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7

8 **Diel behaviour and time budget of the adult pine weevil, *Hylobius abietis***

9

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24 Abstract

25 The pine weevil (*Hylobius abietis* (L.); Coleoptera: Curculionidae) has a high economic impact
26 on forest regeneration in Europe. The general biology of the pine weevil has received
27 considerable attention, but there is insufficient knowledge about its diel behaviour and time
28 budget. Therefore, the feeding and locomotion behaviour of individual adult weevils on Norway
29 spruce (*Picea abies* (L.) Karst.) seedlings is observed for 24 hour-periods in the laboratory. Both
30 girdled and non-girdled seedlings are used to assess how the weevils' behaviour is influenced by
31 the plants physiological response to the girdling. The locomotion pattern shows a distinct
32 maximum during the beginning of the dark phase whereas most feeding occurs during the second
33 half of the dark phase and the first hours of the subsequent light phase. The girdling treatment
34 increase the time that weevils spend on the seedlings during the first part of an observation
35 session, but has no effect on their feeding pattern. The weevils' time budgets on girdled and non-
36 girdled seedlings are similar. On average, they spend 34 % of their time in locomotion and 6 %
37 on feeding. Females spend more time feeding than males (7.1 % vs. 4.2 %), possibly because
38 they have higher food requirements, e.g. for egg production. Females also spend more time in
39 total on the seedlings than males (26.3 % vs. 7.0 %). This study reveals, in high temporal
40 resolution, the diel feeding and locomotion behaviour and time budget of male and female pine
41 weevils.

42

43 Keywords: chronobiology, circadian, conifers, daily rhythms, diurnal, herbivore, plant-insect
44 interactions, photoperiod, sex differences

45

46

47 **Introduction**

48 Diel rhythms allow organisms to prepare for periodic changes in environmental conditions, e.g.
49 onset of darkness. They are also affecting trophic relations and thus influencing survival chances
50 of predators and prey (Harmsen *et al.*, 2011). Even on an intraspecies level synchronisation of
51 diel rhythms is important (Danilevsky *et al.*, 1970). For example, females of different pyralid
52 moth species call during the beginning or end of the dark phase (Zavodska *et al.*, 2012), which
53 are common periods of high insect activity (Danilevsky *et al.*, 1970). The males of these species
54 have high locomotion activity during the respective periods which increases chances in mate
55 locating (Zavodska *et al.*, 2012). Other examples of diel rhythms include, the apple blossom
56 weevil (*Anthonomus pomorum* L.) and Warren rootcollar weevil (*Hylobius warreni* Wood),
57 which are mostly active and feed during dark phases, whereas they rest during light phases
58 (Cerezke, 1994; Duan *et al.*, 1996). In addition, both *H. warreni* and *Hylobius xiaoi* (Zhang)
59 show distinct diel movement patterns, ascending trees at dusk and descending at dawn (Cerezke,
60 1994; Wen *et al.*, 2004).

61

62 In the present study, the diel behaviour of the pine weevil (*Hylobius abietis* (L.) (Coleoptera:
63 Curculionidae)), an economically important forest pest species, is investigated. The pine weevil
64 is distributed over large parts of Europe and Asia. It feeds on tender bark of several conifer
65 species, e.g. Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies*). Pine weevils
66 reproduce in the roots of recently killed or dying conifer trees, which occur at high densities in
67 man-made clear-cuts and after storm fellings or forest fires (Eidmann, 1974). The weevils
68 disperse to suitable areas for reproduction by long-distance migratory flight in spring and early
69 summer (Solbreck & Gyldberg, 1979). Host volatiles emitted from stumps and dying trees guide

70 the flying weevils to suitable sites, and soon after they have settled their wing muscles
71 degenerate (Nordenhem, 1989). Walking weevils use both visual and olfactory cues to locate
72 conifer seedlings (Björklund *et al.*, 2005). Although weevils feed mostly on bark of roots and
73 twigs of mature conifer trees, it is the feeding on the stem bark of seedlings that cause the
74 economic losses (Wallertz *et al.*, 2006; Örlander *et al.*, 2000). Damages to seedlings may cause
75 mortality of up to 90 %, mostly due to girdling (Långström & Day, 2004; Petersson & Örlander,
76 2003).

77

78 Under laboratory conditions locomotion behaviour of pine weevils is highest at the beginning of
79 dark phases and decreases towards the end of dark phases or after the onset of light phases
80 (Merivee *et al.*, 1998; Pszczolkowski & Dobrowolski, 1999). Under field conditions their
81 locomotion is also influenced by ambient temperature and humidity, which are generally most
82 favourable at twilight (Sibul *et al.*, 1999). They feed mainly during dark phases under field
83 conditions, but under semi-field conditions in shaded cages and laboratory conditions their
84 feeding activities has not been demonstrated to show any obvious temporal pattern (Christiansen
85 & Bakke, 1971; Merivee *et al.*, 1998).

