

Meta-analysis reveals that seed-applied neonicotinoids and pyrethroids have similar negative effects on abundance of arthropod natural enemies

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Background. Seed-applied neonicotinoids are widely used in agriculture, yet their effects on non-target species remain incompletely understood. One important group of non-target species is arthropod natural enemies (predators and parasitoids), which contribute considerably to suppression of crop pests. We hypothesized that seed-applied neonicotinoids reduce natural-enemy abundance, but not as strongly as alternative insecticide options such as soil- and foliar-applied pyrethroids. Furthermore we hypothesized that seed-applied neonicotinoids affect natural enemies through a combination of toxin exposure and prey scarcity.

Methods. To test our hypotheses, we compiled datasets comprising observations from randomized field studies in North America and Europe that compared natural-enemy abundance in plots that were planted with seed-applied neonicotinoids to control plots that were either (1) managed without insecticides (20 studies, 56 site-years, 607 observations) or (2) managed with pyrethroid insecticides (8 studies, 15 site-years, 384 observations). Using the effect size Hedge's d as the response variable, we used meta-regression to estimate the overall effect of seed-applied neonicotinoids on natural-enemy abundance and to test the influence of potential moderating factors.

Results. Seed-applied neonicotinoids reduced the abundance of arthropod natural enemies compared to untreated controls ($d = -0.30 \pm 0.10$ [95% confidence interval]), and as predicted under toxin exposure this effect was stronger for insect than for non-insect taxa ($Q_M = 8.70$, $df = 1$, $P = 0.003$). Moreover, seed-applied neonicotinoids affected the abundance of arthropod natural enemies similarly to soil- or foliar-applied pyrethroids ($d = 0.16 \pm 0.42$ or -0.02 ± 0.12 ; with or without one outlying study). Effect sizes were surprisingly consistent across both datasets ($I^2 = 2.7\%$ for no-insecticide controls; $I^2 = 0\%$ for pyrethroid controls), suggesting little moderating influence of crop species, neonicotinoid active ingredients, or methodological choices.

Discussion. Our meta-analysis of nearly 1,000 observations from North American and European field studies revealed that seed-applied neonicotinoids reduced the abundance of arthropod natural enemies similarly to broadcast applications of pyrethroid insecticides. These findings suggest that substituting pyrethroids for seed-applied neonicotinoids, or vice versa, will have little net effect on natural enemy abundance. Consistent with previous lab work, our results also suggest that seed-applied neonicotinoids are less toxic to spiders and mites, which can contribute substantially to biological control in many agricultural systems. Finally, our ability to interpret the negative effect of neonicotinoids on natural enemies is constrained by difficulty relating natural-enemy abundance to biological control function; this is an important area for future study.

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14 **ABSTRACT**

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16 non-target species remain incompletely understood. One important group of non-target species is
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43 little net effect on natural enemy abundance. Consistent with previous lab work, our results also
44 suggest that seed-applied neonicotinoids are less toxic to spiders and mites, which can contribute
45 substantially to biological control in many agricultural systems. Finally, our ability to interpret
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47 natural-enemy abundance to biological control function; this is an important area for future
48 study.

49

50 INTRODUCTION

51 Arthropod natural enemies (predators and parasitoids) contribute considerable value to
52 agriculture by suppressing pests that attack crop plants. For example, biological control of the
53 soybean aphid (*Aphis glycines*) is estimated to be worth at least \$84 million per year in just five
54 U.S. states (Zhang & Swinton 2012). Given their importance to pest management, it is essential
55 to understand how agricultural practices influence natural-enemy communities and their ability
56 to suppress crop pests. Insecticide use is one common agricultural practice that can influence
57 natural-enemy populations and biological control. Insecticides are used to manage pests,
58 however, in some cases they also disrupt biological control, leading to unintended outbreaks of
59 target or non-target pests (Geiger et al. 2010; Settle et al. 1996; Stern et al. 1959). Elucidating

60 how insecticides and natural enemies interact can be useful in designing stable pest management
61 strategies, identifying insecticide products that have greater selectivity for pests versus natural
62 enemies, applying them in ways that conserve natural enemies, and in some cases even using
63 insecticides to enhance the efficacy of biological control (Croft & Brown 1975; Hull & Beers
64 1985).

65 One use of insecticides that is increasingly widespread is planting seeds coated with
66 neonicotinoid insecticides, especially in large-acreage field crops where these seed coatings are
67 ubiquitous in some crops and regions (Simon-Delso et al. 2015). Seed coatings account for at
68 least 80% of neonicotinoids applied in the U.S. (Douglas & Tooker 2015) and accounted for
69 roughly 87% in Britain prior to recent restrictions (Simon-Delso et al. 2015). Through their
70 systemic activity, seed-applied neonicotinoids target some early-season soil and foliar pests, and
71 their relatively low cost and ease of handling makes them an attractive option as ‘insurance’
72 against sporadic pest populations (Douglas & Tooker 2015; Jeschke et al. 2011). The popularity
73 of neonicotinoids is also related to their toxicological profile; their binding behavior at nicotinic
74 acetylcholine receptors confers potent toxicity against a broad array of pest insect species, and
75 simultaneously low acute toxicity to mammals (Tomizawa & Casida 2005).

76 Despite the broad toxicity of neonicotinoids to insects, some researchers reasoned that by
77 applying them in relatively small doses ‘targeted’ to crop seed, seed-applied neonicotinoids
78 should have high ecological selectivity for crop pests and low potential to reduce populations of
79 beneficial insects (Jeschke et al. 2011). The environmental effects of these products have since
80 been called into question by evidence that non-target species, especially bees, can be exposed to
81 seed-applied neonicotinoids via contaminated soil, planting dust, floral resources, and guttation
82 droplets (Godfray et al. 2014; Godfray et al. 2015). This pollinator controversy has spurred new

83 regulations in several jurisdictions (European Commission 2013; Government of Ontario 2015),
84 in turn raising the question of whether other pest management tactics that might replace
85 neonicotinoids, including conventionally applied insecticides, have more or less influence on
86 non-target species. We argue that fully understanding the trade-offs associated with seed-applied
87 neonicotinoids requires attention not only to pollinators but also to natural enemies that suppress
88 crop pests.

