Ecological and pest-management implications of sex differences in scarab landing patterns on grape vines

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Background Melolonthinae beetles, comprising different white grub species, are a globally-distributed pest group. Their larvae feed on roots of several crop and forestry species, and adults can cause severe defoliation. In New Zealand, the endemic scarab pest *Costelytra zealandica* (White) causes severe defoliation on different horticultural crops, including grape vines (*Vitis vinifera*). Understanding flight and landing behaviours of this pest can help inform pest management decisions. **Methods** Adult beetles were counted and then removed from 96 grape vine plants from 21:30 until 23:00 h, every day from October 26 until December 2, during 2014 and 2015. Also, adults were removed from the grape vine foliage at dusk 5, 10, 15, 20 and 25 min after flight started on 2015. Statistical analyses were performed using generalised linear models with a beta-binomial distribution to analyse proportions and with a negative binomial distribution for beetle abundance. **Results** By analysing *C. zealandica* sex ratios during its entire flight season, it is clear that the proportion of males is higher at the beginning of the season, gradually declining towards its end. When adults were successively removed from the grape vines at 5-min intervals after flight activity begun, the mean proportion of males ranged from 6 to 28%. The male proportion suggests males were attracted to females that had already landed on grape vines, probably through pheromone release. **Discussion** The seasonal and daily changes in adult *C. zealandica* sex ratio throughout its flight season are presented for the first time. Although seasonal changes in sex ratio have been reported for other melolonthids, changes during their daily flight activity have not been analysed so far. Sex-ratio changes can have important consequences for the management of this pest species, and possibly for other melolonthids, as it has been previously suggested that *C. zealandica* females land on plants that produce a silhouette against the sky. Therefore, long-term management might evaluate the effect of different plant heights and architecture on female melolonthid landing patterns, with consequences for male distribution, and

subsequently overall damage within horticultural areas.

1 **Ecological and pest-management implications of sex differences in scarab landing patterns**
2 **on grape vines.**

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16 **Abstract**
17 **Background**
18 Melolonthinae beetles, comprising different white grub species, are a globally-distributed pest
19 group. Their larvae feed on roots of several crop and forestry species, and adults can cause
20 severe defoliation. In New Zealand, the endemic scarab pest *Costelytra zealandica* (White)
21 causes severe defoliation on different horticultural crops, including grape vines (*Vitis vinifera*).
22 Understanding flight and landing behaviours of this pest can help inform pest management
23 decisions.
24 **Methods**
25 Adult beetles were counted and then removed from 96 grape vine plants from 21:30 until 23:00
26 h, every day from October 26 until December 2, during 2014 and 2015. Also, adults were
27 removed from the grape vine foliage at dusk 5, 10, 15, 20 and 25 min after flight started on 2015.
28 Statistical analyses were performed using generalised linear models with a beta-binomial
29 distribution to analyse proportions and with a negative binomial distribution for beetle
30 abundance.
31 **Results**
32 By analysing *C. zealandica* sex ratios during its entire flight season, it is clear that the proportion
33 of males is higher at the beginning of the season, gradually declining towards its end. When
34 adults were successively removed from the grape vines at 5-min intervals after flight activity
35 begun, the mean proportion of males ranged from 6 to 28%. The male proportion suggests males
36 were attracted to females that had already landed on grape vines, probably through pheromone
37 release.
38 **Discussion**
39 The seasonal and daily changes in adult *C. zealandica* sex ratio throughout its flight season are
40 presented for the first time. Although seasonal changes in sex ratio have been reported for other

41 melolonthids, changes during their daily flight activity have not been analysed so far. Sex-ratio
42 changes can have important consequences for the management of this pest species, and possibly
43 for other melolonthids, as it has been previously suggested that *C. zealandica* females land on
44 plants that produce a silhouette against the sky. Therefore, long-term management might
45 evaluate the effect of different plant heights and architecture on female melolonthid landing
46 patterns, with consequences for male distribution, and subsequently overall damage within
47 horticultural areas.
48
49 **Introduction**
50 White grubs (Coleoptera: Scarabaeidae: Melolonthinae) are a widely distributed group of
51 herbivorous insects feeding on a variety of plant hosts around the world (Jackson & Klein,
52 2006). Their larvae feed on plant roots, leading to severe damage to commercial crops and
53 pastures (Jackson & Klein, 2006; Frew et al., 2016), as well as to natural forests and forestry
54 plantations (Švestka, 2006, 2010). Some melolontids do not feed as adults, like *Leucopholis*
55 *lepidophora* (Blanchard) (Kalleshwaraswamy et al., 2016) and *Phytholaema herrmanni*
56 (Germain) (Durán, 1954), while others cause minor plant defoliation. These include *Schizonyza*
57 *ruficollis* (F.) (Kulkarni et al., 2007) and *Holotrichia* spp. (Kulkarni et al., 2009) in India, *Hoplia*
58 *philanthus* (Füessly) in Belgium (Ansari et al., 2006), and *Phyllophaga cuyabana* (Moser) in
59 Brazil (Oliveira & García, 2003). However, dramatic defoliation can also occur. In Eastern Asia,
60 *Ectinohoplia rufipes* (Motschulsky) adults can severely defoliate ornamental trees around golf
61 courses, as well as in gardens and parks (Kim et al., 2013). In Central and Western Europe, adult
62 defoliation by *Melolontha melolontha* (L.) and *Melolontha hippocastani* (F.) has led to severe
63 defoliation on different horticultural crops, such as grape vines (*Vitis vinifera* (L.)) and forest
64 trees, respectively (Jackson & Klein, 2006; Reinecke et al., 2006; Švestka, 2006, 2010). Such
65 defoliation also has been recorded in New Zealand, where *Costelytra zealandica* (White) attacks
66 several horticultural crops, including tamarillo (*Solanum betaceum* (Cav.)), avocado (*Persea*
67 *americana* (Mill.)), blueberries (*Vaccinum corymbosum* (L.), strawberries (*Fragaria* x *ananassa*
68 (Duchesne)), kiwifruit (*Actinidia chinensis* (Planch)), and grape vines (Binfield, 1933; Blank &
69 Olson, 1982; Blank, Olson & Bell, 1983; East, Willoughby & Koller, 1983; East & Holland,
70 1984). Sometimes, severe defoliation can occur (Blank, Olson & Bell, 1983; East, Willoughby &
71 Koller, 1983; Blank, 1992), leading in some cases to prophylactic application of synthetic
72 insecticides to reduce *C. zealandica* damage. Nowadays, the use of such an approach is
73 discouraged due to the environmental and human health problems related to their use (Reganold
74 & Wachter, 2016).
75 The importance of the defoliation produced by melolontids has promoted investigation of their
76 plant colonization patterns, subsequently leading to work on their flying and landing behaviour.
77 In Melolonthinae, after landing on their host plants, females attract males by releasing sex
78 pheromones (Henzell & Lowe, 1970) or by the release of green leaf volatiles (GLV) produced
79 after feeding on plant foliage (Harari et al., 1994; Reinecke et al., 2002a), or both acting together
80 (Reinecke et al., 2002b; Reinecke et al., 2006). However, in *Maladera matrida* (Argaman),