86

87 Diel patterns are also expressed in plants, e.g. in photosynthesis activity and volatile emission
88 (Lüttge & Hertel, 2009; Staudt *et al.*, 1997). It is recently reported that the diel regulation of an
89 induced plant's defence is synchronized with temporal patterns of its herbivores' feeding
90 behaviour (Goodspeed *et al.*, 2012). Damages to conifer seedlings, like girdling, a typical
91 damage caused by adult pine weevils (Eidmann, 1974), cause changes in the chemical
92 composition of the stem and in the volatiles that are emitted (Gref & Ericsson, 1985; Martin *et*

93 *al.*, 2003). Such chemical changes in seedlings due to insect damage may also influence pine
94 weevil behaviour (Kännaste *et al.*, 2009).

95

96 Time budgets describe the proportions of time that an animal allocates to different behaviours
97 (Joern *et al.*, 1986). These proportions do not necessarily reflect the importance of the
98 behaviours but indicate the optimal solutions to environmental problems (Joern *et al.*, 1986).

99 Many insects, such as apple blossom weevils, Costa Rican weevils, potato beetles, carabid
100 beetles and grasshoppers, spend most of their time resting (Drees *et al.*, 2008; Duan *et al.*, 1996;
101 Joern *et al.*, 1986; Mitchell & Low, 1994; Wright *et al.*, 1999). Less time is allocated to feeding
102 behaviour, ranging from just 3 % for the Costa Rican weevil up to 27 % for the Apple blossom
103 weevil (Duan *et al.*, 1996; Wright *et al.*, 1999).

104

105 The aim of this study was to describe diel behaviour patterns and time budgets of adult pine
106 weevils. Locomotion and feeding behaviour of adult pine weevils are studied on Norway spruce
107 (*Picea abies* (L.) Karst.) seedlings in the laboratory under an artificial light regime using 24-h
108 video-recordings. Because the behaviour may be influenced by the physiological condition of the
109 seedling, the behaviours on both non-girdled and previously girdled seedlings are investigated.

110 The behaviour of male and female weevils is also compared.

111

112 **Materials and methods**

113 *Insect and plant material*

114 The pine weevils used in the study were collected in central Sweden during spring migration a
115 few weeks before the start of the experiment. After collection, the weevils were fed with

116 branches of Scots pine while kept in darkness at 10°C. These storage conditions interrupt the
117 reproductive development of the weevils (Bylund *et al.*, 2004). About a week prior to their use in
118 the experiment the weevils were transferred to a chamber with room temperature (22°C) and the
119 artificial day-night-cycle (L18:D6) as was used during the experiment (see below). This
120 procedure synchronized the physiological state of the weevils, since mating, oviposition and
121 therewith associated period of high feeding activity starts soon after the transfer from storage in
122 darkness at 10°C (Bylund *et al.*, 2004).

123

124 The plant material consisted of one and a half year old Norway spruce (*Picea abies*) seedlings
125 grown from seeds collected in a seed orchard and produced for plantation in central Sweden by a
126 commercial nursery (Sjögränd, Bergvik Skog AB). The seedlings were kept outdoors and
127 transferred to a chamber providing the experimental conditions a day before use.

128

129 *Experimental procedure*

130 The experiment was conducted during June to September 2011. Six male and six female pine
131 weevils were placed singly in a transparent plastic cylinder (height 8 cm, diameter 11 cm) that
132 was placed around the middle part of a spruce seedling's stem (Figure 1). The stem base and top
133 shoot were not accessible to the weevil to reduce heterogeneity of the feeding source (Kaakinen
134 *et al.*, 2004). Neither the connections between the parts of the cylinder nor the connection to the
135 seedling were airtight. Two video cameras (Sony Handycam HDR-XR550VE, Sony
136 Corporation, Tokyo, Japan; G lense (F 1.8-3.4; focal length: f 3.8-38 mm)) were then focused on
137 the seedling, set at 45 ° to each other to ensure high visibility of weevil activity close to the
138 seedling. However, the weevils could be out of vision when being close to either of the cameras,

139 i.e. away from the seedling. Both before and during the entire experimental period the following
140 artificial light regime was used: 18 h light/6 h darkness with visible light intensities of 59 and 0.1
141 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively (Sky 200 SKP meters; Skye Instruments Ltd., Llandrindod Wells,
142 UK). This light regime was not in phase with the natural light cycle. To avoid that a change in
143 light intensity at the start of the observation sessions influenced the weevils' behaviour, the
144 sessions were started with a 7 h light phase, followed by the 6 h dark phase, and then an 11 h
145 light phase. The experiment was conducted at room temperature (22 °C).

