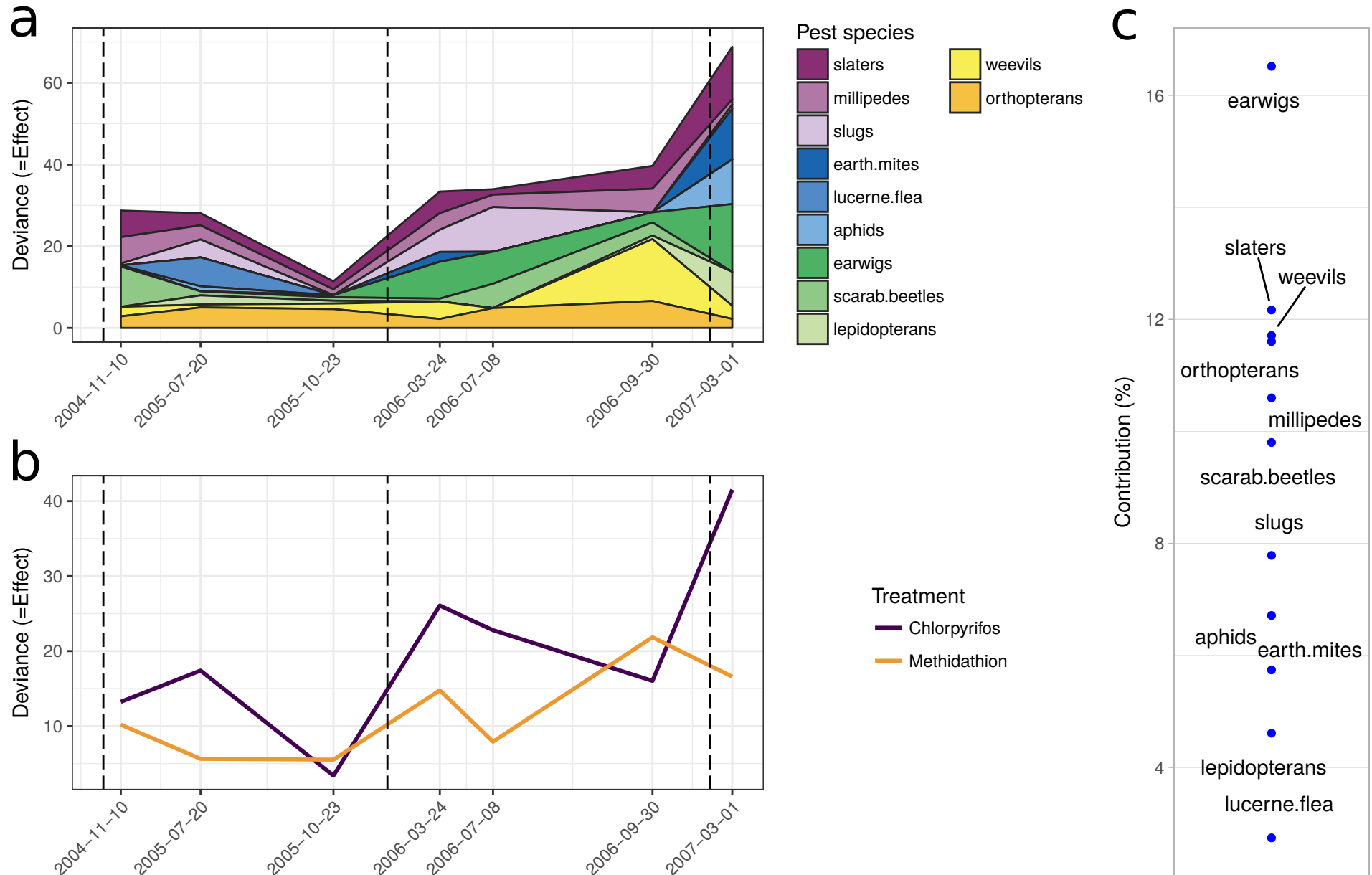
Figure 6(on next page)

Pest community

Effect size through time on the pest community identified in this study. (a) Each species grouping has had individual generalised linear models performed on abundance. The effect size is relative to the control, and the different colours represent the species contribution to that effect size, at that point in time. The three dashed vertical lines represent the application of the pesticide treatments. (b) The overall community response to the application of the treatments through time. The four dashed vertical lines represent the application of the pesticide treatments. (c) The proportional contribution of each species to the overall deviance.

Figure 7(on next page)

Yield

Yield (tonnes per hectare) per crop type and season, treatment and experimental transcects
(blocks 1-3).