89 Seed-applied neonicotinoids can potentially reduce populations of arthropod natural
90 enemies through at least two mechanisms: toxin exposure and prey scarcity. Neonicotinoids are
91 toxic to many natural-enemy species (Cloyd & Bethke 2011; Hopwood et al. 2013), but it is
92 unclear whether under field conditions natural enemies encounter meaningful doses of toxins
93 from seed treatments. What constitutes a ‘meaningful dose’ is likely to vary by taxon. In the
94 laboratory, insects are orders of magnitude more susceptible to neonicotinoids than arachnids
95 (Table 1), and at least some evidence suggests that this difference is based on the molecular
96 structure of arachnid acetylcholine receptors (Meng et al. 2015). If natural-enemy populations
97 were reduced by seed-applied neonicotinoids through toxin exposure, we would expect insects to
98 be more strongly affected than arachnids. Alternatively, neonicotinoids could exert indirect
99 negative effects, for instance by reducing the abundance of prey, leading to less aggregation,
100 persistence, or reproduction of natural enemies in crop fields (Croft & Brown 1975). Under this
101 prey-scarcity scenario, we would expect insects and arachnids to be similarly affected, but
102 functional groups to differ relative to their degree of dependence on pest prey (parasitoid >
103 predator > omnivore). These two types of effects of insecticides on natural enemies are not
104 mutually exclusive, and both can interfere with biological control (Johnson & Tabashnik 1999).

105 Clarifying the mechanisms at play can nonetheless guide researchers and pest managers toward
106 more successful integration of chemical and biological control.

107 Field experiments on the influence of seed-applied neonicotinoids on natural enemies
108 have reached mixed conclusions. Some have found no statistically significant effects (Al-Deeb et
109 al. 2003), some a mix of null and negative effects (Albajes et al. 2003), and others more
110 consistent negative effects (Hallett et al. 2014). Some of this variability may be related to the
111 small sample sizes of most field studies, which limits their statistical power. Conversely,
112 variability in study results may reflect real differences across crop species, natural-enemy taxa,
113 active ingredients, and other factors. One powerful tool to make sense of such apparently mixed
114 results and to untangle the various factors influencing study outcomes is meta-analysis
115 (Cumming 2012). This approach has been fruitfully applied in similar situations, for instance, to
116 estimate the influence of *Bt* transgenic crops on non-target organisms (Marvier et al. 2007;
117 Naranjo 2009; Wolfenbarger et al. 2008). One of the salient benefits of meta-analysis in
118 controversial areas is that it provides a rigorous, quantitative, and replicable method of
119 synthesizing evidence for researchers, policy-makers, and the public (Marvier et al. 2007).

120 Here, we report results from a meta-analysis of studies investigating under field
121 conditions the influence of seed-applied neonicotinoids on arthropod natural enemies. We used a
122 meta-regression approach to test the hypotheses that seed-applied neonicotinoids: (1) negatively
123 affect natural-enemy abundance relative to untreated controls; (2) reduce natural enemy
124 abundance less than conventional foliar- or soil-applied insecticide treatments; and (3) affect
125 natural enemies through a combination of toxin exposure and prey scarcity. Our results taken
126 together should allow researchers and pest managers to better predict the compatibility of seed-

127 applied neonicotinoids and natural enemies, and to more effectively weigh the use of these
128 insecticides against alternative pest-management options.

129

130 **MATERIALS & METHODS**

131 Meta-analysis is a method for synthesizing observations from independent yet similar studies to
132 characterize the size and variability of an effect – in this case the influence of seed-applied
133 neonicotinoids on natural enemies of crop pests. Our meta-analysis was guided to some extent by
134 previous meta-analyses that characterized the influence of *Bt* crops on populations of non-target
135 arthropods (Marvier et al. 2007; Naranjo 2009; Wolfenbarger et al. 2008). We departed from
136 these previous studies in our statistical approach. In particular, as described below, we
137 capitalized on advances in statistical programs over the past several years to better account for
138 the hierarchical nature of the dataset. All of our analyses were conducted within the R statistical
139 program (R Core Team 2015).

140

141 **Analysis Procedure**

142 **1. Searching the literature**

143 Using four databases (ISI Web of Science, Agricola, CAB abstracts, and ProQuest Dissertations
144 & Theses Database), we searched for studies on the influence of neonicotinoids on arthropod
145 natural enemies. We used the following search phrase, adjusting the syntax as necessary for
146 different search engines: “(neonic* OR imidacloprid OR clothianidin OR thiamethoxam) AND
147 (preda* OR enem* OR parasit*) AND seed”. We combed the resulting studies and published
148 reviews (Chagnon et al. 2015; Hopwood et al. 2013; Lundgren 2009; Pisa et al. 2015) for
149 additional references, and found one additional unpublished thesis because a colleague

150 mentioned it at a scientific meeting. Our final literature search for this analysis was conducted on
151 August 7, 2015. The search was designed by both authors and carried out by the first author.

152

153 **2. Building the dataset**

154 We used the following criteria to include a study in the dataset: (1) it compared field plots that
155 were planted with neonicotinoid-treated seed with control plots that were planted with
156 neonicotinoid-free seed of the same crop variety. There were two types of control plots: those
157 that were not treated with any insecticides (testing whether neonicotinoids have any effect on
158 natural enemies), and those that were treated with an alternative insecticide product (testing
159 whether neonicotinoids affect natural enemies more or less than alternatives). Studies also had to
160 (2) measure abundance or activity-density of one or more taxonomic groups of arthropod natural
161 enemy, (3) be replicated, (4) report the data necessary to calculate effect size (means, sample
162 sizes, and standard errors or standard deviations), and (5) be available in English. Where studies
163 met the first three criteria but did not report some necessary data, we contacted authors to obtain
164 that data, although not all responded. Where necessary we extracted data from published figures
165 using the software GraphClick 3.0.3 (Arizona Software).

166 To build the dataset, we recorded for each study the means and variability for natural-
167 enemy abundance in each treatment group, along with a wide variety of supporting information
168 such as author and affiliation, study location and year, crop species, active ingredient of the seed
169 treatment, size of each plot, number of replicates, and other methodological details. While seed-
170 applied neonicotinoids should have their largest effects during the early growing season, we
171 found that studies varied in their emphasis on this early sampling window, and some studies did
172 not even start sampling until mid-season (Table S1). If a taxon was sampled repeatedly over a

173 single season, we used seasonal summary data when available; otherwise we requested seasonal
174 data from the authors. If this failed, we used peak values. We chose to use seasonal and peak
175 values to be consistent with previous meta-analyses (e.g. Marvier et al. 2007), and to ensure a
176 consistent basis for comparing seed-applied neonicotinoids to pyrethroid applications, which
177 sometimes occur later in the season. Nonetheless, we tested for the potential influence of
178 sampling window (% of sample from the early season) in our statistical analysis, as described
179 below in “Fitting meta-regression models.”