146

147 Each weevil was placed in the cylinder surrounding a seedling a day before the start of each
148 observation session to habituate them to the cylinder. To avoid effects of any feeding scars the
149 seedling was then replaced with a new one after the acclimatization period. Video recording was
150 started when the new seedling was installed and ended 24 h later.

151

152 The behaviour of each weevil was observed in two sessions, once with a non-girdled seedling
153 and once with a seedling that had been manually girdled 24 h prior to video recording. The
154 girdling was done by removing a 5 mm ring of outer and inner bark tissues from the stem under
155 the first node. Half of the weevils of each sex were placed in a cylinder surrounding a girdled
156 seedling during the first observation session and the other half in a cylinder surrounding a non-
157 girdled seedling. Cylinders were cleaned with a damp paper towel between observation sessions.
158 Between the observation sessions the weevils were placed for two days in Petri dishes with
159 pieces of Scots pine twigs and a water tube.

160

161 The weevils' behaviours were visually recorded and divided into two mutually exclusive
162 behaviour groups related to feeding and locomotion as listed in Table 1 (The Observer XT 10,
163 Noldus Information Technology, Wageningen, Netherlands). Feeding behaviours were observed
164 continuously in real-time play back speed and the initiation and termination of these behaviours
165 were recorded with the highest possible resolution allowed by the cameras, 0.04 seconds.
166 However, other behaviours were observed continuously in 16x real-time play back speed and
167 short interruptions in these behaviours may therefore have been missed.

168

169 *Statistical analyses*

170 Each 24-h observation session was split into one-hour periods. Then the total time the weevil
171 spent in each of the behaviours and the time it spent on the seedling in each hour were
172 calculated. For comparisons of behaviours in the presence of girdled and non-girdled seedlings
173 data from the 24-h observation sessions were averaged, whereas for comparisons between sexes
174 the data were first averaged per individual and subsequently for all individuals together.

175

176 The times spent on feeding and in locomotion during the light and dark phases were separately
177 summed and divided by the total duration of the respective phase. The significance of differences
178 in these times between light/dark phases with girdled/non-girdled seedlings or male/female
179 weevils was then tested with 2-way ANOVA (Minitab, Version 16.1.0, Minitab Inc., U.S.A.).

180

181 The total time spent on the seedling before and after the first feeding occasion were compared
182 using the Wilcoxon signed ranks test (R, Version 2.12.1, The R Foundation for Statistical
183 Computing, Austria) as the data did not always meet normality of distribution (Kolmogorov-

184 Smirnov, $P < 0.05$) or homogeneity of variance (F -test, $P < 0.05$) requirements for parametric
185 tests. The comparison was made separately for observation sessions with non-girdled and girdled
186 seedlings, and differences in the times spent by male and female weevils on different behaviours.
187 In addition, the time until the first feeding occasion was compared between non-girdled/girdled
188 seedlings and male/female weevils using Wilcoxon signed ranks and Mann-Whitney tests,
189 respectively.

190

191 To calculate time budgets the proportions of the time during an observation session the weevils
192 spent in locomotion and standing while being on the seedling were calculated. The same was
193 done for the time when weevils were not on the seedling but here a category with “out of vision”
194 was also included. The time they spent standing on the seedling was divided into time when they
195 were and were not feeding. The proportions of times spent on the behaviours between non-
196 girdled/girdled seedlings, and between male/female weevils, were compared using Wilcoxon
197 signed ranks and Mann-Whitney tests, respectively.

198

199 In addition, differences in the proportion of times that males and females spent in locomotion or
200 standing when they were not feeding (which met normality of distribution and homogeneity of
201 variance requirements for parametric tests) were assessed using 2-sample t -tests.

202

203 **Results**

204 *General diel activity*

205 The locomotion activity of the weevils was higher during the dark phase than during the light
206 phase ($P < 0.001$; Figs. 2 a and b, 3 a and b), particularly during the first half of the dark phase.
207 Neither sex nor girdling had a significant effect on locomotion ($P > 0.05$).

208

209 The time spent feeding was low from the beginning of the observation sessions to the second half
210 of the dark phase (Fig. 2 c and d, 3 c and d). Feeding time was not influenced by girdled/non-
211 girdled seedlings or light/dark phase ($P > 0.05$). The interactions between the light phase and
212 girdling or the light phase and weevil sex were neither significant ($P > 0.05$). Females, however,
213 spent more time feeding than males ($P = 0.04$).

