

Crop Protection

Top-down and bottom-up regulation of codling moth populations in cider apple orchards

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Abstract:	<p>The success of biological control by natural enemies in agricultural crops relies on an understanding of the trophic interactions between natural enemies, pests and host plants. Top-down and bottom-up trophic effects, together with potential landscape and local-scale factors, may regulate pest populations. For two years, we analyzed codling moth populations (<i>Cydia pomonella</i>), their crop damage and their parasitoid communities in 26 low-input cider apple orchards in northern Spain. Codling moth abundance was estimated from overwintering larvae sampled with cardboard traps on trees, parasitism was estimated from parasitoids emerged from lab-reared moth larvae, and pest damage was assessed in apples before ripening. Codling moth abundance differed between orchards across years, and was positively correlated with apple production and the cover of apple plantations in the surrounding landscape. The effects of the apple production on codling moth abundance suggest bottom-up pest regulation. Apple damage in individual orchards reached 71%, but decreased with apple production, indicating codling moth satiation. Seven parasitoid species were recorded on codling moth larvae. Parasitism rate in individual orchards reached 42.5% of codling moth larvae. The number of parasitized larvae per orchard was positively related to parasitoid richness, but also to codling moth abundance, suggesting simultaneous top-down and bottom-up effects between parasitoids and pest. This study highlights the need to tackle the whole parasitoid-pest-plant system in order to better manage codling moth damage in orchards. The conservation of complementary parasitoid species through biodiversity-friendly actions should be combined with the control of apple production at the orchard- and landscape scale.</p>

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3 Top-down and bottom-up regulation of codling moth
4 populations in cider apple orchards

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15 **Highlights**

- 16 • We explore the ecological forces determining population size of codling moth (CM).
- 17 • CM abundance increased with apple production and surrounding cover of apple trees.
- 18 • CM apple damage decreased with apple production.
- 19 • Number of parasitized larvae increased with parasitoid richness and CM abundance.
- 20 • Tackling the whole parasitoid-pest-plant system is required to better manage CM.

21

22 **Abstract**

23 The success of biological control by natural enemies in agricultural crops relies on an
24 understanding of the trophic interactions between natural enemies, pests and host plants. Top-
25 down and bottom-up trophic effects, together with potential landscape and local-scale factors,
26 may regulate pest populations. For two years, we analyzed codling moth populations (*Cydia*
27 *pomonella*), their crop damage and their parasitoid communities in 26 low-input cider apple
28 orchards in northern Spain. Codling moth abundance was estimated from overwintering larvae
29 sampled with cardboard traps on trees, parasitism was estimated from parasitoids emerged
30 from lab-reared moth larvae, and pest damage was assessed in apples before ripening. Codling
31 moth abundance differed between orchards across years, and was positively correlated with
32 apple production and the cover of apple plantations in the surrounding landscape. The effects of
33 the apple production on codling moth abundance suggest bottom-up pest regulation. Apple
34 damage in individual orchards reached 71%, but decreased with apple production, indicating
35 codling moth satiation. Seven parasitoid species were recorded on codling moth larvae.
36 Parasitism rate in individual orchards reached 42.5% of codling moth larvae. The number of
37 parasitized larvae per orchard was positively related to parasitoid richness, but also to codling
38 moth abundance, suggesting simultaneous top-down and bottom-up effects between
39 parasitoids and pest. This study highlights the need to tackle the whole parasitoid-pest-plant
40 system in order to better manage codling moth damage in orchards. The conservation of
41 complementary parasitoid species through biodiversity-friendly actions should be combined with
42 the control of apple production at the orchard- and landscape scale.

43

44 **Keywords:** apple damage, *Cydia pomonella*, low-input farming, parasitoids, pest control,
45 trophic interactions

46

47 **1. Introduction**

48 The biological control of agricultural pests by natural enemies can offer effective solutions for
49 avoiding crop damages while reducing the negative environmental and health impacts of
50 chemical pesticides (Landis et al. 2000; Crowder and Jabbour 2014; Demestihis et al. 2017).
51 Among these natural enemies, parasitoids are considered a highly effective group for biological
52 control, thanks to their high diversity and specialization degree (Mason and Huber 1993;
53 Godfray 1994). By impairing and eventually killing individual hosts (Godfray 1994), parasitoids
54 can limit pest populations (Waage and Hassell 1982; Gerling et al. 2001), ultimately reducing
55 crop damages and providing an ecosystem service valued at billions of dollars annually (Losey
56 and Vaughan 2006; Crowder and Jabbour 2014). Importantly, the richness of parasitoid
57 assemblages may be important in modulating their potential for biological control, as different
58 species may render additive and complementary roles in hampering pests (Straub and Snyder
59 2006; Peralta et al. 2014). In fact, several studies have evidenced the positive effects of
60 parasitoid richness on both the magnitude and the temporal stability of parasitism rates (Ives et
61 al. 2005; Snyder et al. 2006; Tylianakis et al. 2006).

62 **Pest population dynamics are frequently regulated not only by the top-down forces**
63 **expected in natural enemy-pest interactions but also** by bottom-up forces via their host plants,
64 with the **availability** of food resources (i.e. crops) limiting pest population growth (Singer and
65 Stireman 2005; Walker et al. 2008; Vidal and Murphy 2018). Consequently, a crop management
66 system which decreases the access of pests to specific food resources, as for example by
67 increasing within-crop species or genetic diversity, may lead to reduced pest damage (Root
68 1973; Smith and McSorley 2000). Establishing effective measures for the parasitoid-based
69 biological control of crop pests therefore requires an integrative understanding of both top-down
70 and bottom-up mechanisms simultaneously operating in all three levels of parasitoid-pest-plant
71 interactions (Singer and Stireman 2005; Peralta et al. 2014).

72 Interactions between parasitoids, pests and crops may be shaped by the environmental
73 heterogeneity inherent to agroecosystems at different (i.e. local and landscape) scales. The

74 habitats adjacent to crop fields may be a source of complementary or alternative food sources
75 for parasitoids (Bianchi and Wäckers 2008; Gillespie et al. 2016), or even pests in cases where
76 they are fields of the the same crop type (Ricci et al. 2009). Furthermore, spillover of insects to
77 crops may be affected by not only the quality, but also the connectivity of surrounding habitats
78 (Dennis et al. 2003; Hiebeler and Morin 2007). Thus, by affecting the population size of
79 parasitoids and pests, local- and landscape-scale habitat variability may affect their trophic
80 interactions, and hence the relative strength of both top-down and bottom-up relationships
81 (Hunter and Price 1992; Maalouly et al. 2013; Šigut et al. 2018).

82 Codling moth (*Cydia pomonella* (L.), Lepidoptera: Tortricidae) is one of the most
83 detrimental and economically serious pests in apple (*Malus x domestica* Borkh) orchards
84 worldwide (Blommer 1994; Beers et al. 2003). In the absence of management, codling moth, a
85 multivoltine species that may attack the fruit several times before harvesting, can lead to an
86 almost complete loss of apple crops (Mills 2005). Its control is mostly based on broad-spectrum
87 insecticides and mating disruption (Reyes et al. 2007; Witzgall et al. 2008). Although a varied
88 assemblage of parasitoids has been described for codling moth in apple orchards (e.g.
89 Athanassov al., 1997; Cross et al. 1999; Mills 2005), in general, their effect on biological control
90 in intensive orchards is considered ineffective due to their insufficiency (Thorpe et al. 2016) or
91 dependency on the environmental context (Maalouly et al. 2013). Nevertheless, little is in fact
92 known of the potential for biological control of codling moths in low-input orchards, where use of
93 pesticides is minimal so there is consequently less disruption of natural enemies. **Such a less**
94 **disturbed scenario may allow** to unravel the functioning of the parasitoid-codling moth-apple
95 interaction.