180 For each taxon, we recorded sampling method, life stage, habitat (soil/epigeal or
181 foliar/aerial), functional group, and taxonomic information to the lowest level provided. Many
182 natural enemies consume plant products (pollen, nectar, seeds, etc.) in addition to live prey; we
183 assigned taxa to functional groups using an existing classification (Wolfenbarger et al. 2008),
184 and filling in gaps where necessary based on the scientific literature. We defined ‘natural
185 enemies’ to include the following functional groups of Wolfenbarger et al. (2008): mixed,
186 omnivore, predator, and parasitoid. The ‘omnivore’ group comprises taxa that are believed to
187 rely equally on prey and non-prey foods (e.g., Formicidae, Gryllidae). The ‘mixed’ group refers
188 to taxonomic units that contain species in multiple functional groups (e.g., Carabidae). We
189 classified natural enemies into functional groups based on the taxonomic level at which the data
190 were reported, which varied from class to species (see Data S1 for a full list of functional group
191 classifications).

192

193 **3. Defining the scope of the study**

194 While our initial intent was to include studies from all geographic regions, we restricted the
195 current analysis to North America and Europe because most of the studies from other regions (8

196 of 11 studies, all from South Asia) lacked sufficient details for us to interpret reported measures
197 of variation. For the part of our analysis comparing neonicotinoids to alternative insecticides, we
198 restricted the analysis to pyrethroids, the only insecticide class that was compared to seed-
199 applied neonicotinoids in at least three independent studies (Table S1). Because seed-application
200 is far more common for neonicotinoids than other classes of insecticides (Douglas & Tooker
201 2015), we expected pyrethroid comparisons to be applied using traditional broadcast methods
202 such as foliar sprays or granular soil applications. This was mostly true, but there was one study
203 that applied pyrethroids as a seed treatment (Baker et al. 2002). For completeness, we included
204 this study in our analyses as a ‘soil-based’ pyrethroid, but also tested the sensitivity of our results
205 to its inclusion.

206

207 **4. Calculating effect size: Hedge’s d**

208 The response variable in our analysis is Hedge’s d , the mean abundance in the control group
209 minus the mean abundance in the treatment group, divided by the pooled standard deviation, and
210 corrected for small sample size (Koricheva et al. 2013). In this case d measures the difference in
211 natural-enemy abundance between control plots and plots planted with neonicotinoid-treated
212 seed, with negative values reflecting lower abundance of natural enemies in neonicotinoid-
213 treated plots compared to controls. We used the ‘escalc’ function (‘metafor’ package) to
214 calculate d and its associated variance (σ^2) for each observation in the dataset.

215

216 **5. Addressing non-independence**

217 Typical studies in our dataset contributed numerous observations resulting from multiple taxa,
218 sampling methods, life stages, site-years, and other factors. As a result we could not assume that

219 all of the observations were statistically independent. As in past meta-analyses (Marvier et al.
220 2007), we addressed this problem in part by eliminating redundant observations, as follows.
221 When results were reported at varying levels of taxonomic resolution, we used only the results at
222 the finest taxonomic level. When multiple life stages were sampled for a given taxon, we used
223 only the observations from the least mobile, but still feeding life stage (Wolfenbarger et al.
224 2008). When a given taxon was sampled in multiple ways, we included results from the sampling
225 method with the highest precision (lowest relative standard deviation). We made an exception to
226 this rule for studies that sampled soil and foliar habitats for Araneae, “predatory mites” and
227 Carabidae, because for these broad taxonomic groups, these habitats are likely to contain mostly
228 non-overlapping taxa. Even after taking these steps to reduce redundancy, our dataset still
229 contained numerous observations per study as a result of multiple taxa, site-years, and crossed
230 factors such as crop varieties and insecticide active ingredients. We accounted for the remaining
231 non-independence through the structure of the meta-regression models as described in the next
232 section.

233

234 **6. Fitting meta-regression models**

235 To estimate the influence of seed-applied neonicotinoids on natural enemies and to test the
236 influence of agroecological or methodological moderating variables on the size of this effect, we
237 employed a mixed effects meta-regression approach using the package ‘metafor’ in R (R Core
238 Team 2015; Viechtbauer 2010). Meta-regression is analogous to multiple regression, but differs
239 in that observations are weighted relative to their precision (typically $1/\text{variance}$). The strength of
240 the meta-regression approach is that it allows us to investigate the influence of multiple

241 moderators at once, while also using random effects to control for the hierarchical nature of the
242 dataset (observations nested in site-years nested in studies).

243 We split our larger dataset into two parts: one for observations that compared seed-
244 applied neonicotinoids to an insecticide-free control and a second for observations that compared
245 seed-applied neonicotinoids to pyrethroids (Data S1). For each of these datasets we fit three
246 models, all estimated using restricted maximum likelihood in ‘metafor’ (Code S1, S2). First we
247 fit a ‘null’ model that did not include random effects of site-year or study, nor fixed effects of
248 moderators. This model mainly serves as comparison to results of previous meta-analyses on
249 non-target effects of agricultural technologies, many of which did not account for nesting of
250 observations within studies. We next fit a ‘site-year/study’ model that included only random
251 effects of site-year and study, but no fixed effects (i.e., no moderators). From these two initial
252 sets of models we generated 95% confidence intervals for the overall influence of seed-applied
253 neonicotinoids on natural enemies, and also characterized variability in the effect sizes using
254 ‘heterogeneity’ as measured by the Q statistic. Q is the weighted sum of squared differences of
255 effect sizes from the mean, and can be used to test whether variability among effect sizes is
256 greater than would be expected by sampling error alone (Viechtbauer 2007b). In addition to the
257 Q test, we calculated $I^2 = 100\% \times (Q - df) / Q$, where df = degrees of freedom ($k - 1$; k = the total
258 number of observations), which estimates the percentage of variability in effect sizes that is due
259 to true heterogeneity rather than sampling error (Higgins et al. 2003). Finally, to assess whether
260 it was necessary to include the study and site-year effects, we examined the variance components
261 associated with these effects in the ‘site-year/study’ model using profile plots.