214

215 *Diel activity on the seedling*

216 Both males and females started feeding a similar time after the beginning of the experiment (11.3
217 ± 0.6 h and 7.6 ± 1.2 h, respectively; $P = 0.13$; $N = 12$). On non-girdled seedlings feeding started
218 later than on girdled seedlings (11.3 ± 1.1 h and 7.6 ± 1.2 h, respectively; $P = 0.02$; $N = 24$).

219

220 Weevils spent more time on the seedlings during the second half of the dark phase than in the
221 first half (Fig. 2c and d). They also spent significantly less ($P < 0.001$) time on non-girdled
222 seedlings before (1.7 ± 0.6 min h^{-1}) than after (17.0 ± 4.3 min h^{-1} ; Fig. 2c) the first feeding
223 occasion, but not on girdled seedlings (6.5 ± 4.5 min h^{-1} before, 11.9 ± 2.8 min h^{-1} after; $P =$
224 0.052 ; Fig. 2d). Thus, the weevils spent more time on seedlings that had been injured by either
225 manual girdling or weevil feeding.

226

227 Female weevils spent more time than males on the seedlings both in total and relative to the time
228 spent feeding (Fig. 3c and d). Males spent more time on the seedlings after the first feeding
229 occurrence ($1.0 \pm 0.2 \text{ min h}^{-1}$ before, $7.1 \pm 1.3 \text{ min h}^{-1}$ after; $P = 0.03$), but not females (6.8 ± 4.3
230 min h^{-1} before, $21.7 \pm 4.0 \text{ min h}^{-1}$ after; $P = 0.06$). The time on the seedling that was spent not
231 feeding was not significantly different before (females $6.8 \pm 4.3 \text{ min h}^{-1}$; males $1.0 \pm 0.2 \text{ min h}^{-1}$)
232 and after (females $14.9 \pm 3.7 \text{ min h}^{-1}$; $P = 0.31$; males 2.0 ± 0.6 ; $P = 0.31$) the first feeding
233 occurrence for both sexes.

234

235 *Time Budget*

236 Weevils spent on average 6 % (range, 2.1 % to 13.8 %) of the day feeding (Fig. 4). Most of the
237 time they were not on the seedling and during this time they spent a larger proportion of the time
238 standing than in locomotion.

239

240 The girdling treatment did not affect the total amount of time allocated to any tested behaviour
241 ($P > 0.05$; Fig. 4 a and b).

242

243 Females spent significantly more of the time on the seedling than males (26 % and 7%,
244 respectively; $P = 0.01$). They also spent significantly more time than males in locomotion ($4.4 \pm$
245 0.4% and $1.5 \pm 0.1 \%$, respectively; $P = 0.01$; Fig. 4 d and c) and standing without feeding (14.8
246 $\pm 2.0 \%$ and $1.3 \pm 0.2 \%$, respectively; $P = 0.01$) on the seedling. However, females did not
247 spend more time than males standing while feeding ($7.2 \pm 0.5 \%$ and $4.2 \pm 0.2 \%$, respectively; P
248 $= 0.07$). When not on the seedling, there was no significant difference between females and
249 males in time spent in locomotion ($24.3 \pm 1.1 \%$ and $37.8 \pm 2.8 \%$, respectively; $P = 0.09$) or in

250 time spent standing (42.8 ± 2.6 % and 42.5 ± 2.2 %, respectively; $P = 0.81$). However, females
251 were standing for a higher proportion of the time when they were not feeding than males
252 (61.9 ± 4.8 % and 45.7 ± 5.2 %, respectively; $P = 0.049$).

253

254 The weevils fed predominantly on the stems of the seedlings (96.0 ± 1.2 %, see “food intake”;
255 table 1), but also on needles (4.0 ± 1.2 %).

256

257 **Discussion**

258 This study investigated the diel behaviour pattern and time budget of adult pine weevils with
259 high temporal resolution under laboratory conditions. Feeding on seedlings and locomotion both
260 on and away from seedlings and the time allocated to these behaviours are examined in detail.