96 In the present study, we **explored** parasitoid top-down and apple bottom-up effects on
97 codling moth abundance and the damage it **caused** in low-input cider apple orchards in northern
98 Spain. Both trophic forces **were** evaluated taking into account the potential effects of landscape-
99 and local-scale factors. Specifically, we **sought** to explore: (1) codling moth abundance and its
100 associated damage to apple production across years and orchards; (2) the bottom-up effects of
101 the host apple plant on codling moth abundance and its associated damage; (3) the
102 composition of the parasitoid assemblage attacking the codling moth across years and
103 orchards; and (4) the top-down effects of parasitoid richness, **and the bottom-up effects of**

104 **codling moth abundance**, on parasitized codling moth larvae. We then interpreted these findings
105 in terms of management **recommendations** for promoting pest control by natural enemies.

106

107 **2. Materials and methods**

108 *2.1. Study system and spatial sampling design*

109 The study was conducted in the cider apple crop area of Asturias (N Spain) (**Fig. A1**). In this
110 region, cider is a valuable traditional product, which is strongly ingrained in society, linked to
111 tourism, gastronomy, leisure and an important source of income. The majority of cider apple
112 orchards are relatively small (most cover between 0.5 and 4 ha), are extensively or semi-
113 extensively managed and comprised of local cultivars. Extensive traditional orchards of
114 randomly distributed cultivars are grown on seedling rootstocks (100-250 trees/ha), but new
115 orchards are grown on semi-dwarfing rootstocks with cultivars distributed in rows (500-650
116 trees/ha). **Apple trees in these production systems have large, dense canopies, reaching**
117 **frequently more than five meters high**. Apple orchards in Asturias experience notable biennial
118 bearing, with heavy yields in the odd years and lower yields in the even ones.

119 Orchards are embedded in a highly variegated traditional landscape, containing a fine-
120 grained mosaic of land-uses, such as orchards, livestock pastures, other fruit plantations (e.g.
121 blueberry, kiwifruit), timber (mainly eucalyptus) plantations, semi-natural woody vegetation
122 patches (e.g. temperate broad-leaved forest, riparian forest), and human infrastructures. At the
123 small scale of their immediate neighbourhoods, apple orchards are typically surrounded, either
124 totally or partially, by natural woody vegetation in the form of hedgerows. Farmers use local
125 cultivars tolerant to most common apple diseases (e.g. canker, scab, powdery mildew) and
126 **have high tolerance to pests, as most of them are not perceived as severe threats to**
127 **productivity (Martínez-Sastre et al. 2020). As a consequence, farmers control pests according to**
128 **their own perceptions and following personal schemes and, as** a result, the use of pesticides in
129 these orchards is very low, and often even null. We were able to collect data on sprayings from
130 some of the orchards studied, and the total annual number of pesticide sprayings per orchard
131 ranged from 0 to 2 (except in one organic orchard that sprayed six times; four of which with
132 granulosis virus against codling moth). In non-organic orchards spraying against codling moth
133 was with difubenzuron sprayings. Only one orchard applied a broad-spectrum insecticide

134 (lambda-cyhalothrin). Nevertheless, the high number of codling moth recorded in almost all the
135 orchards suggests limited and weak control of codling moth by farmers. In the region, the
136 codling moth has two generations, the second being incomplete (Miñarro 2006).

137 **Sampling was conducted in the years 2015 and 2016** in 26 semi-extensive cider apple
138 orchards distributed over 600 km² (**Fig. A1**). Orchards were chosen to represent a gradient of
139 variability in the environmental conditions within apple orchards (i.e. tree trunk diameter,
140 orchard size, canopy cover) and in the surrounding landscape (i.e. cover of semi-natural
141 habitats, pastures, apple orchards). In each orchard, we established a 25 m radius sampling
142 station within the plantation, **close but** at least 25 m away from the orchard edge. **Each sampling**
143 **station comprised around 100 trees of which ten were randomly selected (hereafter, focal trees)**
144 **among those with medium to high apple load and with the criterion of comprising trees from**
145 **several cultivars, in order to minimize a potential cultivar effect on the results.**

146

147 *2.2 Sampling of codling moth and parasitoids and estimation of crop damage and production*

148 We sampled codling moth larvae using traps made of 10cm wide corrugated cardboard bands
149 wrapped around the trunk of selected trees (40cm above the ground and always under the first
150 branch) (**Fig. B1**). The traps intercept larvae when they are moving from apples to the ground,
151 and they use the spaces provided by the corrugated cardboard as shelter for pupation and
152 overwintering. We covered all traps with a plastic mesh until collection, to protect them from
153 moisture and animals (e.g. snails). Traps were installed in mid-July and collected in mid-
154 December, and then stored at 5°C until mid-February allowing larvae to diapause.

155 We counted codling moth larvae collected, differentiating between males and females
156 by the presence or absence of male gonads, visible through the tegument (MacLellan 1972). In
157 addition, we identified a group of codling moth larvae that were conspicuously small (hereafter,
158 small size larvae). In such larvae, development has been blocked due to parasitism (Reed-
159 Larsen and Brown 1990). In addition to the codling moth larvae cocoons, ectoparasitoid
160 cocoons were also identified in the cardboard traps and were counted, collected and stored in
161 individual vials for rearing.

162 In each orchard, the average number of codling moth larvae per tree per orchard
163 (hereafter, *CM abundance*) was estimated across cardboard traps from the sum of alive codling

164 moth larvae (females, males and small size larvae), dead codling moth larvae (killed by various
165 unknown causes), ectoparasitoid cocoons (as each ectoparasitoid comes from a codling moth
166 larva), and marks indicating pecking by birds. Although signs of pecking were scarce, **we**
167 **estimated** the number of larvae that could have been eliminated **by birds. For that**, we
168 calculated the number of larvae per unit area in the undamaged part of the cardboard trap and
169 then estimated the number of bird-predated larvae by considering the size of the area damaged
170 by pecks.

171 To estimate apple damage rate caused by codling moth (hereafter, *CM damage*) per
172 orchard and year, we randomly collected from each focal tree 10 apples from the tree canopy
173 and 10 apples from the ground below the tree **around one week before harvest**. All apples were
174 cut in half *in situ* to look for signs of codling moth damage (e.g. larvae inside, galleries, frass),
175 allowing us to calculate the average percentage of apples damaged per tree.

176 We quantified apple production per orchard per year, estimated as the average **number**
177 **of apples** of the focal trees within each sampling station. **Number of apples per tree** were
178 estimated before harvest from the average number of apples on 10 branches extrapolated to
179 the total number of branches per tree.

180 In order to determine the level of parasitism (i.e. the number of larvae attacked by
181 parasitoids) we selected, from the codling moths collected from traps in each orchard, a
182 subsample of 50 females, 50 males and 50 small size larvae per orchard. The three types of
183 larvae were placed in different plastic containers with new corrugated cardboard bands for
184 shelter, and reared at $25 \pm 1^\circ\text{C}$ and a 16h/8h light/dark photophase. Samples were monitored
185 periodically for the emergence of both codling moth and parasitoid adults. Emerged parasitoids
186 were kept individually in Eppendorf tubes at -20°C until species identification (Athanasov et al.
187 1997; Graham 1969; Peters and Baur 2011).

188 We estimated the total number of parasitized codling moth larvae per orchard
189 (hereafter, *number of parasitized larvae*). For this, firstly, we extrapolated the proportion of
190 parasitoids emerged from the reared male and female codling moth subsamples (number of
191 parasitoid emerged / parasitoid emerged + codling moth emerged) (Miñarro and Dapena 2004;
192 Maalouly et al. 2013) to the number of female and male codling moth larvae counted in that
193 orchard. Secondly, all small size larvae were also considered as parasitized codling moth

194 (Reed-Larsen and Brown 1990), irrespective of parasitoid emergence or not from each
195 subsample after rearing. We quantified the total number of parasitized codling moth larvae per
196 orchard as the cumulative number of estimated male and female parasitized larvae, plus the
197 number of small size larvae, plus the number of recorded ectoparasitoids. We also estimated a
198 *parasitism rate*, i.e., the percentage of parasitized larvae in relation to the total number of
199 overwintering codling moth larvae per orchard. Finally, the richness of parasitoids per orchard
200 and year (hereafter, *parasitoid richness*) was estimated as the cumulative number of parasitoid
201 species emerged from larvae samples and the ectoparasitoid species found in each orchard.