262 The third model we fit for each dataset was a ‘moderator’ model designed to test whether
263 agro-ecological or methodological variables influenced the effect of seed-applied neonicotinoids

264 on natural enemies. Along with random effects of site-year and study, these models included
265 fixed effects of potential moderating variables that we identified *a priori* (See Table 2 for
266 details): broad taxonomic group (insect or non-insect), functional group (predator, parasitoid,
267 omnivore, or mixed), habitat (soil-associated or foliage-associated), neonicotinoid active
268 ingredient group (imidacloprid or clothianidin/thiamethoxam), crop species (maize, soybean, or
269 other), publication status (peer-reviewed or dissertation/thesis/other), pyrethroid application
270 method (where applicable; soil or foliar application), plot size, and proportion of samples
271 collected during the first 40 days of crop growth, when neonicotinoids typically have activity
272 against target pests (Magalhaes et al. 2009; Seagraves & Lundgren 2012). For the pyrethroid
273 analysis, to reduce the risk of drawing spurious conclusions we left out crop species, publication
274 status, and functional group, because some levels of these moderators were not supported by at
275 least three independent studies (Table 2). We transformed continuous moderators (plot size,
276 proportion early samples) where necessary and centered them on a mean of zero to facilitate
277 interpretation. Categorical variables were converted to effects coding by employing the
278 ‘contr.sum’ option in ‘contrasts.’ This made the intercept of the fitted model reflect the mean
279 value across the means of all moderator variables, and the slopes reflect the difference associated
280 with the level of each moderator from the overall mean. We first tested for the significance of all
281 the moderators combined (using an omnibus Q test for moderators); when that was significant,
282 we went on to test the significance of individual moderators.

283 We tested the moderators that we expected to have the greatest likelihood of influencing
284 effect size, while limiting the total number of moderators to preserve the power of the analysis.
285 As described in the introduction, effects of taxonomic group and functional group have
286 implications for whether natural enemies are affected by neonicotinoids through toxin exposure

287 (insects > arachnids) and/or prey scarcity (parasitoid > predator > omnivore). Habitat, active
288 ingredient, and crop species may mediate the influence of neonicotinoids on natural enemies
289 because these factors likely correspond to differences in exposure, toxicity, and prey
290 communities. We included publication status because a relationship between publication status
291 and effect size could indicate publication bias (Koricheva et al. 2013). Finally, we included plot
292 size, early-season sampling, and pyrethroid application method in the model to control for
293 methodological variables that we suspected might influence research outcomes.

294

295 **7. Assessing statistical assumptions, potential biases, and robustness of results**

296 As in multiple regression, in meta-regression correlations among moderator variables
297 (collinearity) can render estimates and tests of model parameters unreliable (Kutner et al. 2005;
298 Viechtbauer 2007a). Before fitting the ‘moderator’ models, we first calculated and examined
299 pairwise correlations among all our moderators. We also examined generalized variance inflation
300 factors (GVIF) by fitting linear models using the ‘lme’ function (nlme package) with our
301 moderators in the models as fixed effects and site-year nested in study as random effects. We
302 used the ‘vif’ function (car package) to calculate GVIF values.

303 To further assess the fit of our models, we examined the standardized residuals versus fits
304 and inspected the normality of the residuals using a QQ plot. To screen for influential
305 observations, we plotted leverage values on their own and relative to residuals. When we found
306 potential outliers, we re-fit our models without them to assess their influence on our conclusions.

307 We tested for publication bias in part through the ‘publication status’ moderator in the
308 meta-regressions, as discussed above. Additionally we examined a weighted histogram of the
309 effect sizes for evidence of ‘missing’ observations near zero, and used the ‘trimfill’ and ‘funnel’

310 functions ('metafor' package) on our null models to generate funnel plots and test for 'missing'
311 observations in the distributions. Finally to test the robustness of the overall effect size estimates,
312 when they differed from zero, we calculated Rosenberg's 'fail safe N', that is, the number of null
313 observations necessary to make the observed effect size non-significant. We note that most of
314 these methods for testing publication bias do not take into account the hierarchical nature of our
315 dataset; there do not appear to be tools available that explicitly account for this data structure.

316 To test the sensitivity of our results to inclusion of particular studies, we conducted a
317 'leave one out' analysis in which we removed the observations associated with each study from
318 each dataset one by one, and then re-fit all three models. We assessed the consistency of the
319 confidence intervals for the overall effects, as well as the fitted slopes and hypothesis tests for
320 the 'moderator' models. Where eliminating a study changed the interpretation of our analysis, we
321 noted this in the results.

322

323 **8. Predator-prey ratios**

324 Because insecticides can affect pest and predator populations differently, predator-prey ratios
325 may be more reflective of biological control function than predator abundance alone (e.g. Croft
326 & Nelson 1972; Naranjo 2005; Ooi 1982). Unfortunately most studies did not report predator-
327 prey ratios or sufficient data to calculate them, but we were able to perform a preliminary
328 summary based on soybean studies, which more often reported cumulative abundance of both
329 pests and relevant predators. For the soybean studies that reported both the cumulative
330 abundance of a pest taxon and the cumulative abundance of the predator guild for that pest, we
331 calculated predator-prey ratios for neonicotinoid treatments and controls. Without access to the
332 original data it was impossible to estimate a variance for the predator-prey ratios, so we did not

333 perform a formal meta-analysis; instead we discuss them qualitatively to lend preliminary insight
334 into the relative impact of neonicotinoids on predator and pest populations. To facilitate this
335 summary, we calculated the percent change in the predator-prey ratio in each neonicotinoid
336 treatment relative to each control. Negative values indicate that seed-applied neonicotinoids
337 reduced the predator-prey ratio relative to the control, while positive values indicate the opposite.
338

339 RESULTS

340 1. Results of the literature search & characteristics of the meta-analysis dataset

341 In total we screened 921 titles and abstracts, yielding 62 candidate reports (Fig. 1). After
342 assessing eligibility, filtering for relevant functional groups and geographic regions (North
343 America and Europe), and reducing redundant observations, our final dataset for the no-
344 insecticide controls comprised 607 observations collected over 56 site-years and 20 independent
345 studies (Table S1, References S1). For the pyrethroid controls, our final dataset comprised 384
346 observations collected over 15 site-years and 8 independent studies (Table S1, References S1).
347 Corn (*Zea mays*) and soybean (*Glycine max*) were the dominant crops, and insects were better
348 represented in the dataset than non-insect arthropods (arachnids and chilopods; Table 2).
349 Unsurprisingly, given the focus of our study, predators were the dominant functional group (67-
350 68% of observations), although parasitoids, omnivores, and mixed functional groups were also
351 represented. Observations were spread fairly equally among soil and foliar habitats, different
352 active ingredients, and pyrethroid application methods, and most observations were derived from
353 peer-reviewed studies (76-93%; Table 2). Plot size ranged widely from 1 to 110,000 m², and
354 proportion of early season sampling ranged from zero to 100 percent (Table S1). Most studies
355 had a sample size of three to six replicate plots per treatment (Table S1).