261 The effects of girdling seedlings on the weevils’ behaviour are also investigated.

262

263 Both locomotion and feeding behaviour show a diel rhythm. Most locomotion occurs during the
264 first half of the dark phase, then locomotion decreases and feeding activity increases during the
265 second part of the dark phase and into the light phase. The increase in locomotion activity in
266 connection with the dark phase is consistent with previous reports on pine weevils and many
267 carabid beetles (Merivee *et al.*, 1998; Pszczolkowski & Dobrowolski, 1999; Thiele & Weber,
268 1968). Merivee and co-workers (1998) report that the increase of pine weevil locomotion occurs
269 before the onset of the dark phase, which is not clearly seen in the present study. The reduction
270 in locomotion behaviour in the second half of the dark phase is consistent with the pattern
271 described by Pszczolkowski & Dobrowolski (1999). In the field, other factors affect the weevils’
272 behaviour as well. Locomotion activity is most frequent at dusk and dawn, which coincide with

273 periods of favourable weather conditions, i.e. moist and moderately warm (Sibul *et al.*, 1999).
274 Other weevil species, such as the Warren rootcollar weevil, pine root collar weevil, *H. xiaoi*, and
275 apple blossom weevil, also express higher locomotion activity during the night (Cerezke, 1994;
276 Duan *et al.*, 1996; Wen *et al.*, 2004; Wilson, 1968). This study shows that changes in light
277 intensity *per se* can be enough to trigger a diel behaviour pattern, since the present study uses an
278 artificial light and dark cycle, which is not in phase with the natural day-night cycle.

279

280 The pine weevils feed mostly during the second half of the dark phase and the beginning of the
281 following light phase, somewhat in accordance with findings by Pohris (1983) that pine weevils
282 feed more during constant darkness than during constant light. In addition, the diel feeding
283 pattern observed in the present study is more strongly expressed in males, possibly because they
284 spend less time feeding than females (present study and Merivee *et al.*, 1998). This may reflect
285 females' need to spread their feeding behaviour over longer time periods than males as females
286 have to digest larger amounts of food but their digestion times are similar (Nuorteva, 1972).

287

288 In contrast to the pine weevil, feeding activity of the apple blossom weevil increases sharply
289 directly after the onset of the dark phase, possibly because its locomotion activity is more evenly
290 distributed during the dark phase (Duan *et al.*, 1996). In the present study, some of the reduction
291 in the pine weevils' locomotion during the second half of the dark phase may have been due to
292 the onset of feeding behaviour. However, as these behaviours are mutually exclusive it is
293 difficult to determine if the pine weevil's feeding is concentrated in the second part of the dark
294 phase because locomotion activity predominates in the first part or *vice versa*.

295

296 When they are not feeding, males spent less of the time standing than females, in line with
297 previous findings that males have higher locomotion activity than females (Merivee *et al.*, 1998).
298 A possible explanation for this pattern is that the males may search for mates more actively than
299 females, which is common in insects that produce no long-range signals for attracting mates
300 (Brown *et al.*, 1997). Seedlings and other host material have their own attraction potential and
301 males might find females easier in the vicinity of those.

302

303 Discrepancies in pine weevil feeding patterns observed in different studies might be partly due to
304 differences in the types of food sources used. Living plants may differ in their responses to
305 weevil damage, e.g. due to their induced defences (Heijari *et al.*, 2005), whereas cut-off twigs, as
306 used by Merivee and co-workers (1998), are already severely damaged. In addition, there are diel
307 patterns in the expression of living plants' defences, which may as well influence diel patterns of
308 feeding behaviour (Goodspeed *et al.*, 2012). Even minor damage to seedlings may trigger
309 changes in their volatile emissions and make them more attractive to pine weevils (Nordlander,
310 1991). However, the pine weevil generally responds to these volatiles within a short range from a
311 seedling (Björklund *et al.*, 2003). In the present study, weevils spent more time on both girdled
312 seedlings and non-girdled seedlings after the first feeding occasion than on non-girdled seedlings
313 before the first feeding on them, indicating that they were attracted to damaged seedlings. After
314 the first feeding on non-girdled seedlings their behaviour patterns were similar to those on
315 previously girdled seedlings. Therefore, some of the behavioural patterns we observed on non-
316 girdled seedlings would probably not be observed on cut-off twigs (see Merivee *et al.*, 1998). No
317 differences in time spent feeding on girdled and non-girdled seedlings were observed, maybe due
318 to that the defensive response to the damage had not been completed before the use in the

319 experiment. Conifers may respond quickly to damage, by e.g. up-regulating the defensive gene
320 expression within a few hours (Ralph *et al.*, 2006) and increase the resin content within 48 hours
321 (Sampedro *et al.*, 2011). However it may take up to a week before responses reach maximum
322 level (Lewinsohn *et al.*, 1991). In the present study, a relatively short period is used between the
323 first and second damage, because this resembles common patterns of weevil attack when
324 seedlings are planted on clear cuts (Björklund *et al.*, 2005; Nordlander, 1991).