202

203 *2.3 Landscape and local-scale features*

204 Landscape structure was quantified by means of a Geographic Information System of the study
205 area (GIS, ArcGIS9.3) based on 1:5000- scale orthophotographs (2014). Different types of
206 cover were digitized in order to include four main habitats assumed to potentially affect codling
207 moth and parasitoids: (1) semi-natural woody vegetation, (2) pastures and meadows, (3) apple
208 plantations and (4) exotic tree plantations (mainly eucalyptus). Semi-natural woody vegetation
209 included forest patches of variable size, hedgerows, and isolated trees, but excluded scrubland
210 patches. We estimated the availability of the different habitats within a 1km-radius circular plot
211 centered on the sampling station of each orchard. This spatial scale fits with the long flight
212 distances of parasitoids and codling moth adults (Yu et al. 2009; Pajač et al. 2011).

213 As local-scale features that can affect codling moth and parasitoids, we measured: (1)
214 orchard size, (2) the amount of cover by apple tree canopy in each sampling station (hereafter,
215 apple canopy cover; from a GIS layer of apple canopy projection), (3) apple tree trunk diameter
216 (average from 25 trees within each sampling station), (4) the proportion of surrounding
217 hedgerows and, (5) the proportion of apple plantations, both (4) and (5) being estimated within a
218 circular plot of 125 m radius centered on the sampling station (hereafter, hedgerows R125 and
219 apple plantation R125 respectively; from a GIS layer).

220

221 *2.4. Statistical analysis*

222 We compared CM abundance and CM damage among orchards by using Kruskal-Wallis tests
223 (trees as sampling units) and between years with paired t-tests (orchards as sampling units).

224 Parasitoid richness and parasitism rate per orchard were compared between years with paired
225 Wilcoxon tests, whereas the number of parasitized larvae per orchard was compared between
226 years with a paired t-test. All variables were checked for normality prior to tests, and CM
227 abundance (sqrt) and number of parasitized larvae (log) were transformed. Analyses were
228 performed using functions in the package *stats* (R Core Team 2013).

229 We sought to analyze the different trophic interactions between apple crop, codling moth
230 and parasitoids, taking into account potential environment effects. To do this, we applied
231 Generalized Linear Mixed Models (GLMMs; Bolker et al. 2009), considering CM abundance
232 (sqrt), CM damage and number of parasitized larvae (log) as different response variables (all of
233 which were checked for normality, and thus models considered Gaussian distribution and
234 identity link). Trophic interactions were first analyzed by means of simple “trophic models”,
235 searching for observational evidence of bottom-up and top-down forces. These simple trophic
236 models included a specific pest-plant or parasitoid-pest interaction and the potential effects of
237 landscape- or local-scale features. Hence, one model searched for bottom-up (plant→pest)
238 effects on codling moth by checking the relationships between apple production per orchard
239 (predictor) and CM abundance and CM damage (response variables). Another model searched
240 for bottom-up (pest→parasitoids) and top-down (parasitoids→pest) effects between codling
241 moth and parasitoids by checking the relationships between the number of parasitized larvae
242 per orchard (response) and CM abundance (bottom-up predictor) and parasitoid richness (top-
243 down predictor).

244 To evaluate the role of the environment, we widened the previous trophic models by
245 incorporating, as additional predictors, landscape- and local-scale variables. Because of the
246 large number of environmental variables, we did not include all variables at once in single
247 extended models (Frost 2019; see Ricci et al. 2009, for a similar rationale). Thus, for each
248 response variable, a “local-scale” extended model included, as additional predictors, orchard
249 size, hedgerows R125, apple plantation R125, apple canopy cover, tree trunk diameter, and
250 apple production. Similarly, a “landscape” extended model included, as additional predictors,
251 the covers of apple plantation, semi-natural habitat, pastures and exotic trees. All main
252 predictors were initially included in the full extended models, but, to avoid over-parameterization
253 and overfitting, those terms that were non-significant ($P > 0.05$) were excluded in a backward

254 stepwise procedure to select the simplest model, using likelihood ratio tests. Finally, we
255 combined, for each trophic interaction, the selected “local-scale” and “landscape-scale”
256 variables which were significant in a last GLMM.

257 All GLMMs included year as a fixed factor and orchard identity as a random factor
258 given that all orchards were replicated across all years (Bolker et al., 2009). The marginal and
259 conditional R^2 were calculated to assess the amount of variance explained by the fixed and
260 random effects, respectively (Nakagawa and Schielzeth 2013). Means \pm Standard Deviation
261 (SD) are shown throughout the text. GLMMs were performed using the package *nlme* (Pinheiro
262 et al. 2020).

263 All statistical analyses were performed using the software package R, version 3.5.3.

264

265 **3. Results**

266 *3.1. Codling moth abundance and crop damage*

267 A total of 16,536 larvae were collected from the cardboard traps (7,618 in 2015, 8,918 in 2016).
268 CM abundance per cardboard trap ranged from 1 to 99 (32.43 ± 27.00) in 2015 and 1 to 89
269 (37.26 ± 25.87) in 2016. While CM abundance was **not significantly different** between years, it
270 did vary significantly between orchards each year (Fig. C1A; Table D1).

271 Codling moth damage to apples varied significantly between orchards and years (Fig.
272 C1B; Table D1). CM damage per orchard ranged from 1.50% to 49.00% in 2015 and from
273 14.58% to 70.93% in 2016. Damage, overall, was greater in 2016 ($42.75\% \pm 16.39$; mean \pm SD)
274 than in 2015 ($21.40\% \pm 15.76$).

275

276 *3.2. Determinants of codling moth abundance and crop damage*

277 The best model in terms of bottom-up effects on codling moth populations, after taking into
278 account local- and landscape-scale factors (Table E1 and E2), showed a positive significant
279 response of CM abundance to apple production, apple plantation R125 and year (2016) (Table
280 1, Fig. 1 A and B).

281 In contrast, the best model for bottom-up effects on codling moth damage to apples
282 (Table E1 and E2) found a negative significant effect of apple production (Table 1, Fig. 1C), i.e.

283 higher yields lead to lower damage rates. Again, a positive significant effect of year (2016) on
284 CM damage was found (Table 1).

285

286 3.3. *Codling moth parasitoid assemblage*

287 We found seven parasitoid hymenoptera species from four families (Table 2). Three species
288 accounted for 94.9% of individuals of the parasitoid assemblage and were widespread across
289 orchards: *Ascogaster quadridentata* (Wesmael) (1,148 individuals, 66.3% of individuals, 20
290 orchards in 2015 and 22 in 2016); *Pristomerus vulnerator* (Panzer) (298 and 17.2% of
291 individuals, 9 orchards in 2015 and 14 in 2016); *Trichomma enecator* (Rossius) (197 and
292 11.4%, 13 orchards in 2015 and 11 in 2016) (Figs. F1-F2). Less frequent species, which
293 included *Liotryphon caudatus* (Ratzeburg) (45 individuals), *Nippocryptus vittatorius* (Jurine)
294 (31), *Dibrachys cavus* (Walker) (5) and *Perilampus tristis* (Mayr) (8), accounted in total for 5.1%
295 of individuals (Table 2, Fig. F2).

296 We found a total of 1,732 parasitized larvae (641 in 2015, 1,091 in 2016) (Table 2). The
297 number of parasitized larvae ranged from 0 to 190 per orchard and year (35.44 ± 43.85) (Fig.
298 F2). No differences in the number of parasitized larvae were detected between years (Table
299 D1). The number of parasitoid species found in 2015 and 2016 was 6 and 7, respectively. There
300 were no significant differences between years in parasitoid richness (Table D1, Fig. F1), but
301 parasitism rate was significantly higher in 2016 ($14.55 \pm 11.91\%$; range: 0 – 42.45%) than in
302 2015 ($8.57 \pm 7.15\%$; 0 – 24.12%) (Table D1; Fig. C1C).

303

304 3.4. *Determinants of the number of parasitized larvae*

305 The number of parasitized larvae per orchard was positively affected by parasitoid richness as
306 well as CM abundance (Table 3, Fig. 2). No effects of local- or landscape-scale variables were
307 detected (Table E1 and E2).