356

357 **2. Seed-applied neonicotinoids negatively affected natural enemies compared to no-**
358 **insecticide controls**

359 Consistent with our hypothesis, seed-applied neonicotinoids reduced abundance of arthropod
360 natural enemies relative to untreated plots (Fig. 2). The mean effect size (d) was -0.30 or -0.26,
361 for the ‘site-year/study’ and ‘null’ models respectively. For context, an effect size of -0.30 would
362 correspond to an approximate reduction of 16% in natural-enemy abundance ($-0.30 \times$ median
363 relative standard deviation [RSD] of 0.53). The estimates of the variance components from the
364 fitted model suggested that site-year explained most of the shared variation among observations
365 within studies (study $\sigma^2 = 0.0035$, site-year $\sigma^2 = 0.08$). The negative effect of seed-applied
366 neonicotinoids on natural enemies appeared to be homogenous ($Q = 622.5$, $df = 606$, $P = 0.31$),
367 with an I^2 indicating that all but 2.7% of variation in effect sizes could be explained by random
368 sampling error.

369 Despite the low heterogeneity identified in the initial analysis, we proceeded with the
370 ‘moderator’ analysis to test the influence of various factors on the effect of seed-applied
371 neonicotinoids on natural enemies. We did this because we had planned the moderator analysis *a*
372 *priori*, and because the Q test, despite being the most powerful test available, still has low power
373 to detect heterogeneity in datasets like ours where the within-study sample sizes are small
374 (Viechtbauer 2007b). Indeed, when we fit the meta-regression model with our eight moderator
375 variables, the omnibus test suggested that the moderators taken together did explain significant
376 variation in effect size ($Q_M = 21.5$, $df = 11$, $P = 0.029$). Broad taxonomic group apparently drove
377 this result, as it was the only moderator that was significant when tested individually (Table 3).
378 As predicted under toxin exposure, the negative effect of seed-applied neonicotinoids on natural
379 enemies was stronger for insects than for non-insect taxa (mostly spiders and mites; Fig. 2).

380 When estimated separately, the negative effect of neonicotinoids on insects remained significant,
381 while the effect on non-insect taxa did not differ significantly from zero (Fig. 2). Although
382 functional group did not significantly moderate the influence of seed-applied neonicotinoids on
383 natural enemies ($P = 0.13$), the fitted slopes for this moderator were fairly large and followed a
384 trend consistent with indirect effects via prey scarcity (parasitoid > predator > omnivore, Table
385 3). Surprisingly, effect size was not influenced to a significant extent by crop species,
386 neonicotinoid active ingredient, habitat, or any of the methodological variables (Table 3).

387

388 **3. Seed-applied neonicotinoids reduced natural enemy abundance similarly to pyrethroid** 389 **insecticides**

390 Contrary to our prediction, the effect size for pyrethroids versus seed-applied neonicotinoids did
391 not differ significantly from zero (Fig. 3), suggesting that these two groups of insecticides reduce
392 natural-enemy abundance to a similar extent. The sensitivity analysis revealed that one study
393 (Ohnesorg et al. 2009) had a large influence on effect sizes and confidence intervals, so we
394 present results both with and without this study (Fig. 3).

395 With all studies in the dataset, the mean effect size (d) was 0.16 or 0.07, for models that
396 did or did not include random effects of site-year nested in study (Fig. 3). Including random
397 effects for site-year and study made the confidence intervals very wide, because of the influence
398 of Ohnesorg et al. (2009). Similarly, the estimates of the variance components from the fitted
399 model suggested that study explained most of the shared variability among observations (study
400 $\sigma^2 = 0.58$, site-year $\sigma^2 = 0.04$). The effects of seed-applied neonicotinoids on natural enemies
401 compared to pyrethroid controls appeared to be homogenous ($Q = 369.6$, $df = 383$, $P = 0.68$),

402 with an I^2 indicating that 100% of the variation in effect sizes could be explained by random
403 sampling error.

404 When Ohnesorg et al. (2009) was excluded from the dataset, the confidence intervals
405 were smaller but the conclusion remained the same: seed-applied neonicotinoids and pyrethroids
406 had similar influences on natural-enemy populations (Fig. 3). The overall mean effect size was
407 very close to zero, -0.02 or 0.03, for models that did or did not include random effects of site-
408 year nested in study. The variance components for study and site-year were small in this model
409 (study $\sigma^2 = 0.01$, site-year $\sigma^2 = 0.0008$). Consistent with the first analysis, there was no evidence
410 of heterogeneity in these effects ($Q = 316.9$, $df = 375$, $P = 0.99$, $I^2 = 0$).

411 Again, we proceeded with the ‘moderator’ model to test whether various factors
412 influenced the magnitude of effect size. In this case, the omnibus test suggested that the
413 moderators did not explain significant variation in effect size (all studies: $Q_M = 4.50$, $df = 6$, $P =$
414 0.61 ; excluding Ohnesorg et al. 2009: $Q_M = 3.40$, $df = 6$, $P = 0.76$). This result is not surprising
415 given the zero estimate of heterogeneity in this dataset, and suggests that the effect size of seed-
416 applied neonicotinoids compared to pyrethroids is fairly consistent across the dataset, except for
417 the observations associated with one outlying study.

418

419 **4. Statistical assumptions, potential biases, and robustness of results**

420 We found little evidence of collinearity among our moderators. Pairwise correlations among
421 moderators were centered near zero and mostly small (No-insecticide control: 84% < 0.2 ,
422 median: -0.03, mean: -0.003, range: -0.56 to 0.52; Pyrethroid control: 80% < 0.2 , median: -0.05,
423 mean: -0.05, range: -0.51 to 0.34). Generalized variance inflation factors for moderators in both

424 analyses were all less than two, again suggesting that collinearity among our predictors was
425 minimal (Kutner et al. 2005).

426 For each of the datasets, diagnostic plots identified a handful of outliers with large
427 standardized residuals (absolute value > 3). However, these outliers had little leverage, and
428 removing them did not appreciably change parameter estimates or the outcome of significance
429 tests (data not shown; results can be replicated using Code S1 & S2).