325

326 Pine weevils only allocate about 6 % of their time to feeding behaviour, but this is twice as much
327 as Costa Rican weevils spend on feeding (Wright *et al.*, 1999), whereas apple blossom weevils
328 may spend up to 27 % of their time feeding (Duan *et al.*, 1996). The variation between species
329 can be due to differences in their biology, differences in the nutritional value of their host plants
330 or differences in the defence reactions of the host plants (Harrison, 1987).

331

332 Most of the feeding observed was on the stem bark, where the pine weevil generally causes
333 damage. A smaller proportion of feeding was observed on needles. Some of the needles were not
334 used as a food resource, but rather the weevils bit them off and then fed on the bark in the
335 vicinity of the removed needle. However, other needles were partly eaten (data not shown),
336 possibly due to differences in nutrition composition or water content between needles and bark
337 (Kaakinen *et al.*, 2004), which may make needles attractive as a supplementary food source.

338

339 In this study, a detailed description is presented of diel activity patterns of both male and female
340 pine weevils on both girdled and non-girdled seedlings. The sex of the weevils affects the diel
341 patterns of both feeding and locomotion behaviour as well as the time budget, but the girdling

342 did not. On average, weevils spent 6 % of their time feeding and 34 % in locomotion and the rest
343 of the time standing. The results of this study, which are the outcome of the interaction between
344 the pine weevil and a conifer seedling, provides basic knowledge for future studies that in the
345 end may enable development of new methods for increasing seedling survival, hence improving
346 the regeneration of managed conifer forests.

347

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354

355

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357

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471

472

473 Table 1: Ethogram of observed behaviours

Behaviour	Definition	Comment
Feeding	Merged group of food intake and biting needles	
Food intake	Manipulation of bark with the mouth parts	Periods of bark removal and ingestion were not distinguished
Biting needles	Manipulation of needles with the mouth parts	Amount could not be measured
Locomotion	Any behaviour that transports the weevil from one position to a position more than one weevil length away	Includes short periods of standing (e.g. stop-and-go periods)
Standing	Remaining within a radius of one weevil length	Includes local movements and feeding
Out of vision	Not visible on video recording	Weevil may be performing locomotion or standing, but not feeding behaviours

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478 **Figure legends**

479 Figure 1: Experimental set-up. A small water tube and a dark coloured shelter for weevils to hide
480 under inside the cylinder are not shown.

481

482 Figure 2: Diel behaviour patterns of pine weevil with non-girdled (N = 12) and previously
483 girdled seedlings (N = 12) in total (a, b) and while on the seedlings (c, d). Shaded areas represent
484 the dark phase. Values are means \pm one standard error.

485

486 Figure 3: Diel behaviour patterns of male (N = 6) and female (N = 6) pine weevil in total (a, b)
487 and while on the seedlings (c, d). Shaded areas represent the dark phase. Values are means \pm one
488 standard error.

489

490 Figure 4: Time budget of both sexes of pine weevils with access to a non-girdled (a) and a
491 previously girdled (b) seedling (each N = 12) and time budget of male (c) and female (d) weevils
492 (each N = 6) with access to both kinds of seedlings. Values are average percentages of time over
493 the entire 24-hours observation sessions.