308

309 4. **Discussion**

310 In this work, we disentangle the trophic interactions between apple and codling moth and its
311 parasitoids in cider apple orchards while simultaneously searching for landscape- and local-
312 scale features that might affect these interactions. In the 26 cider-apple orchards monitored for

313 two years, codling moth populations were able to reach high densities per tree, damaging up to
314 70.9% of the apple crop. Seven parasitoid species attacked codling moth, parasitism rates
315 reaching 42.5%. By analyzing each trophic interaction we detected positive effects of resource
316 availability (i.e. apple production and apple plantations) on codling moth abundance. However,
317 damage by this pest proportionally decreased with apple production, suggesting the satiation of
318 the pest under high resource availability. Moreover, codling moth parasitism increased with
319 parasitoid richness and codling moth abundance. Our study thus suggests that simultaneous
320 top-down and bottom-up forces across the interaction triad parasitoids-pest-plant could be
321 important and even necessary to control codling moth in apple crops. We discuss below the
322 possible consequences of these interactions in low-input systems, in relation to the promotion of
323 biological control by parasitoids with potential benefits for cider apple production.

324

325 *4.1 Codling moth abundance and crop damage*

326 Average codling moth damage to apples per year was 21% in 2015 and 43% in 2016, and more
327 than 30 larvae on average were trapped on each tree. These high values are inconceivable in
328 intensive orchards producing dessert apples, where the abundance of codling moth is less than
329 a few larvae per tree (Ricci et al. 2009, Monteiro et al. 2013) and apple damage is typically
330 below the economic threshold of 1% (Cross et al. 1999). However, the high codling moth
331 abundances and damage levels recorded in our cider orchards are not surprising taking into
332 account the high tolerance level of local farmers to pests and the lack of regular applications of
333 pest control techniques in the region.

334 The population of codling moth was stable across years but highly variable among
335 orchards each year. Thus, orchards with a higher proportion of apple orchards in the immediate
336 surroundings (125 m radius) and larger apple yields had higher numbers of codling moth. Both
337 these factors can be considered **an estimate for** host density (i.e. availability of resources), at
338 different spatial scales, and taken together suggest a bottom-up regulation of codling moth
339 populations. We did not detect landscape effects at the larger scale (1 km radius), supporting
340 the results of Ricci et al. (2009), who found major environmental effects on codling moth
341 abundance at distances below 150 m.

342 Despite a stable codling moth population across years, apple damage in 2016 (42.7%)
343 was double that of 2015 (21.3%). As commented earlier, apple orchards in Asturias experience
344 notable biennial bearing, and 2015 was a year of heavier yields than 2016 (we counted 2.5
345 times more apples per tree in 2015 (689 ± 346) than in 2016 (270 ± 212); paired t-test for
346 difference between years; $P < 0.001$). This decrease in pest attack rate associated with the year
347 of high yield seems to be the result of a satiation process by which the population of the
348 specialist herbivore is unable to respond numerically or functionally to resource overyielding
349 (Kelly 1994; Kelly and Sork 2002). In our case, codling moth abundance and, hence, the
350 estimated amount of apples damaged, remained stable across years, indicating that changes in
351 percentage of damage depended on yield dynamics (i.e. the total number of apples) rather than
352 on changes in pest population size. Thus, the results here demonstrate that although biennial
353 bearing led to a dilution of codling moth attack, this reproductive strategy did not seem to
354 ultimately regulate pest population size. A similar pattern was found in these orchards for the
355 apple blossom weevil (*Anthonomus pomorum* L.), a specialist pest also totally dependent on
356 apple trees (in this case flowers) for reproduction (Miñarro and García 2018). Anyway, the
357 present study was restricted to two years, thus covering only one cycle of biennial fruit
358 production. It would be interesting to see if the observed pattern is confirmed over a longer time.

359

360 4.2 Codling moth parasitism

361 The parasitoid richness in these low-input orchards was high in comparison with that recorded
362 in more intensive orchards (e.g. Maalouly et al. 2013; 2015). We found a parasitoid assemblage
363 of seven species attacking codling moth that was stable across years and sites. All the
364 parasitoid species found are among those known to attack codling moth in Europe, with the
365 three dominant parasitoids in the study area (*A. quadridentata*, *P. vulnerator*, *T. enecator*) also
366 being the most frequent across European orchards (Athanasov et al. 1997; Cross et al. 1999;
367 Mills 2005). *Dibrachys cavus* and *P. tristis* can also act as hyperparasitoids but their disturbing
368 effect on overall parasitism is unlikely in the study area given their low occurrence in the
369 sample (<1%). The community of codling moth parasitoids was highly heterogeneous not only in
370 taxonomic but also in functional terms. As such, these seven species (belonging to different
371 genus) represent a wide gradient of morphological, behavioural, and physiological variability

372 observed in body size (e.g. the four ichneumonid species are clearly larger), adult emergence
373 phenology (large species emerge before codling moth whereas small species emerge later
374 (Miñarro and Dapena 2004)) or, interestingly, host stage attacked: egg (*A. quadridentata*),
375 young larvae (*P. vulnerator*, *P. tristis*), older larvae (*T. enecator*) and cocoon (*L. caudatus*, *D.*
376 *cavus*) (Athanassov et al. 1997; Mills 2005). This variability suggests high functional diversity
377 and explicit niche segregation that can partially avoid spatial and temporal competition among
378 parasitoid species and so enhance pest control (Finke and Snyder 2008; Cancino et al. 2014).

379 The number of parasitized larvae per orchard was positively related to not only
380 parasitoid richness, but also to codling moth abundance, suggesting simultaneous top-down
381 and bottom-up effects between parasitoids and pest. Regarding the top-down effects, we can
382 assume that the biological control of codling moth increases as the result of the additive effects
383 of the incorporation of different parasitoid species with complementary roles (Finke and Snyder
384 2008; Peralta et al. 2014). As mentioned above, such complementarity arises from the
385 segregation of ecological niches between parasitoids in order that they can exploit the trophic
386 resource (i.e. pest) while avoiding interspecific competition (Finke and Snyder 2008; Poisot et
387 al. 2013). The bottom-up effects of pest abundance on number of parasitized larvae are not
388 surprising given the high dependence of parasitoids on host abundance (Hassell 2000) and the
389 fact that more available hosts would enable more parasitized larvae.

390 Parasitoids provided a parasitism rate that ranged from 0 to 42.5% across orchards and
391 averaged 14.5% or 8.6%, depending on the year. **This means a reduction of up to 42.5% in the**
392 **number of emerged moths in the next generation.** Studies in high-input orchards have reported
393 very low parasitism rates (<5% on average) (Maalouly et al. 2013; Monteiro et al. 2013),
394 probably as a consequence of pesticide use, not only in the orchard itself but also in
395 surrounding ones (Ricci et al. 2009; Mates et al. 2012; Monteiro et al. 2013). This indicates that,
396 as a general recommendation, the use and spectrum of pesticides should be reduced for the
397 conservation of parasitoid communities.

398

399 *4.3 Conclusions and implications for management*

400 Populations of codling moth in the study area remained stable across years and were favoured
401 by the host plant: number of apples at the orchard scale and cover of apple orchards at the

402 surrounding-landscape scale. However, damage by this pest depended on yield dynamics and
403 proportionally decreased with apple production, suggesting a satiation of the pest under high
404 resource availability. In line with this, farmers should, evidently, first try to reduce codling moth
405 populations, preferably with techniques compatible with biological control. Importantly,
406 neighbouring farmers should coordinate their actions to reduce codling moth spreading between
407 orchards. Second, farmers face the challenge of trying to increase and to stabilize apple yields
408 in order to have a high and predictable percentage of apples undamaged every year as a
409 consequence of the satiation process.

410 Our results show that, undoubtedly, parasitoids can exert a notable reduction in the
411 codling moth population (up to 42% in certain orchards). This top-down effect is enhanced by
412 parasitoid species richness providing more functionally diverse communities and, hence, apple
413 crops should be managed accordingly to ensure such parasitoid diversity. We did not detect any
414 landscape- or local factors driving parasitoid communities that allow us to make specific
415 management recommendations. Nevertheless, it is well known that general biodiversity-friendly
416 actions, such as promoting environmental heterogeneity, floral resources or alternative hosts at
417 local- and landscape scales, help the conservation and promotion of parasitoid communities
418 (MacFadyen et al. 2011; Gillespie et al. 2016).