81   males land on their plant hosts before females. By feeding on their host plant, GLV are released
82   attracting the females to where feeding occurs (Harari, Ben-Yakir & Rosen, 1994). Observations
83   on *C. zealandica* flying behaviour have suggested that females land before males on shrubs and
84   trees (Farrell & Wightman, 1972). After landing, males are attracted to those plants by the
85   female pheromone, identified as phenol (Henzell & Lowe, 1970). Although the effects of phenol
86   on male attraction are well understood for *C. zealandica* (Henzell & Lowe, 1970; Unelius et al.,
87   2008; Marshall et al., 2016), *M. melolontha*, *M. hippocastani* (Reinecke et al., 2006), and *P.*
88   *cuyabana* (Zarbin et al., 2007), there are no studies quantifying adult *C. zealandica* sex ratio at
89   the landing phase of its daily plant colonising activity. In addition, no research has reported the
90   trends in sex ratio throughout the entire *C. zealandica* flight season for any agricultural or natural
91   system. We hypothesized that females arrive on *V. vinifera* before males. Therefore, the aims of
92   this work were: i) to quantify *C. zealandica* sex ratio throughout its seasonal and daily flight
93   period; and ii) to correlate adult abundance with sex ratio through its seasonal and daily flight
94   activity.
95
96   **Material and Methods**
97   *Study sites*
98   This work was conducted in the Marlborough region of New Zealand. In this area, two
99   commercial vineyards were chosen. One in the Awatere Valley (41°44'S; 173°52'E) owned by
100   Kono Beverages, and another close to Blenheim city (41°33'S; 173°55'E) that belongs to Wither
101   Hills. These locations are situated in homogeneous landscapes generally dominated by
102   conventionally-managed vineyards. To reduce the effect of conventional management practices
103   on the interpretation of the observations described below, organically-managed vineyard blocks
104   (cv. Pinot Noir) were chosen at both sites. The sizes of the blocks were 6.12 and 4.58 ha in the
105   Awatere Valley and Blenheim, respectively. Grape vines were 14 and 15 yr old in the Awatere
106   Valley and Blenheim, respectively. Plant spread along the vine row was 1.8 m between each
107   other. During the study period, from late October to late November 2014 and 2015, no pesticides
108   or herbicides were applied on those blocks, apart from sulphur, which was applied to control
109   fungal diseases. In New Zealand, organic vineyard areas, including headlands, inter-row and
110   under-vine areas are covered by a mixture of ground covers that mainly comprise ryegrass
111   (*Lollium perenne* (L.)), white clover (*Trifolium repens* (L.)), and fescue (*Festuca* spp.).
112
113   *Adult C. zealandica sampling*
114   Sampling was carried out every day during *C. zealandica* flight season. In the Marlborough area,
115   individuals start flying from the end of October until the end of November (Farrell & Wightman,
116   1972). Within the October to November period, adults initiate flight 20 min after sunset, flying
117   for approximately 26 min (González-Chang, Unpublished data). During their flight activity,
118   adults land on grape vine foliage and then feed and mate, staying on the grape vines for at least 3
119   h (González-Chang, Unpublished data). Based on this landing behaviour, adults were counted
120   and then removed from 96 plants at both study locations from 21:30 until 23:00 h. These plants

121   had a mean height of 1.5 m, and were consecutively sampled along the vine row over three
122   consecutive rows. Sampling started along the row at the edge of a vineyard block to 50 m from
123   the edge, sampling 32 plants on each row. The row sampling method was performed at the
124   Awatere Valley during 2014 and 2015 and in Blenheim only on 2015.
125