430 We found no evidence of publication bias in our datasets. The distributions of effect sizes
431 were bell-shaped with no evidence of an absence of observations near zero (Fig. S1, S2).
432 Furthermore, publication status was not a significant moderator of effect size in the no-
433 insecticide comparison (Table 3). Rosenberg's fail-safe N suggested that over 10,000 null
434 observations would be necessary to render non-significant the difference between seed-applied
435 neonicotinoids and insecticide-free controls. The 'trimfill' analysis estimated zero missing
436 observations for each of the datasets, lending further support to the absence of publication bias.

437 The 'leave one out' analyses showed that our results for the no-insecticide comparison
438 were fairly robust to the exclusion of particular studies. The estimated intercepts, slopes, and
439 confidence intervals were quite similar across the analyses, and the overall effect of
440 neonicotinoids on natural enemies was consistently negative (data not shown; results can be
441 replicated using Code S1). In two out of twenty cases (Ohnesorg et al. 2009; Sotelo-Cardona
442 2010), leaving a study's observations out of the analysis changed the omnibus test of moderators
443 from significant to non-significant. This is perhaps not surprising given that the heterogeneity in
444 this dataset was generally low. Overall, the sensitivity analysis suggested that no particular study
445 was overly influential in the finding of a negative effect of seed-applied neonicotinoids on

446 natural enemies compared to insecticide-free controls, but that the difference in this effect
447 between insects and other taxa should be tested in future studies.

448 As discussed previously, for the effect of seed-applied neonicotinoids versus pyrethroids,
449 the ‘leave one out’ analysis revealed that one study (Ohnesorg et al. 2009) had a fairly large
450 influence on the width of confidence intervals. Nonetheless, we reemphasize that regardless of
451 the inclusion of this study or the model used to estimate effect sizes, the confidence interval for
452 this comparison always enclosed zero, suggesting little to no difference in the influence of seed-
453 applied neonicotinoids and pyrethroids on natural-enemy abundance. Excluding one study that
454 used seed-applied pyrethroids as the comparison group (Baker et al. 2002) did not change our
455 results. It is notable that there were fewer studies available that investigated pyrethroid
456 insecticides versus no-insecticide controls (8 studies versus 20 studies), and very few studies
457 investigating other insecticide classes (Table S1), a discrepancy that could be addressed in future
458 research.

459

460 **5. Effect of seed-applied neonicotinoids on predator-prey ratios in soybeans**

461 Seven soybean studies reported sufficient information to calculate predator-prey ratios. The focal
462 prey in five of the studies was the soybean aphid (*Aphis glycines*), while a sixth study focused on
463 herbivorous thrips and a seventh focused on pest slugs (mainly *Deroceras* spp.). Aphids and
464 thrips are listed on the neonicotinoid label for soybeans and so could be considered ‘target pests’,
465 though in practice soybean aphids are often not controlled sufficiently with seed-applied
466 neonicotinoids (Myers & Hill 2014). Slugs are non-target pests because they are generally not
467 susceptible to neonicotinoids (Douglas et al. 2015; Simms et al. 2006).

468 For studies focusing on soybean aphids, plots planted with neonicotinoid-coated seeds
469 had numerically lower predator-prey ratios than plots treated with foliar insecticides
470 (neonicotinoids, pyrethroids, or pymetrozine) in 13 out of 16 comparisons (Fig. S3). In contrast,
471 plots planted with neonicotinoid-coated seeds had numerically higher predator-prey ratios than
472 untreated controls in 11 out of 16 comparisons. For the studies focusing on non-aphid prey
473 (thrips or slugs), all three predator-prey ratios were numerically lower in neonicotinoid-treated
474 plots than untreated controls (Fig. S3). These results suggest that seed-applied neonicotinoids
475 have a stronger effect on aphids than on natural enemies, and that the tested foliar insecticides
476 are even more selective. On the other hand, the limited data available for non-aphid pests suggest
477 that seed-applied neonicotinoids reduce predator-prey ratios, which could signal a disruption of
478 biological control. We caution that these conclusions are based on relatively few studies and
479 pest/predator combinations, and lack an estimate of variability. Moreover, most of them are
480 based on the ratio of a focal pest to the summed abundance of a relevant guild of generalist
481 predators, and so do not take into account differences between natural enemy taxa in predation
482 rates.

483

484 **DISCUSSION**

485 We performed a meta-analysis of field studies to determine the influence of seed-applied
486 neonicotinoids on arthropod natural enemies of crop pests in North America and Europe. After
487 gathering and synthesizing results from almost 1,000 observations gleaned from 20 studies, we
488 found that seed-applied neonicotinoids: (1) reduced natural-enemy abundance and (2) reduced
489 natural enemies similarly to foliar or soil-applied pyrethroids. Furthermore, the influence of
490 seed-applied neonicotinoids on natural enemies differed by broad taxonomic group: insects were

491 more strongly affected than non-insect taxa such as spiders and mites. This last result suggests
492 that reductions in natural-enemy populations associated with seed-applied neonicotinoids are at
493 least partly a result of toxin exposure, rather than prey scarcity alone.

494 Seed-applied neonicotinoids reduced the abundance of natural enemies relative to no-
495 insecticide controls, with an effect size ($d = -0.30$) corresponding to roughly 16% reduced
496 abundance. This result was robust to different modeling choices and unexpectedly consistent
497 across crop species and neonicotinoid active ingredients. For comparison, the mean effect of
498 organic farming (versus conventional farming) on predatory insect abundance was estimated to
499 be $d = 0.49$ (Bengtsson et al. 2005). Both effect sizes suggest that insecticides can undermine
500 natural-enemy populations, but the consequences of these reductions for ecosystem services are
501 hard to predict given a lack of research relating predator abundance to biological control function
502 and its economic value (Naranjo et al. 2015). The one study in our dataset that explicitly related
503 predator abundance to crop yield was our previous study in a no-till soybean system (Douglas et
504 al. 2015). In that study, a 31% reduction in early season abundance of slug predators in
505 neonicotinoid-treated plots corresponded to a 67% increase in slug abundance and an eventual
506 5% reduction in soybean yield. Incidentally, the season-long reduction in slug-predator
507 abundance was 16%, very similar to the mean effect identified in this meta-analysis, suggesting
508 that a reduction of this magnitude can have economic consequences. Future efforts to relate
509 natural-enemy abundance to crop yield could also make use of the concept of ‘natural-enemy
510 units,’ which help to consolidate diverse natural enemies into a single measure of pest-
511 suppression potential (Bahlai et al. 2010; Hallett et al. 2014). Ultimately, it would be valuable to
512 build the knowledge base necessary to fit models relating natural-enemy abundance to pest

513 abundance and ultimately crop productivity, analogous to those recently developed for
514 pollination services (Koh et al. 2016).