419 Codling moth feeds directly on the commercial product (i.e. apples) and, consequently,
420 the tolerance threshold for this pest in dessert apple crops is very low (usually <1% of damage;
421 Cross et al. 1999). Although this threshold would evidently be higher in the case of cider apple,
422 the high values of abundance and damage of codling moth reflect that biological control, by
423 itself, is not strong enough to maintain codling moth populations and crop damage below
424 reasonable thresholds. This happens even despite the high biodiversity not only of parasitoids
425 but also of generalist predators, both arthropods (Miñarro et al. 2011) and birds (García et al.
426 2018), attacking codling moth in these low-input cider orchards. Thus the promotion of biological
427 control should be combined with other control strategies compatible with natural enemies (e.g.
428 mating disruption, granulosis virus or cultural practices like post-harvest recovery of attacked
429 fruit; Judd et al. 2005; Witzgall et al. 2008; Wearing et al. 2012) to ensure the most effective and
430 sustainable control of this key pest.

431

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441

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443

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445 collection. RMS analyzed the data and wrote the manuscript, with guidance from DG and MM.
446 MM, DG and RP revised the manuscript.

447

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- 593

594 **Figure legends**

595 **Figure 1.** Significant effects of apple production and local-scale variables on codling moth
596 abundance and codling moth damage. Colours indicate different years, 2015 (black) and 2016
597 (white). Linear fits predicted by Generalized Linear Mixed Models are shown for each
598 combination of predictor and response variables.

599

600 **Figure 2.** Significant effects of codling moth abundance (A) and parasitoid richness (B) on the
601 number of parasitized larvae. Colours indicate different years, 2015 (black) and 2016 (white).
602 Linear fits predicted by Generalized Linear Mixed Models are shown for each combination of
603 predictor and response variables.

604

605

606 **Table 1.** Final Generalized Linear Mixed Models evaluating bottom-up effects on codling moth
 607 abundance and damage taking into account local- and landscape-scale factors (Gaussian
 608 distribution, identity link). The variance (\pm SD) estimate for orchard identity, considered as a
 609 random factor, is also shown.

CM abundance		R²m 0.282; R²c 0.630		
Predictors	Estimate\pm SE	t	p	
Intercept	1.192 \pm 1.132			
Apple production	0.003 \pm <0.001	3.469	0.002	
Apple plantationR125	3.993 \pm 1.833	2.179	0.039	
Year (2016)	1.805 \pm 0.566	3.188	0.005	
Orchard (random factor)	1.317 \pm 1.360			

CM damage		R²m 0.400; R²c 0.721		
Predictors	Estimate\pm SE	t	p	
Intercept	0.343 \pm 0.058			
Apple production	-0.001 \pm <0.001	-2.727	0.013	
Year (2016)	0.143 \pm 0.043	3.320	0.003	
Orchard (random factor)	0.111 \pm 0.103			

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613

614 **Table 2.** Number of codling moth larvae (percentage of relative abundance in brackets)
 615 parasitized by different parasitoid species per year and in total.

Species	Family	2015	2016	Total
<i>Ascogaster quadridentata</i>	Braconidae	318 (49.6%)	830 (76.1%)	1148 (66.3%)
<i>Pristomerus vulnerator</i>	Ichneumonidae	152 (23.6%)	146 (13.4%)	298 (17.2%)
<i>Trichomma enecator</i>	Ichneumonidae	128 (20.1%)	69 (6.4%)	197 (11.4%)
<i>Liotryphon caudatus</i>	Ichneumonidae	13 (2.0%)	32 (2.8%)	45 (2.6%)
<i>Nippocryptus vittatorius</i>	Ichneumonidae	26 (4.1%)	5 (0.5%)	31 (1.7%)
<i>Perilampus tristis</i>	Perilampidae	0 (0.0%)	8 (0.7%)	8 (0.5%)
<i>Dibrachys cavus</i>	Pteromalidae	4 (0.6%)	1 (0.1%)	5 (0.3%)
Total		641 (100%)	1091 (100%)	1732 (100%)

616

617 **Table 3.** Final Generalized Linear Mixed Model evaluating bottom-up and top-down effects on
 618 number of parasitized larvae taking into account local-scale and landscape effects (Gaussian
 619 distribution, identity link). The variance (\pm SD) estimate for orchard identity, considered as a
 620 random factor, is also shown.

Number of parasitized larvae		R²m 0.680; R²c 0.680	
Predictors	Estimate\pm SE	t	p
Intercept	0.638 \pm 0.258		
Parasitoid richness	0.701 \pm 0.099	7.110	<0.001
CM abundance	0.014 \pm 0.005	2.744	0.013
Orchard (random factor)	0.003 \pm 0.803		

621

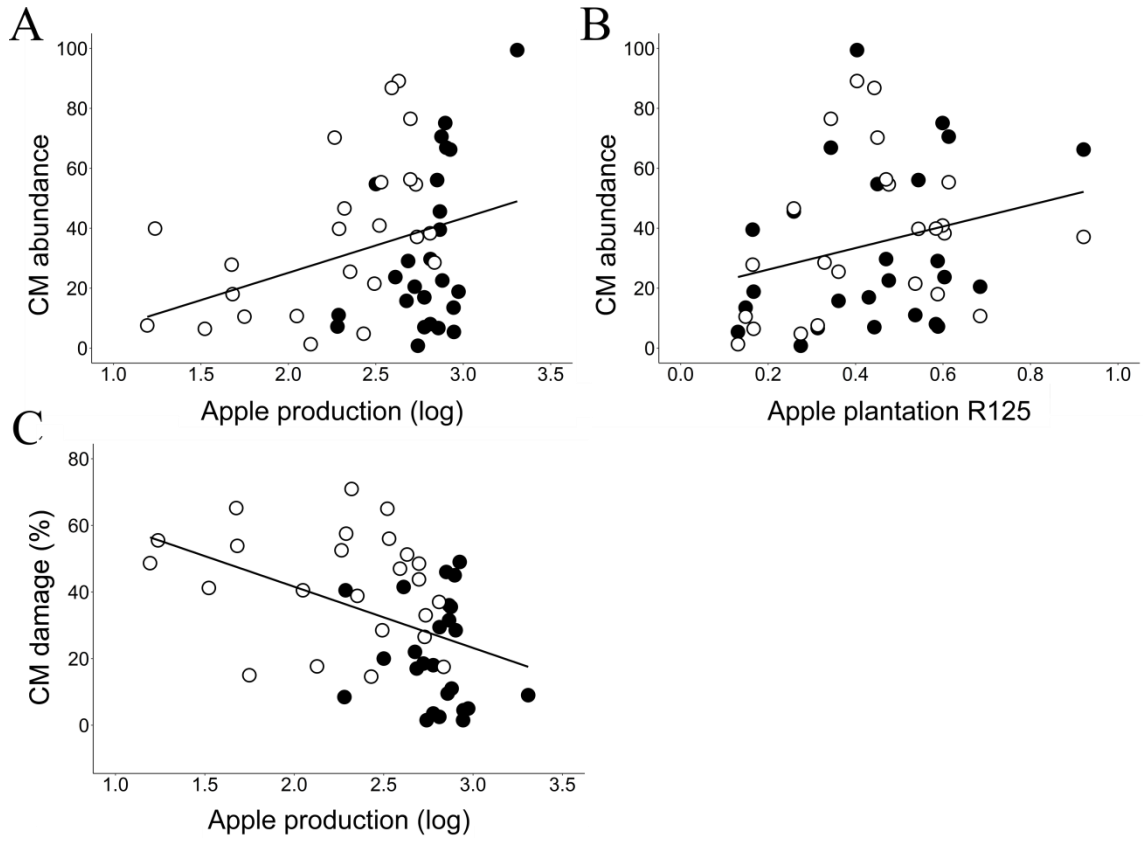
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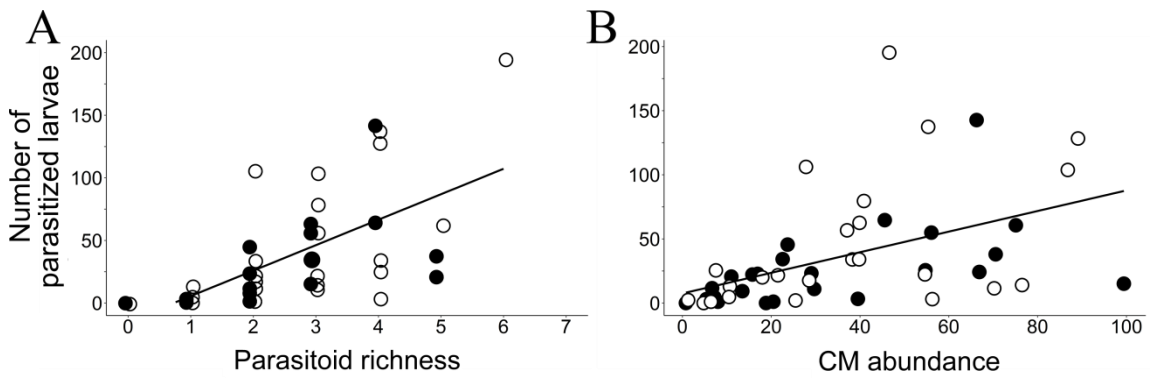
628 **Figure 1.**