126   *Seasonal sex ratio*
127   Daily during the 2014 flight season from October 27th until November 30th, beetles from the
128   Awatere Valley were collected by hand. During the 2015 flight season, beetle collection started
129   in the Awatere Valley from November 7th until December 1st, while in Blenheim from October
130   26th until December 1st. Individual polyethylene bags were used to collect the beetles, so samples
131   were not mixed between days or locations. Using a 50 ml cylindrical plastic container, a sub-
132   sample was taken from each bag for sex ratio analysis. Within this sub-sample the ratio was
133   analysed by randomly sexing 20 individuals. This procedure was repeated three times for each
134   day. The proportion of males (M) over females (F) for each sampled day was obtained after
135   taking a mean of the three measurements. This proportion was calculated as M/(M+F). Days
136   without flight activity were removed from further sex-ratio analysis. The separation of males
137   from females was carried out using the morphological characteristics proposed by Kain (1972)
138   and Kelsey (1965). Using a stereomicroscope (Carl Zeiss), the distal-ventral section of the
139   abdomen was examined for male characters, including a shallow depression in the centre of the
140   sixth abdominal sternite (Kelsey, 1965), and the presence of parameres through the pygdium
141   (Kain, 1972). Female characters included the presence of colleterial glands, as two spherical dots
142   in the fifth sternite, and two genital sclerites situated at each side of the vulva (Kain, 1972).
143

144   *Adult C. zealandica removal to determine daily sex-ratio patterns*
145   To investigate the dynamics of sex-ratio changes on grape vine foliage during the flight season,
146   adult beetles were successively removed from the grape vines at different periods during 2015.
147   At both sites, adults were removed from the grape vine foliage at dusk at 5, 10, 15, 20 and 25
148   min after flight started. One plant at the edge of each vineyard block at both locations was used
149   for this experiment. This plant was not included in the 96 plants used in the seasonal sex-ratio
150   determination explained above. Adults were visually counted on the selected grape vine and then
151   removed by hand at each time period. When the number of adults collected exceeded 60, 20
152   individuals were sub-sampled three times from those samples. The mean of the sub-sampled was
153   used for further statistical analyses. This daily sequential adult removal was performed from
154   November 2 to 28 and November 14 to 28 at Blenheim and the Awatere Valley, respectively.
155   Days without flight activity, because of adverse weather, were removed from further sex-ratio
156   analysis. The mean proportion of males and total adult numbers at each sampling day at each
157   time interval were considered as replicates.
158

159   *Statistical analyses*

160 The proportions obtained for each day during the sampling season, and those calculated at each
161 evaluated time period, were analysed using a generalised linear model (GLM), with a beta-
162 binomial distribution and "logit" as the link function (Cribari-Neto & Zeileis, 2010). It has been
163 previously suggested that the beta regression approach is inherently heteroscedastic and easily
164 accommodates asymmetries typically found in rates and proportions (Cribari-Neto & Zeileis,
165 2010; Grün, Kosmidis & Zeileis, 2012). Beta-binomial GLMs were calculated with the R
166 package "betareg" (Cribari-Neto & Zeileis, 2010). Spearman's rank correlation was used to
167 assess the correlation between adult abundance and sex ratio through the flight season (Crawley,
168 2007). The same analysis was used to evaluate the correlation between adult abundance and the
169 sex ratio at each time period studied. Differences in adult abundance between periods were
170 analysed using Tukey's multiple contrasts post-hoc analysis (Venables & Ripley, 2002) after
171 fitting a GLM with a negative binomial distribution and a logarithmic link function at each
172 sampling site. Negative binomial GLM and Tukey's tests were performed using the R packages
173 "MASS" and "multcomp", respectively. All statistical analyses were performed using the
174 statistical software R v.3.2.5 (R Core Team, 2016).
175
176 **Results**
177 *Seasonal sex ratio*
178 In the Awatere Valley in 2014, a total of 36,369 adults were sampled, while in 2015 only 6,111
179 were collected. During 2015 in Blenheim, 14,731 adults were collected. Sex-ratio was not
180 statistically correlated with beetle abundance through the flight season in the Awatere Valley
181 during 2014 (φ=0.02; p=0.92) nor in 2015 (φ=-0.35; p=0.12). However, at the Blenheim site, this
182 correlation was positive and significant (φ=0.51; p<0.01). In the Awatere Valley the proportion
183 of males decreased through time during 2014 (slope=-0.08; z=-4.8; p<0.001) and in 2015
184 (slope=-0.02; z=-2.6; p<0.01). A similar result was observed in Blenheim during 2015 (slope=-
185 0.03; z=-4; p<0.001). Seasonal sex-ratio results are shown in Figs. 1-3.
186
187 *Adult C. zealandica removal to determine daily sex-ratio patterns*
188 When adults were removed from the grape vine foliage every 5 min after flight activity started,
189 the proportion of males increased through the 5 to 25 min time interval at Blenheim (slope=0.06;
190 z=2.12; p<0.05), but not at the Awatere Valley (slope=0.001; z=0.03; p=0.98) (Fig. 4). Tukey
191 contrasts showed that adult numbers significantly increased between 10 and 15 min after flight
192 occurred at both sites, and then decreased to initial levels between 20 and 25 min after it started
193 (Fig. 5). In the Awatere Valley, flight activity completely ceased 25 min after begun, while in
194 Blenheim it stopped after 30 min. No correlation was found between adult abundance and the
195 sex-ratio during each time period evaluated at Blenheim (φ=-0.1; p=0.87) or in the Awatere
196 Valley (φ=-0.8; p=0.2).
197
198 **Discussion**