515 Our finding that seed-applied neonicotinoids can in some cases increase predator-prey
516 ratios further highlights that natural-enemy abundance is not equivalent to biological control
517 function. We stress that a formal analysis of predator-prey ratios was not possible, but generally
518 seed-applied neonicotinoids tended to have a smaller effect on natural enemies than on pest
519 aphids, and a relatively larger effect on natural enemies than on other pest taxa (slugs and thrips).
520 This pattern is further supported by case studies in the literature. We are not aware of any
521 systems in which seed-applied neonicotinoids have been associated with resurgence of target
522 pests such as aphids; however, there are several examples where these seed treatments have been
523 associated with increased abundance and sometimes economic outbreaks of non-target pests,
524 including spider mites (Smith et al. 2013), slugs (Douglas et al. 2015), and late-season stem-
525 boring caterpillars (Pons & Albajes 2002).

526 Our finding that insects were more strongly affected by seed-applied neonicotinoids than
527 were non-insect groups (mainly spiders and mites) suggests that toxin exposure is at least partly
528 responsible for the overall negative effect we observed, and raises the question of how insect
529 natural enemies are being exposed to these seed-applied toxins. Neonicotinoids can poison
530 natural enemies through ingestion as well as contact with sprays or residues (Lucas et al. 2004;
531 Torres & Ruberson 2004; Wang et al. 2008). Possible exposure pathways include contact with
532 soil or planting dust (Goulson 2013), ingestion of contaminated prey (Douglas et al. 2015;
533 Szczepaniec et al. 2011), and for some natural enemies, ingestion of pollen, nectar, or other plant
534 products (Lundgren 2009; Moser & Obrycki 2009). The relative importance of the various
535 exposure pathways in the field is unclear, but we did see a non-significant trend for soil-dwelling

536 taxa to be more strongly affected than foliar-dwelling taxa. Typically ~ 90% of seed-applied
537 neonicotinoids remain in soil, rather than entering the growing crop plant (Goulson 2013), and
538 recent findings reveal a layer of elevated residues on the soil surface where many species are
539 active (Limay-Rios et al. 2016). Soil exposures, therefore, appear to be an important area for
540 future research, particularly because previous research has leaned toward foliar-dwelling taxa.
541 Finally, although the pattern we observed is consistent with the toxin exposure hypothesis, we
542 cannot rule out that different responses to seed-applied neonicotinoids by insects and non-insect
543 taxa reflect differences in ecology rather than (or in addition to) toxin susceptibility. For
544 instance, many spider species can endure very long periods of starvation (e.g. Anderson 1974),
545 and there are also important differences in mobility between insects and arachnids. The toxin
546 exposure hypothesis could be tested more directly using semi-field enclosure studies that hold
547 immigration and prey availability constant while changing the exposure of insects and other taxa
548 to neonicotinoid residues.

549 In contrast to toxin exposure, there was insufficient evidence to conclude that prey
550 scarcity contributed to reductions in natural-enemy abundance by seed-applied neonicotinoids,
551 although this result may change with additional research. While functional group was not a
552 significant moderator of natural-enemy response to seed-applied neonicotinoids, there was a
553 trend in the direction we would expect if prey scarcity were involved (parasitoid > predator >
554 omnivore). The prey scarcity hypothesis is also supported by a case study on the multicolored
555 Asian lady beetle, *Harmonia axyridis*. This species is an important predator of the soybean
556 aphid, and its population dynamics in the American Midwest over the past two decades
557 correlated with changes in abundance of its soybean aphid prey, which in turn correlated with
558 use of seed-applied neonicotinoids (Bahlai et al. 2015). More generally, although seed-applied

559 neonicotinoids do not always provide economic control of aphids, they do sometimes reduce
560 their seasonal populations (Hallett et al. 2014; Heidel-Baker 2012; Johnson et al. 2009; Ohnesorg
561 et al. 2009; Tinsley et al. 2012). In turn, aphids are key prey for many generalist predators in
562 agricultural systems (Donaldson et al. 2007; Symondson et al. 2002). Future research could test
563 the relative importance of prey scarcity versus toxin exposure through field studies that
564 manipulate prey density independently of neonicotinoid treatment.

565 We expected seed-applied neonicotinoids to reduce populations of natural enemies less
566 than foliar or soil-applied pyrethroids, but aside from one outlying study (Ohnesorg et al. 2009),
567 this was not the case. The limited number of independent studies (8) comparing neonicotinoids
568 to pyrethroids may have affected this result, and we encourage more research in this area. That
569 said, our finding is consistent with previous meta-analyses (Naranjo 2009; Wolfenbarger et al.
570 2008) that found a negative effect of pyrethroids on predatory arthropods (versus transgenic *Bt*
571 varieties) of similar magnitude to the negative effect we found for seed-applied neonicotinoids
572 (versus untreated controls). Pyrethroids are the second most widely used class of insecticides in
573 the world after neonicotinoids (Sparks 2013), and are important alternatives to seed-applied
574 neonicotinoids in North American and European field crops (Budge et al. 2015; Douglas &
575 Tooker 2015; Furlan & Kreutzweiser 2015). Their use is therefore likely to increase if, when,
576 and where neonicotinoid use is restricted. Foliar and some soil-applied pyrethroids have the
577 advantage that they can be applied in response to economic pest populations, and can therefore
578 be more compatible with integrated pest management than seed treatments, which are typically
579 applied to the seed months before planting (Furlan & Kreutzweiser 2015; Johnson et al. 2009). It
580 is also worth noting that pyrethroids and neonicotinoids overlap in their acute toxicity to
581 mammals (Tomizawa & Casida 2005), although foliar or soil applications would likely also

582 entail different exposures than seed applications. Additionally, in some cases seed-applied
583 neonicotinoids may be replaced by cultural management tactics or nothing at all. While the full
584 economic, human health, and environmental trade-offs of neonicotinoids versus pyrethroids and
585 other pest management strategies are beyond the scope of this study, our results do suggest that
586 seed-applied neonicotinoids are neither uniquely risky nor benign to an important group of non-
587 target invertebrates.