629

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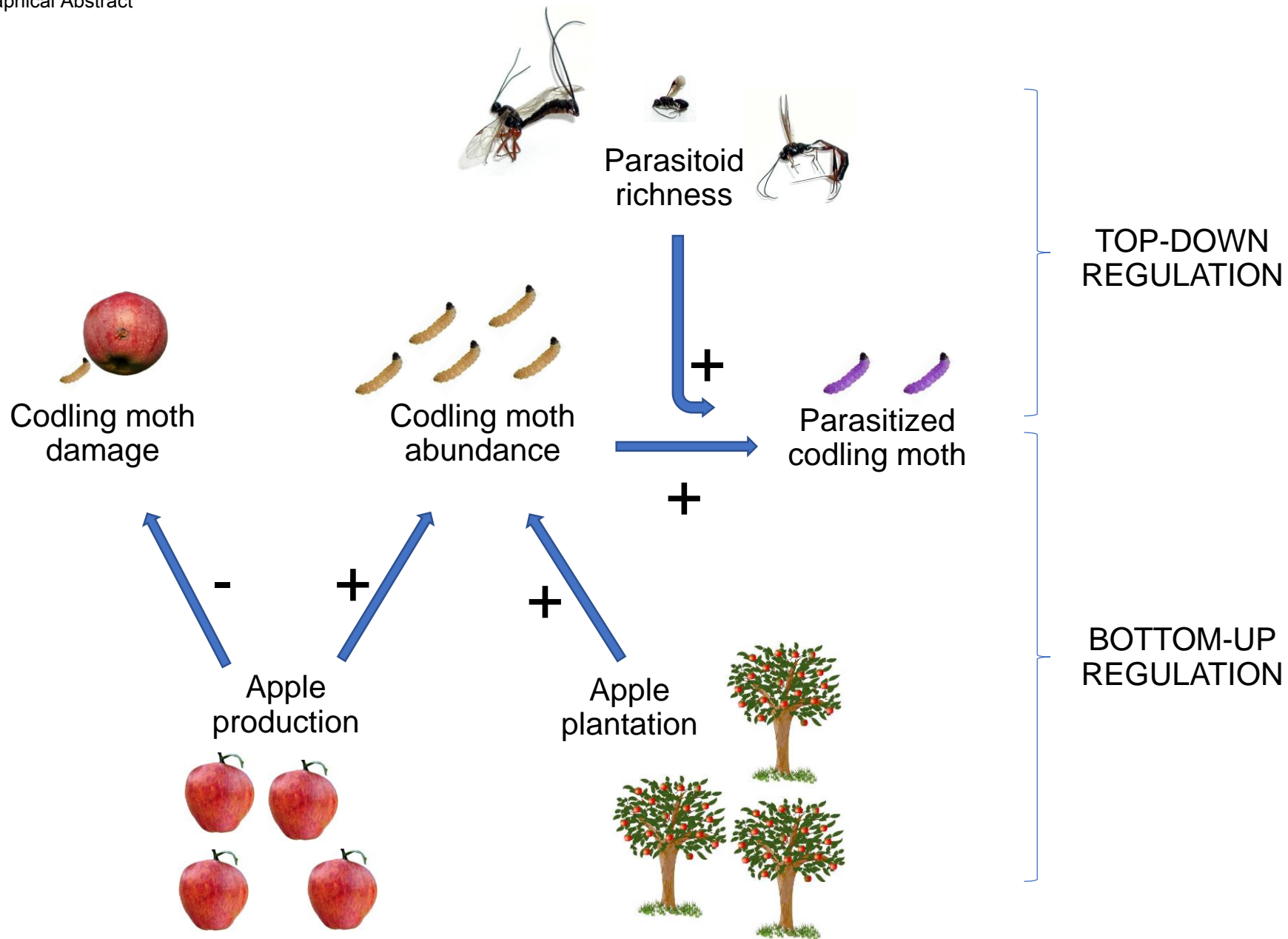
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634 **Figure 2.**



Supplementary material

Top-down and bottom-up regulation of codling moth populations in cider apple orchards

Rodrigo Martínez-Sastre, Rocío Peña, Alejandro González-Ibáñez, Daniel García,
Marcos Miñarro

Appendix A. Study area

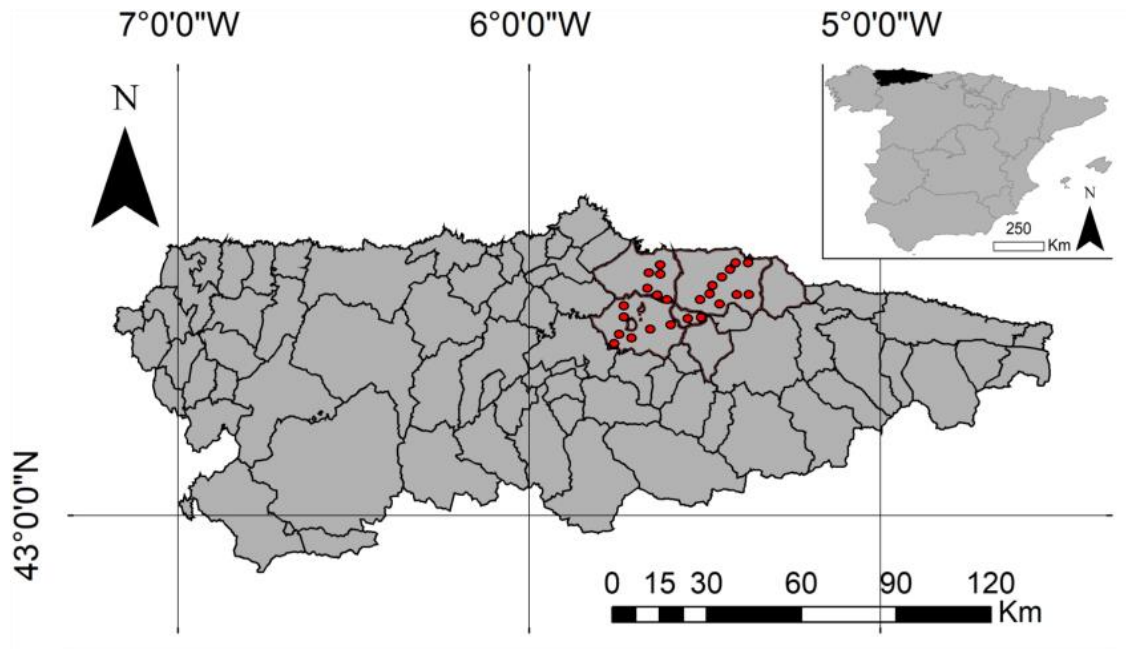


Figure A1. Study area. Inset shows location within Spain of Asturias region. Larger image shows Asturias, with the cider-apple orchards selected for this study depicted as red spots.