199   In this work, seasonal and daily changes in adult *C. zealandica* sex-ratio throughout its flight
200   season are presented for the first time. The proportion of males significantly decreased towards
201   the end of the season at both studied locations. However, when adults were removed from the
202   grape vine foliage at several time periods during the same day, trends in the sex-ratio differed
203   between the two locations. These results are discussed in terms of this insect's behaviour. The
204   implications of these results on sustainable *C. zealandica* control in vineyards also are discussed.
205

206   *Seasonal sex-ratio trend*
207   A widely-accepted sex-ratio theory based on natural selection proposed by R.A. Fisher, suggests
208   that sex-ratio is equilibrated on a 1:1 proportion to promote species survival (Fisher, 1930).
209   However, many species do not follow the 1:1 theoretical proportion as they respond to their
210   changing local environmental conditions (Hamilton, 1967). In Melolonthinae sex-ratio though
211   their flight season can be female-biased (Méndez-Aguilar et al., 2005; Kalleshwaraswamy et al.,
212   2016), male-biased (Ansari et al., 2006; Švestka, 2006, 2010) or at a 1:1 ratio (Harari, Ben-Yakir
213   & Rosen, 1994; Méndez-Aguilar et al., 2005). However, it is worthwhile noting that sex-ratio
214   can vary between those proportions every day during the flight season (Švestka, 2006, 2010;
215   Kalleshwaraswamy et al., 2016), as shown in this study (Figs. 1-3). In this work, seasonal sex-
216   ratio varied between years and locations, being male-biased (64% males) and female-biased
217   (47% males), in the Awatere Valley in 2014 and 2015, respectively. In Blenheim, the seasonal
218   sex-ratio was 1:1 (50% males). The sex-ratio differences between sites in this study highlights
219   the temporal and spatial variability of melolontid sex-ratio, which might be determined by
220   environmental conditions (Sánchez, 2008; Gempe & Beye, 2011). Previous studies suggested
221   that *C. zealandica* males appear seasonally before females do (Kelsey, 1951; Pottinger, 1968;
222   Farrell & Wightman, 1972). Early male appearance might have important evolutionary
223   consequences for the survival of *C. zealandica* because as soon as the females emerge, males are
224   already present for mating (Kelsey, 1951; Durán, 1954; Harari, Ben-Yakir & Rosen, 1994;
225   Kalleshwaraswamy et al., 2016). The evolutionary advantage of early male emergence is in
226   agreement with the results presented in this work, as a higher proportion of males were found at
227   the beginning of the flight season at both studied years and locations. The reduction in male
228   numbers through time might be associated with a reduction in female pheromone production
229   towards the end of the flight season, as pheromone effects on male attractiveness have been well
230   studied in *C. zealandica* (Henzell, 1970; Unelius et al., 2008; Marshall et al., 2016). Recently, it
231   has been suggested that phenol, the main sex pheromone in *C. zealandica* (Henzell, 1970), is
232   produced by a mutualistic bacterium *Morganella morganii* (Brenner) in its colleterial gland,
233   which is biosynthesized from the amino acid tyrosine (Marshall et al., 2016). In general, insect
234   protein synthesis decreases with aging (Levenbook, 1986), and tyrosine in particular has been
235   shown to decrease with age in other insects like *Bombyx mori* (L.) (Lepidoptera: Bombycidae)
236   (Osanai & Kikuta, 1981). Considering that the *C. zealandica* flight season lasted for around 4
237   wk, from late October to late November at the studied vineyards, females that landed on the

238 grape vine foliage at the end of that period might already have ceased to synthesize their
239 pheromone due to aging, reducing the number of males attracted to them.
240 In the Awatere Valley, no correlation was found between adult numbers throughout the flight
241 season and changes in the sex-ratio. However, in Blenheim, the correlation was positive and
242 significant. A positive correlation suggests that an increase in adult numbers is due an increase in
243 the number of males, because in the Awatere Valley 2014 flight season, 63% of the sampled
244 adults at their November 14 peak in abundance (6,968 individuals) were males. Conversely, at
245 the same location during 2015, only 37% of the adults were males at their November 19 peak
246 (1,039 individuals). In Blenheim, 55% of them were males at their November 6 peak (1,966
247 individuals).
248
249 *Adult removal and daily sex-ratio trends*
250 *Costelytra zealandica* flight activity has been divided in two phases (Kelsey, 1951; Pottinger,
251 1968; Farrell & Wightman, 1972). Firstly, males emerge from the ground and hover over the
252 grass. When females emerge, they climb to the top of a grass blade and release their sex
253 pheromone to attract males. Once mating has occurred, females return to the ground to lay eggs.
254 Secondly, females fly from the grass, searching for silhouettes in the sky, landing on trees,
255 hedgerows and shrubs (Farrell & Wightman, 1972). After landing occurs, pheromones are
256 released, attracting males from the surrounding areas again. Then, males and females drop to the
257 ground and females again lay eggs, although in fewer numbers compared to their first
258 oviposition made close to their initial emergence sites (Farrell & Wightman, 1972). The results
259 presented in this work are in agreement with the colonization sex-driven dynamics for the second
260 flight phase observed during 1970s (Farrel & Wightmann, 1972), in which females land first on
261 the vegetation; as the proportion of males found when adults were removed from the grape vines
262 never exceed 28%. In fact, 10-min after flight begun, only 9 and 18% of the removed adults were
263 males at the Awatere Valley and Blenheim, respectively. Remarkably, an identical two-phase
264 flight behaviour has been described before for *Phyllopertha horticola* (L.) (Coleoptera:
265 Rutelinae) (Schneider, 1962) and for *P. herrmanni* (Durán, 1954). Also, similar landing
266 behaviour where female melolontids arrive before males on plant foliage has been reported for
267 *M. melolontha*, *M. hippocastani* (Reinecke et al., 2002a) and *P. cuyabana* (Oliveira & García,
268 2003). In the Awatere Valley (2015), the proportion of males did not change throughout their
269 daily flight activity, which lasted for 25 min. However, in Blenheim (2015) male proportion
270 increased throughout their daily flight period, which totally ceased 30 min after flight begun.
271 These differences in adult abundance and flight length at dusk could be related to the two-fold
272 increase in adult numbers found in Blenheim compared to the Awatere Valley.
273
274 *Female behaviour and plant silhouettes*
275 Considering that males are attracted to females that have already landed on their host plant,
276 through pheromone communication (Henzell & Lowe, 1970) and/or plant volatiles (Reinecke et
277 al., 2002a), the mechanisms by which females locate host plants have relevance to pest