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495

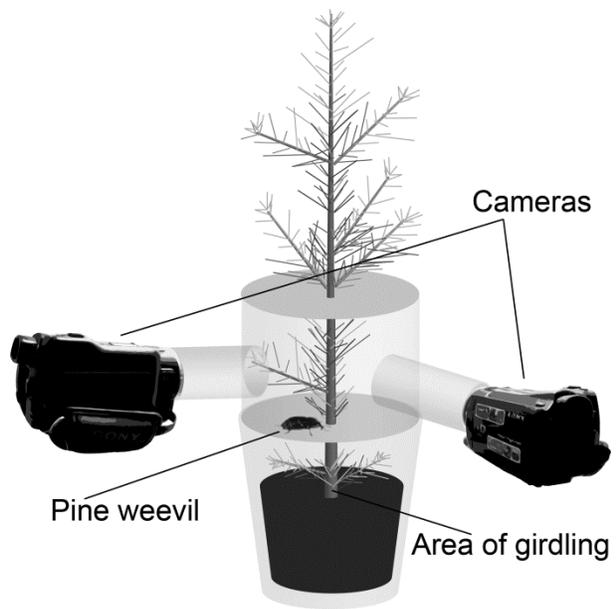


Figure 1

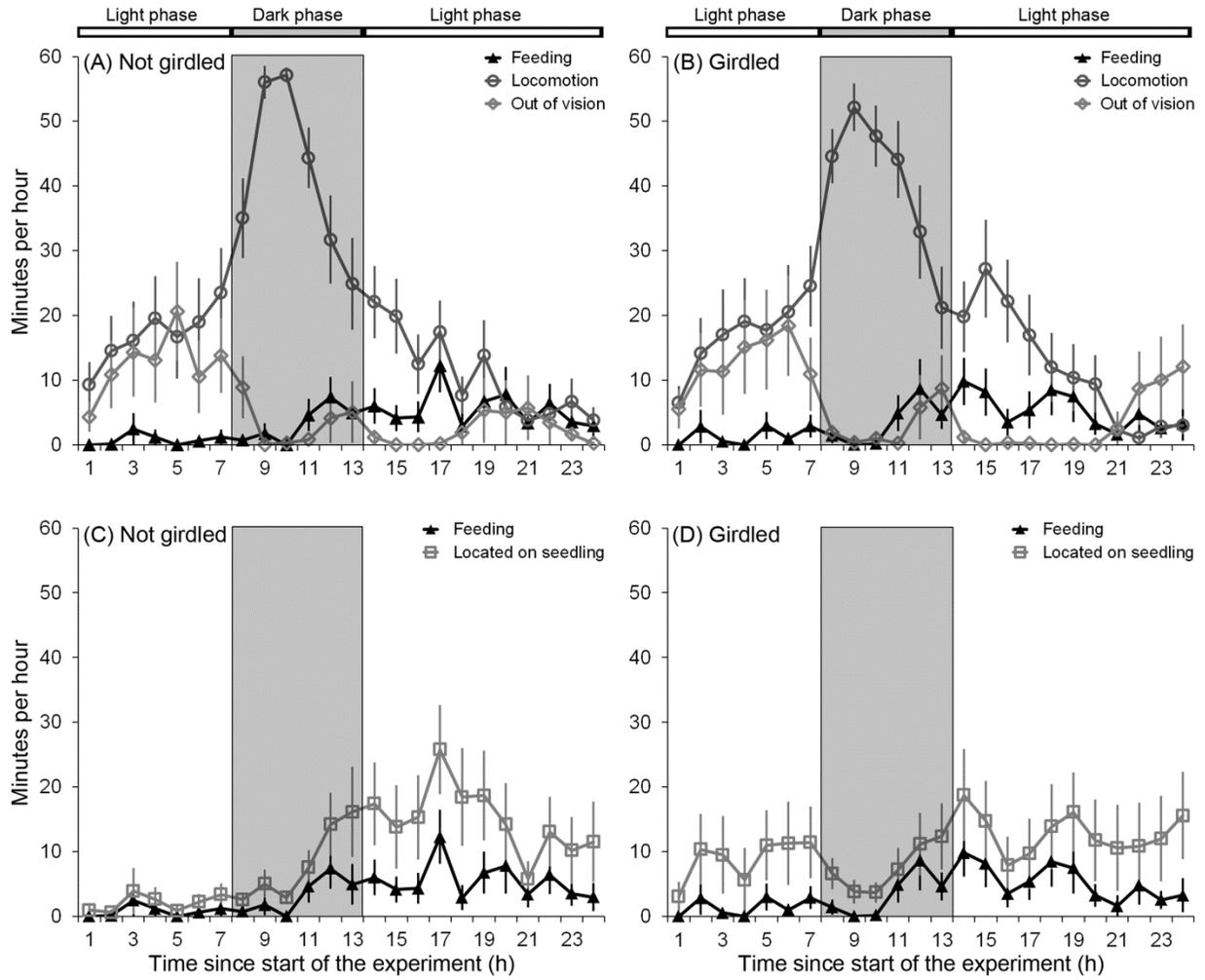


Figure 2

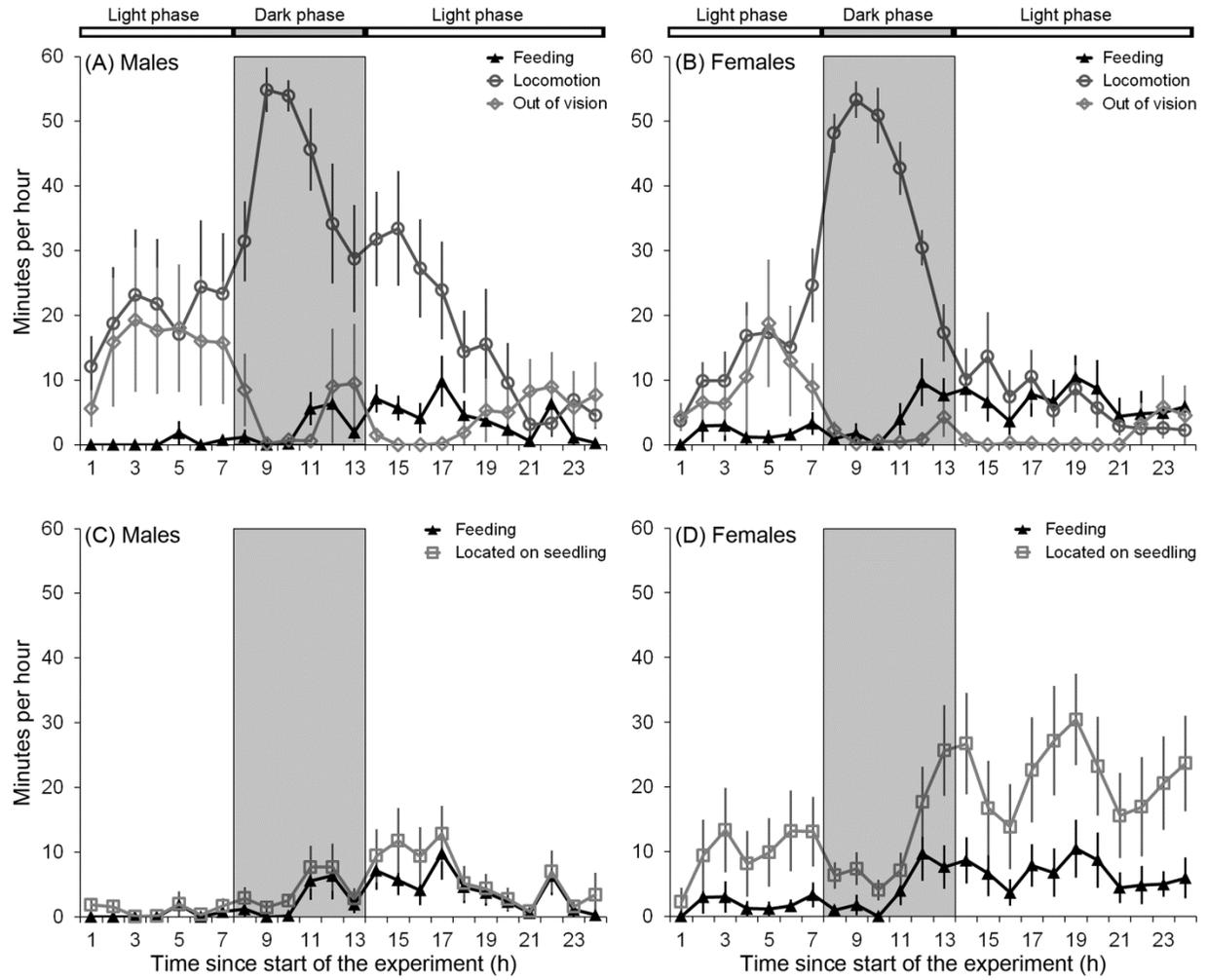
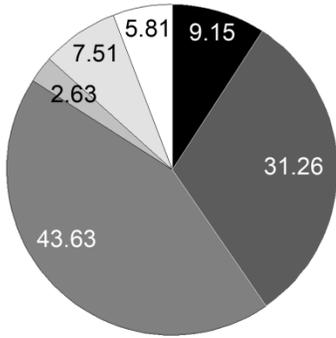
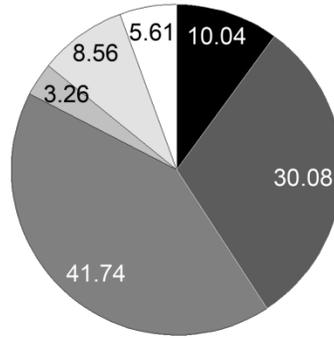


Figure 3

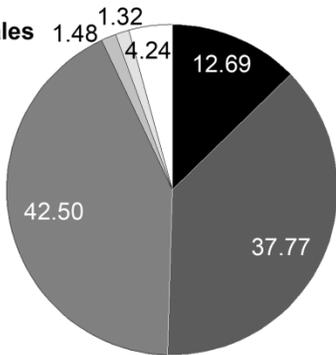
(A) Not girdled



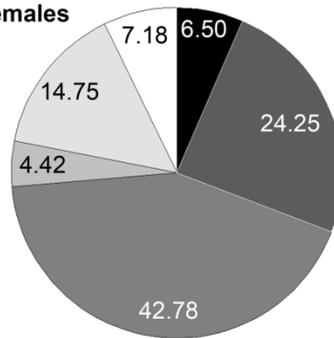
(B) Girdled



(C) Males



(D) Females



Behaviour not on seedling

- Out of vision
- Locomotion
- Standing

Behaviour on seedling

- Locomotion
- Standing without feeding
- Standing and feeding

Figure 4