Appendix B. Details of cardboard trap experiment



Figure B1. Cardboard trap positioned below the first branch and 40 cm above the ground.

Image by Daniel García.

Appendix C. Codling moth abundance, crop damage and parasitism rate between years

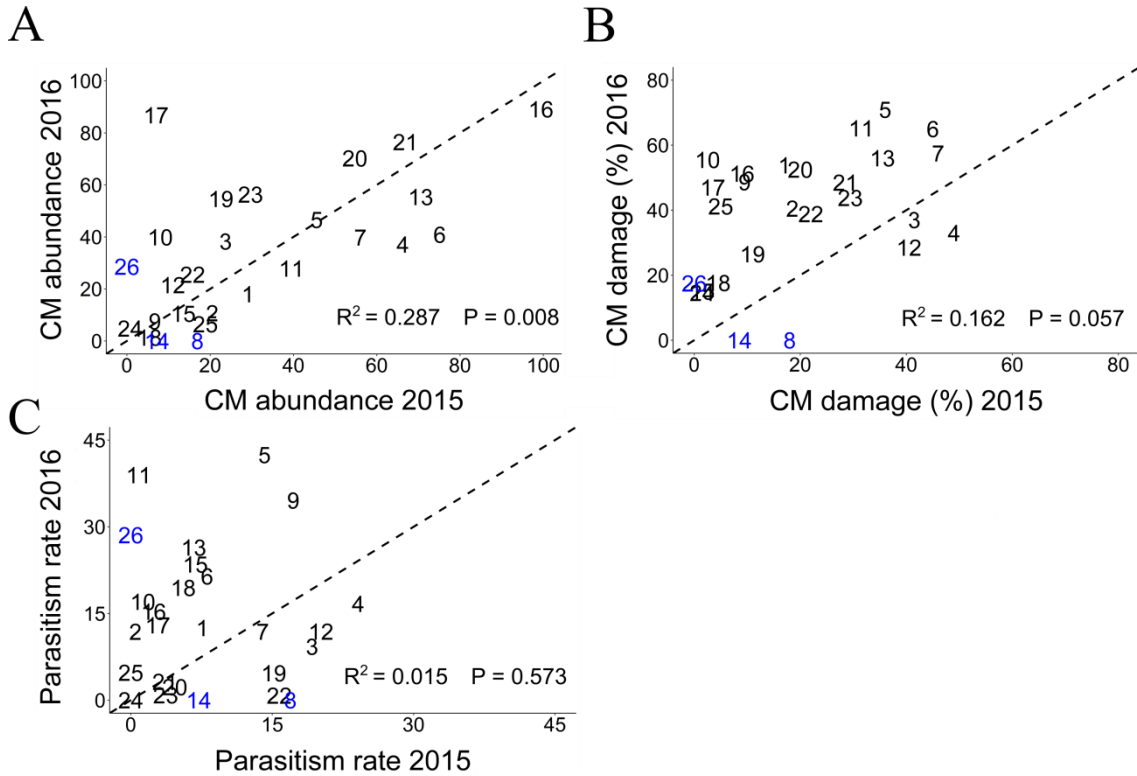


Figure C1. Relationship between the average of codling moth abundance per cardboard trap (A), codling moth damage (B) and parasitism rate (C) in the 26 cider apple orchards studied in 2015 and 2016 (black numbers: cider apple orchards sampled in both years; blue numbers: cider apple orchards sampled in only one year). Coefficient of determination and significance level from correlation tests between years are also shown. The dashed lines represent bisectors.

Appendix D. CM abundance, CM damage and number of parasitized larvae across years and orchards

Table D1. Differences in CM abundance, CM damage, number of parasitized larvae and parasitoid richness across years and orchards. T-tests were performed on CM abundance, CM damage and number of parasitized larvae to compare between years. Kruskal-Wallis tests were performed on CM abundance and CM damage among orchards. A Wilcoxon test was performed on parasitoid richness between years.

	Variable	Df	Statistical test value	p-value
CM abundance	Year	22	t = -0.791	0.437
	Orchard (2015)	24	$\chi^2 = 166.600$	<0.001
	Orchard (2016)	22	$\chi^2 = 159.230$	<0.001
CM damage	Year	22	t = -5.955	<0.001
	Orchard (2015)	24	$\chi^2 = 178.230$	<0.001
	Orchard (2016)	22	$\chi^2 = 119.520$	<0.001
Number of parasitized larvae	Year	22	t = -1.523	0.142
Parasitism rate	Year	22	z = -6.026	<0.001
Parasitoid richness	Year	22	z = -1.625	0.104

Appendix E. Model selection process following a step-wise procedure

Table E1. Models that were included in the procedure for the backward step-wise deletion of non-significant ($p > 0.05$) fixed factors from full local-scale models, for response variables of CM abundance, CM damage and number of parasitized larvae. Values of Akaike Information Criterion (AIC) for the various full- and nested models, and the results of likelihood ratio tests comparing nested models to their corresponding full model are shown. Non-significant predictors that were detected and removed in the step-wise process are shown in bold.

CM abundance(local-scale model)	df	AIC	BIC	logLik	L.Ratio	p-value
Apple production + hedgerow R125 + apple plantation R125 + Orchard size + Apple canopy cover + Diameter + Year	10	210.372	229.084	-95.185		
Apple production + hedgerow R125 + apple plantation R125 + Orchard size + Diameter + Year	9	208.375	225.215	-95.187	0.003	0.957
Apple production + apple plantation R125 + Orchard size + Diameter + Year	8	206.390	221.360	-95.195	0.019	0.991
Apple production + apple plantation R125 + Orchard size + Year	7	205.057	218.155	-95.528	0.685	0.877
Apple production + apple plantation R125 + Year	6	203.617	214.845	-95.809	1.246	0.871
CM damage (local-scale model)	df	AIC	BIC	logLik	L.Ratio	p-value
Apple production + hedgerow R125 + apple plantation R125 + Orchard size + Apple canopy cover + Diameter + Year	10	-40.251	-21.539	30.125		
Apple production + apple plantation R125 + Orchard size + Apple canopy cover + Diameter + Year	9	-42.155	-25.314	30.077	0.096	0.756
Apple production + apple plantation R125 + Apple canopy cover + Diameter + Year	8	-43.663	-28.693	29.831	0.588	0.745
Apple production + apple plantation R125 + Diameter + Year	7	-44.592	-31.494	29.296	1.658	0.646
Apple production + apple plantation R125 + Year	6	-43.425	-32.198	27.713	4.826	0.306

Apple production + Year	5	-42.466	-33.110	26.233	7.785	0.169
Number of parasitized larvae (local-scale model)	df	AIC	BIC	logLik	L.Ratio	p-value
Parasitoid richness + CM abundance + hedgerow R125 + apple plantation R125 + Orchard size + Apple canopy cover + Diameter + Year	11	129.202	149.785	-53.601		
Parasitoid richness + CM abundance + hedgerow R125 + apple plantation R125 + Orchard size + Diameter + Year	10	127.325	146.037	-53.663	0.123	0.725
Parasitoid richness + CM abundance + hedgerow R125 + apple plantation R125 + Diameter + Year	9	126.172	143.013	-54.086	0.971	0.616
Parasitoid richness + CM abundance + apple plantation R125 + Diameter + Year	8	124.877	139.846	-54.438	1.675	0.643
Parasitoid richness + CM abundance + apple plantation R125 + Diameter	7	123.662	136.760	-54.831	2.460	0.652
Parasitoid richness + CM abundance + Diameter	6	124.097	135.324	-56.048	4.895	0.429
Parasitoid richness + CM abundance	5	125.105	134.461	-57.552	7.903	0.245

Table E2. Models that were included in the procedure for the backward step-wise deletion of non-significant ($p > 0.05$) fixed factors from full landscape models, for response variables of CM abundance, CM damage and number of parasitized larvae. Values of Akaike Information Criterion

(AIC) for the different full- and nested models, and the results of likelihood ratio tests comparing nested models to their corresponding full model are shown. Non-significant predictors that were detected and removed in the step-wise process are shown in bold.