278 management. Different authors have proposed that female host finding in Melolonthinae is
279 through the visual recognition of the tallest plant silhouettes in the sky (Durán, 1954; Schneider,
280 1962; Farrell & Wightman, 1972; Oliveira & García, 2003). A study in Brazil showed that *P.*
281 *cuyabana* females preferred tall soybean and corn plants for landing and mating (García, Oliveira
282 & de Oliveira, 2003). Recent work suggests that plant location by *C. zealandica* females might
283 be mediated by the contrast that plant silhouettes produce in the sky (González-Chang,
284 Unpublished data). Males also might use visual cues in the sky for orientation at some extent, but
285 more research is needed, as no evidence for the latter exist so far. Thus, additional research
286 investigating the addition of tall plant species at the edge of crops could have management
287 values, especially if the tall plants can divert females from landing further into the crop area, and
288 in turn, deterring males from the crop.
289
290 *Implications for sustainable C. zealandica management in vineyards*
291 Recently, it has been suggested that a "push-pull" strategy (Khan et al., 2011) might contribute to
292 the control of white grub populations (Dynastinae, Rutelinae, Melolonthinae) around the world
293 (Frew et al., 2016). In the context of *C. zealandica* pest control in grape vines, it seems
294 straightforward to use the sex attractant pheromone (phenol) to "pull" the adults away from the
295 grape vines. Phenol, combined with another management intervention which "pushes" the adults
296 away, as recently shown when crushed mussel shells were applied on the under-vine vineyard
297 areas (González-Chang, Unpublished data), might arise as a novel variant on the "push-pull"
298 approach to control this New Zealand pest in several horticultural crops. However, the use of
299 phenol in such a way has some shortcomings. Although phenol was identified as the pheromone
300 for *C. zealandica* more than 40 years ago (Henzell & Lowe, 1970), it has not been widely used to
301 control this pest due constraints in trap design that led to low capture efficiency (Unelius et al.,
302 2008). Another negative factor of using phenol is its high toxicity for humans (Shadnia &
303 Wright, 2008; Unelius et al., 2008). Instead, if females can be attracted to a host plant outside the
304 crop, the overall numbers of adult *C. zealandica* landing on the crop could be reduced, along
305 with subsequent reduction in plant damage (González-Chang, Unpublished data). The
306 mechanisms behind female *C. zealandica* host location remain unknown with literature only
307 suggesting that females might locate host plants by their contrast against the sky at dusk (Farrell
308 & Wightman, 1972). Despite those assumptions, it cannot be ignored that females can also be
309 attracted to different floral plant volatiles, as demonstrated for *P. horticola* (Ruther, 2004).
310 Furthermore, the potential contribution of GLV, released from adult feeding (Reinecke et al.,
311 2002b), may influence seasonal *C. zealandica* peak activity and abundance, because peak
312 damage coincides with peak flight activity in the middle of the flight season (East et al., 1983).
313 In this work, the peak of abundance was registered during the middle of the flight season. Thus,
314 the chemical ecology underlying *C. zealandica* reproductive behaviour needs further
315 investigation. Another approach could evaluate the contribution of native New Zealand
316 vegetation within or outside the vineyard (Shields et al., 2016), as non-crop vegetation could
317 provide visual silhouettes and organic volatiles to further attract males or females. Although the