CM abundance (landscape model)	df	AIC	BIC	logLik	L.Ratio	p-value
Apple production + apple plantation1000 + snwh1000 + pasture1000 + exotic1000 + Year	9	210.761	227.602	-96.380		
Apple production + snwh1000 + pasture1000 + exotic1000 + Year	8	208.851	223.821	-96.425	0.090	0.764
Apple production + pasture1000 + exotic1000 + Year	7	207.050	220.149	-96.525	0.289	0.865
Apple production + pastures1000 + Year	6	207.435	218.662	-97.718	2.674	0.445
Apple production + Year	5	206.311	215.667	-98.156	3.551	0.470
CM damage (landscape model)	df	AIC	BIC	logLik	L.Ratio	p-value
Apple production + apple plantation1000 + snwh1000 + pasture1000 + exotic1000 + Year	9	-37.836	-20.995	27.918		
Apple production + apple plantation1000 + pasture1000 + exotic1000 + Year	8	-39.002	-24.033	27.501	0.835	0.361
Apple production + pasture1000 + exotic1000 + Year	7	-40.064	-26.966	27.032	1.772	0.412
Apple production + exotic1000 + Year	6	-41.107	-29.880	26.553	2.729	0.435
Apple production + Year	5	-42.466	-33.110	26.233	3.370	0.498
Number of parasitized larvae (landscape model)	df	AIC	BIC	logLik	L.Ratio	p-value
CM abundance + parasitoid richness + apple plantation1000 + snwh1000 + pasture1000 + exotic1000 + Year	10	131.112	149.824	-55.556		
CM abundance + parasitoid richness + apple plantation1000 + snwh1000 + exotic1000 + Year	9	129.126	145.967	-55.563	0.0142	0.905
CM abundance + parasitoid richness + apple plantation1000 + exotic1000 + Year	8	127.643	142.613	-55.822	0.531	0.767
CM abundance + parasitoid richness + apple plantation1000 + exotic1000	7	126.411	139.510	-56.206	1.299	0.729

CM abundance + parasitoid richness + exotic1000	6	125.268	136.495	-56.634	2.156	0.707
CM abundance + parasitoid richness	5	125.105	134.461	-57.552	3.993	0.551

Appendix F. Codling moth parasitoids and parasitism rate among orchards and years

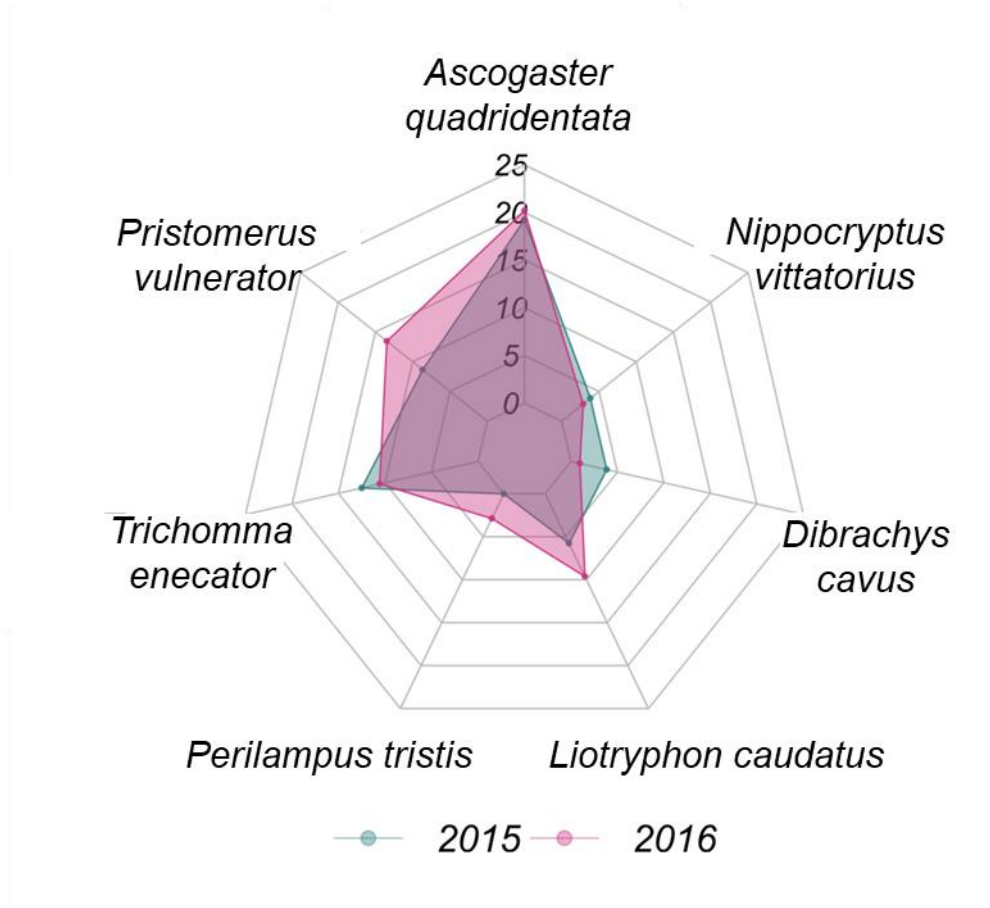


Figure F1. Number of orchards in which each parasitoid occurred.

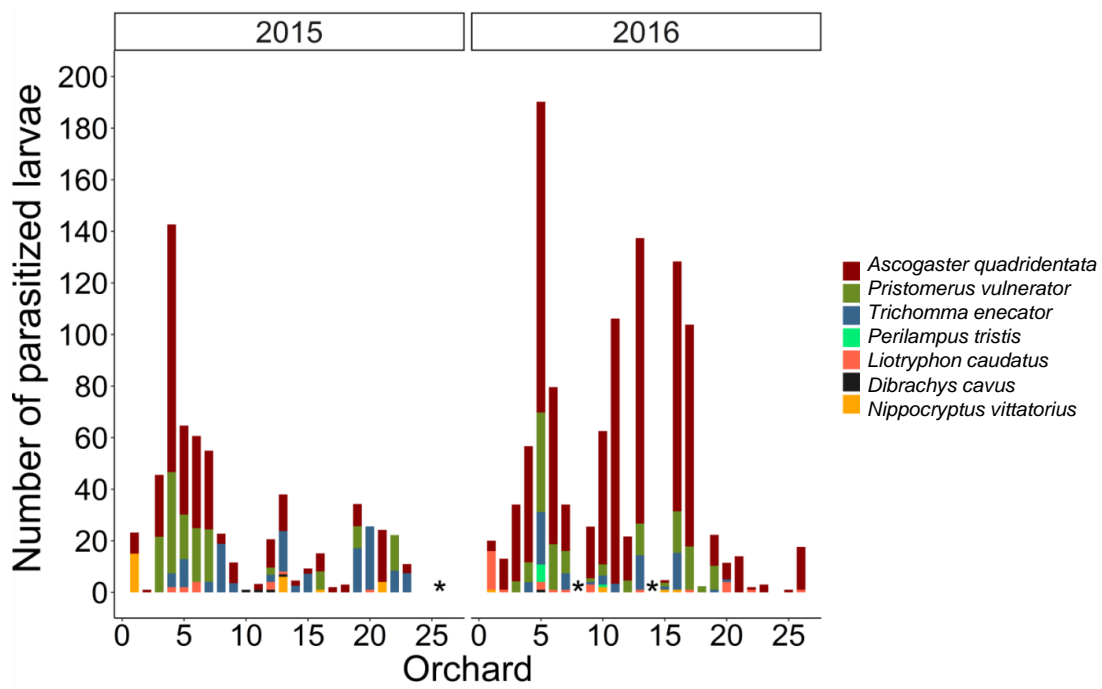


Figure F2. Number of parasitized larvae per year (2015, 2016) by species of parasitoid in the twenty-six cider apple orchards studied.

* indicates orchards not sampled that year.