318 establishment and growth of those native plants in horticultural areas will take time, native plants
319 could contribute to a long-term sustainable *C. zealandica* pest management strategy, with
320 potential enhancement of local functional biodiversity and biological conservation that need to
321 be further addressed (Isaacs et al., 2009).
322
323 **Conclusions**
324 The proportion of *C. zealandica* males landing on the grape vine foliage decreased throughout
325 the flight season during 2014 and 2015 at two New Zealand locations. Blenheim (2015) was the
326 only site where increases in sex-ratio (male-biased) were correlated with increases in overall
327 adult abundance. The sex-ratio correlation with adult abundance might be related to spatial and
328 temporal landscape variations in sex-ratio between Blenheim and the Awatere Valley, although
329 landscape factors were not measured here. When adults were removed daily from the grape vines
330 at several time periods after their flight activity begun, a female-biased sex-ratio was recorded.
331 The female-based sex-ratio suggests that females land on the plant foliage before males,
332 subsequently attracting them by the release of their phenol sex pheromone. The grape vine
333 colonization sex-driven dynamics during adult *C. zealandica* flight activity presented in this
334 work might contribute to management useful in reducing the damage of this endemic New
335 Zealand pest on several horticultural crops, and eventually in other scarabaeids with similar
336 flight behaviour around the world.
337
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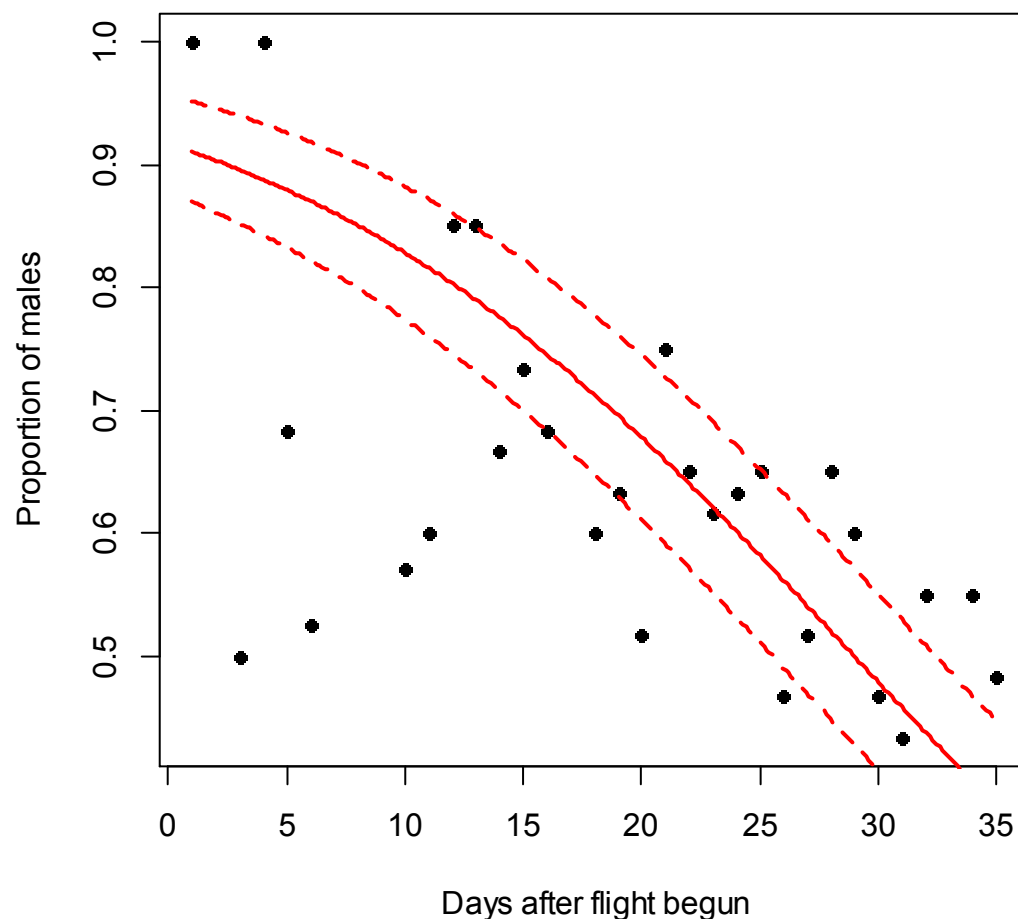
494   **Figure 1** *Costelytra zealandica* sex-ratio (proportion of males) on 35 d after its flight begun,
495   from October 27th to November 30th at the Awatere Valley in 2014. The beta-binomial GLM
496   (solid line) adjusted to each proportion during the flight season is presented with its respective
497   confidence intervals (dashed lines). The proportion of males is reduced through time (slope=-
498   0.08; z=-4.8; *p*<0.001). Days without flight activity during the season were removed from this
499   analysis.

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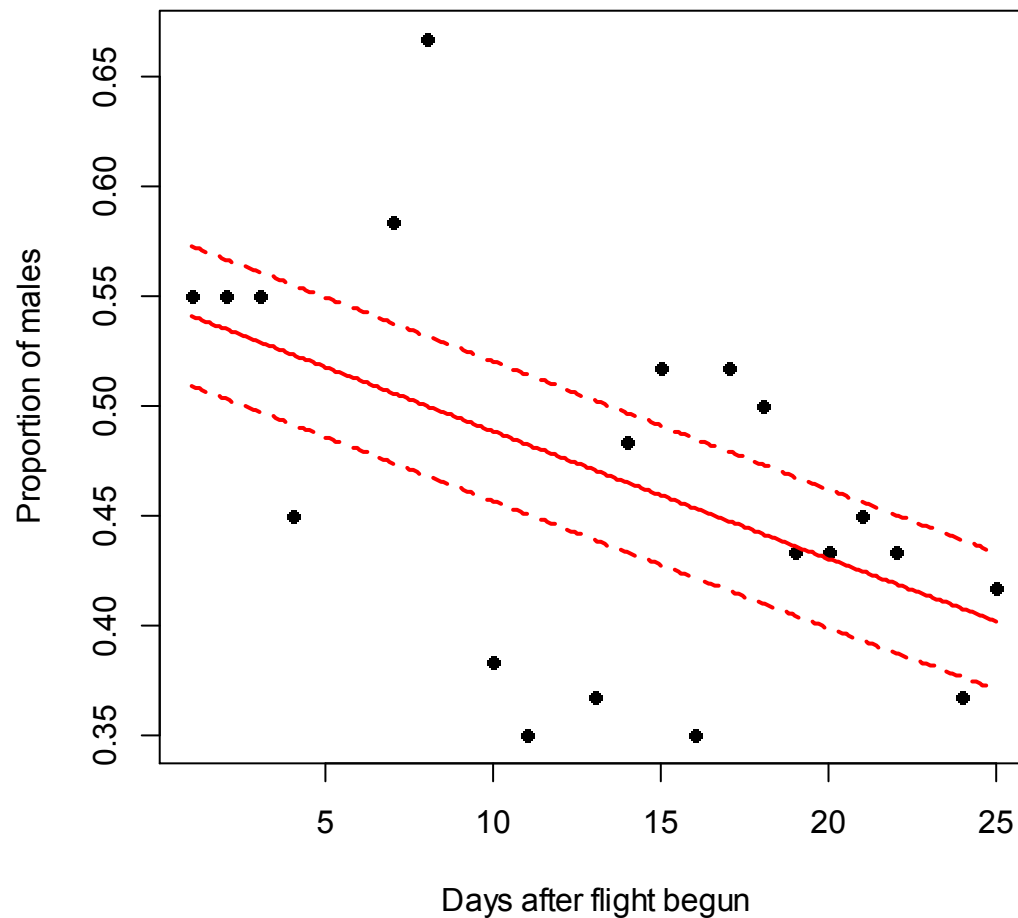
Figure 2 *Costelytra zealandica* sex-ratio (proportion of males) on 25 d after its flight begun, from November 7th to December 1st at the Awatere Valley in 2015. The beta-binomial GLM (solid line) adjusted to each proportion during the flight season is presented with its respective confidence intervals (dashed lines). The proportion of males is reduced through time (slope=-0.02; z=-2.6; p<0.01). Days without flight activity during the season were removed from this analysis.

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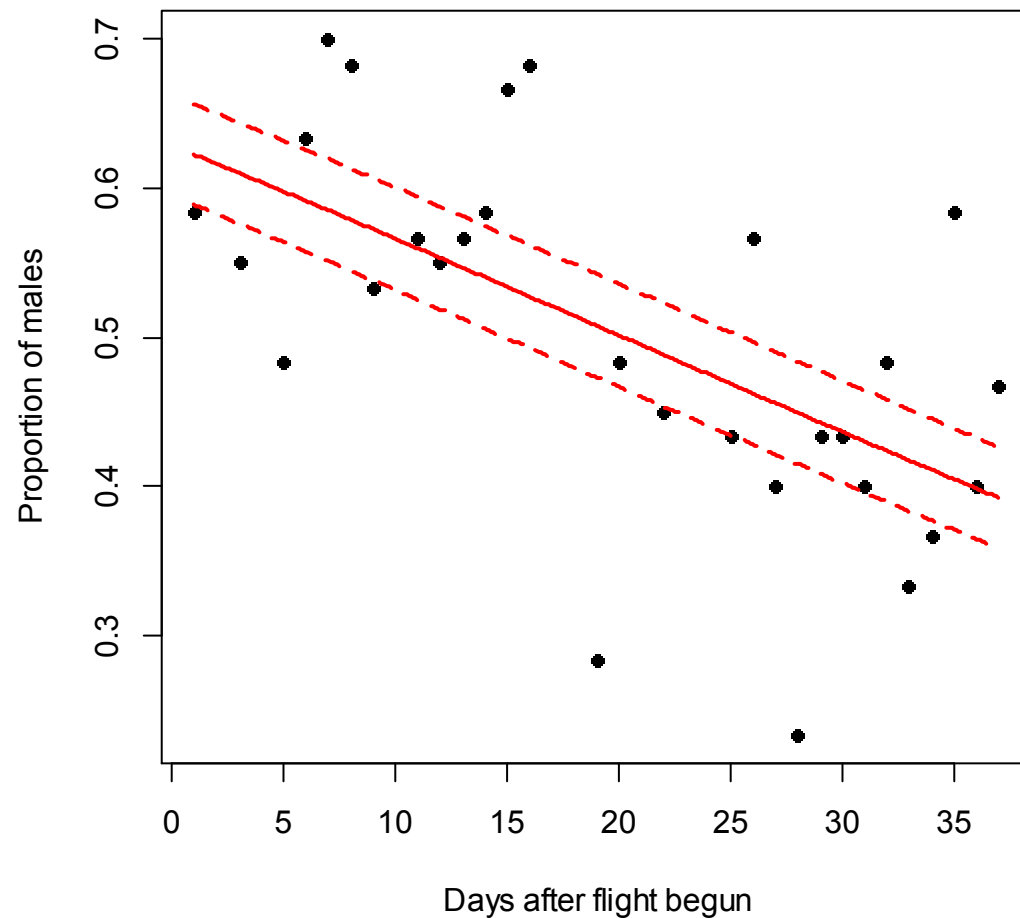
Figure 3 *Costelytra zealandica* sex-ratio (proportion of males) on 37 d after its flight begun,
from October 26th to December 1st close to Blenheim city in 2015. The beta-binomial GLM
(solid line) adjusted to each proportion during the flight season is presented with its respective
confidence intervals (dashed lines). The proportion of males is reduced through time (slope=-
0.03; $z=-4$; $p<0.001$). Days without flight activity during the season were removed from this
analysis

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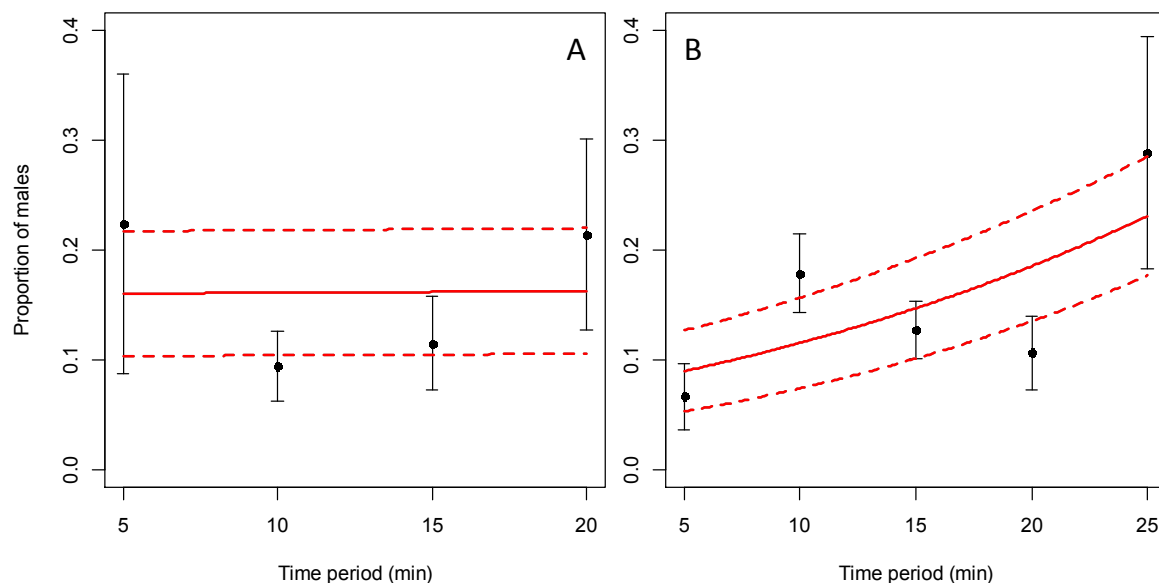
Figure 4 Proportion of *C. zealandica* males, after adult removal from a single grape vine plant at 5 min periods after daily flight activity begun, at the Awatere Valley (A) and Blenheim (B). The beta-binomial GLM (solid line) adjusted to each proportion during those time periods is presented with its respective confidence intervals (dashed lines). In the Awatere Valley (right), male proportion did not change during *C. zealandica* daily flight activity (slope=0.001; z=0.03; p=0.98), but in Blenheim (left), it increased through the daily flight time period (slope=0.06; z=2.12; p<0.05). Error bars are two-standard errors.

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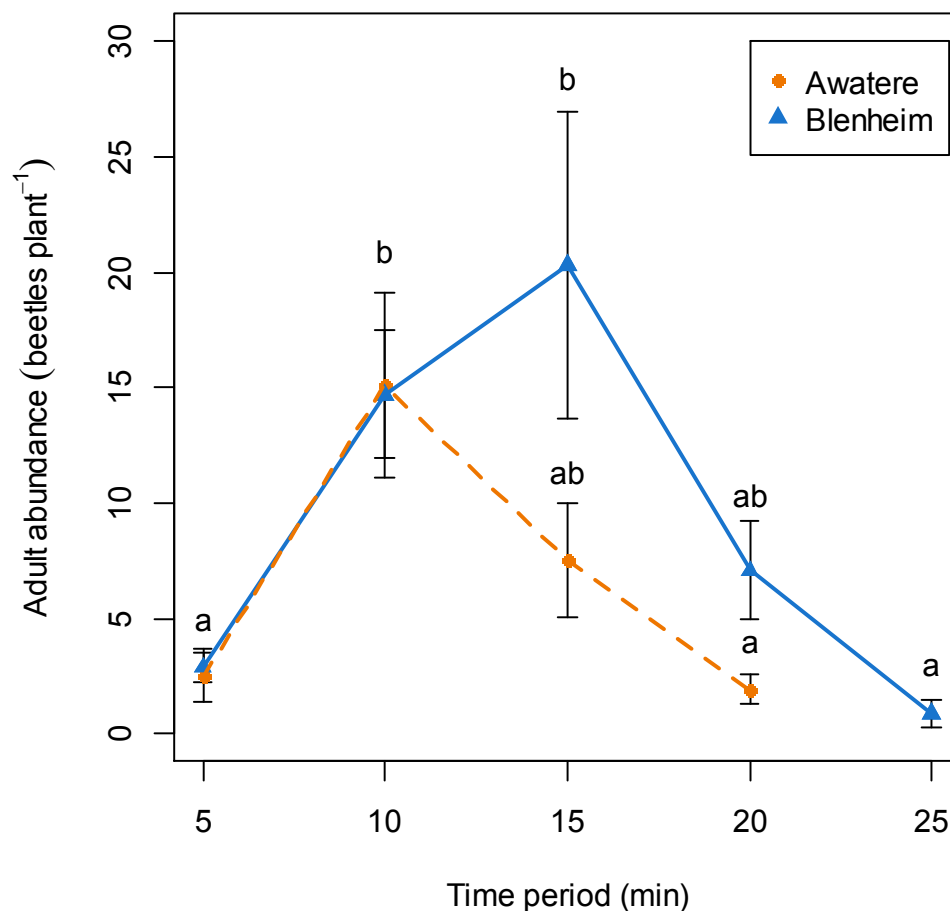
524 **Figure 5** Adult abundance during *C. zealandica* daily flight activity at different 5-min time
525 periods, for beetles that landed on a single grape vine plant at the Awatere Valley (dashed line)
526 and Blenheim (solid line) sites. Tukey contrasts are presented as different letters above each dot.
527 Different letters represent significantly differences among the different time periods, but not
528 between studied locations. Error bars are two-standard errors.

529