588 Prior to our meta-analysis, the statistical results within and across studies in our dataset
589 appeared highly variable, and a narrative review of these findings could characterize them as
590 mixed. In fact, their measured effects were largely consistent with one another, as reflected in the
591 low heterogeneity of effect sizes across our datasets. This apparent contradiction results from the
592 modest size of the effect combined with the high variability of measurements in field studies, and
593 emphasizes the importance of considering statistical power during ecological risk assessment.
594 Detecting a 20% reduction in natural-enemy abundance with 80% probability requires at least 15
595 plots per treatment for many predatory arthropod taxa (Prasifka et al. 2008), far more than most
596 studies contributing to our dataset (typically three to six replicates per treatment). While
597 increasing sample size is an obvious solution, logistical and funding constraints make this a
598 challenge. We suggest that researchers interpret null results conservatively in light of statistical
599 power. Periodic meta-analyses may be useful for drawing broader conclusions, as has been the
600 case for transgenic *Bt* crops (Marvier et al. 2007; Naranjo 2009). The datasets we compiled for
601 this study and a dataset of neonicotinoid effects on bees (Lundin et al. 2015) together provide a
602 foundation for ongoing meta-analyses on the influence of neonicotinoids on non-target species.

603 There are several important limitations of our meta-analysis that stem from constraints of
604 the dataset we compiled. Because most studies measured natural-enemy abundance within a

605 single field season, our results do not address the influence of seed-applied neonicotinoids on
606 other important metrics like species diversity, sublethal effects on behavior, reproduction of
607 long-lived species, or long-term effects on natural-enemy populations associated with chronic
608 exposure. Furthermore, our dataset comprises manipulative plot studies that by their nature do
609 not account for movement of natural enemies across landscapes. By focusing on seasonal mean
610 abundance, we may have underestimated important but transient effects that occur only during
611 the period soon after planting. And finally, our study was not able to address the influence of
612 seed-applied neonicotinoids in cropping systems outside of North American and Europe. It is our
613 hope that future meta-analyses will benefit from increased research in these areas.

614

615 **CONCLUSION**

616 Using meta-analysis to synthesize the results from field studies in North American and Europe,
617 we found that seed-applied neonicotinoids reduced natural-enemy populations similarly to foliar-
618 or soil-applied pyrethroids. The negative effect of neonicotinoids on natural enemies was $d = -$
619 0.30 ± 0.10 [95% CI], corresponding to a reduction of ~16%. The patterns we observed suggest
620 that seed-applied neonicotinoids exert their effects mainly on insect (versus arachnid) natural
621 enemies, at least partly through toxin exposure. If restrictions on neonicotinoid use encourage
622 substitution with pyrethroids, our results suggest that there will be little net effect on natural-
623 enemy populations. In fact, the results of neonicotinoid restriction for natural enemies are likely
624 to be complex, particularly because some pyrethroids can more easily be saved for those
625 situations in which economically damaging pest populations occur. Finally, translating natural-
626 enemy abundance into biological control function is not possible given current knowledge, and is
627 an important area for future study.

628

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635 improved the manuscript.

636

637 TABLES

638 **Table 1.** LC₅₀ results from two laboratory studies that compared imidacloprid toxicity to insect and arachnid predators.

Study	Class	Order	Family	Species	Life stage	LC ₅₀ (ppm)
Mizell & Sconyers 1992*	Arachnida	Mesostigmata	Phytoseiidae	<i>Neoseiulus collegae</i>	Adult females	>12744
				<i>Phytoseiulus macropilus</i>	Adult females	3561
				<i>Proprioseiopsis mexacanus</i>	Adult females	>1274
	Insecta	Coleoptera	Coccinellidae	<i>Olla v-nigrum</i>	Adults	3.07
					Last instar larva	2.62
		Hemiptera	Miridae	<i>Deraeocoris nebulosus</i>	Adults	0.0163
					Neuroptera	Chrysopidae
		Adults (pop 2)	155			
				Eggs	20.2	
Tanaka et al. 2000§	Arachnida	Araneae	Linyphiidae	<i>Gnathonarium exsiccatum</i>	1st instar nymphs	801
				<i>Ummeliata insecticeps</i>	1st instar nymphs	995
				<i>Pardosa pseudoannulata</i>	1st instar nymphs	440
				<i>Tetragnatha maxillosa</i>	1st instar nymphs	136
	Insecta	Hemiptera	Miridae	<i>Cyrtorhinus lividipennis</i>	Adult females	0.36
					Hymenoptera	Dryinidae

639 * Residual toxicity; predators were exposed to imidacloprid residues on petri dishes for 48 to 72 hours

640 § Contact toxicity; predators were immersed in insecticide solution and mortality was measured after 24 to 48 hours

641

642

643 **Table 2.** Description of the dataset used in a meta-analysis of seed-applied neonicotinoid effects on natural enemies of crop pests.

Variable	Levels	No insecticide control			Pyrethroid control		
		Studies	Site-years	Obs. (%)*	Studies	Site-years	Obs. (%)*
Taxonomic group	Insects	20	56	493 (81%)	8	15	313 (82%)
	Non-insect arthropods	14	30	114 (19%)	6	11	71 (18%)
Habitat	Soil-associated	11	26	189 (31%)	6	10	156 (41%)
	Aboveground	15	48	418 (69%)	5	11	228 (59%)
Functional group	Omnivore	6	13	39 (6%)	4	8	41 (11%)
	Mixed	12	32	79 (13%)	5	8	46 (12%)
	Predator	17	48	408 (67%)	8	15	262 (68%)
	Parasitoid	7	27	81 (13%)	2	6	35 (9%)
Active ingredient	Imidacloprid	11	29	336 (55%)	6	12	279 (73%)
	Clothianidin/Thiamethoxam	13	35	271 (45%)	6	10	105 (27%)
Crop species	Corn	7	20	300 (49%)	4	10	244 (64%)
	Soybeans	7	22	200 (33%)	2	5	114 (30%)
	Other	6	14	107 (18%)	2	3	26 (7%)
Publication status	Peer-reviewed journal	13	36	459 (76%)	6	12	358 (93%)
	Diss./Thesis/Other	7	20	148 (24%)	2	3	26 (7%)
Pyrethroid application	Soil-based	-	-	-	5	8	159 (41%)
	Foliar	-	-	-	4	10	225 (59%)
TOTAL		20	56	607 (100%)	8	15	384 (100%)

644 * Number of observations in each category, followed by the percentage of values in the dataset in that category.

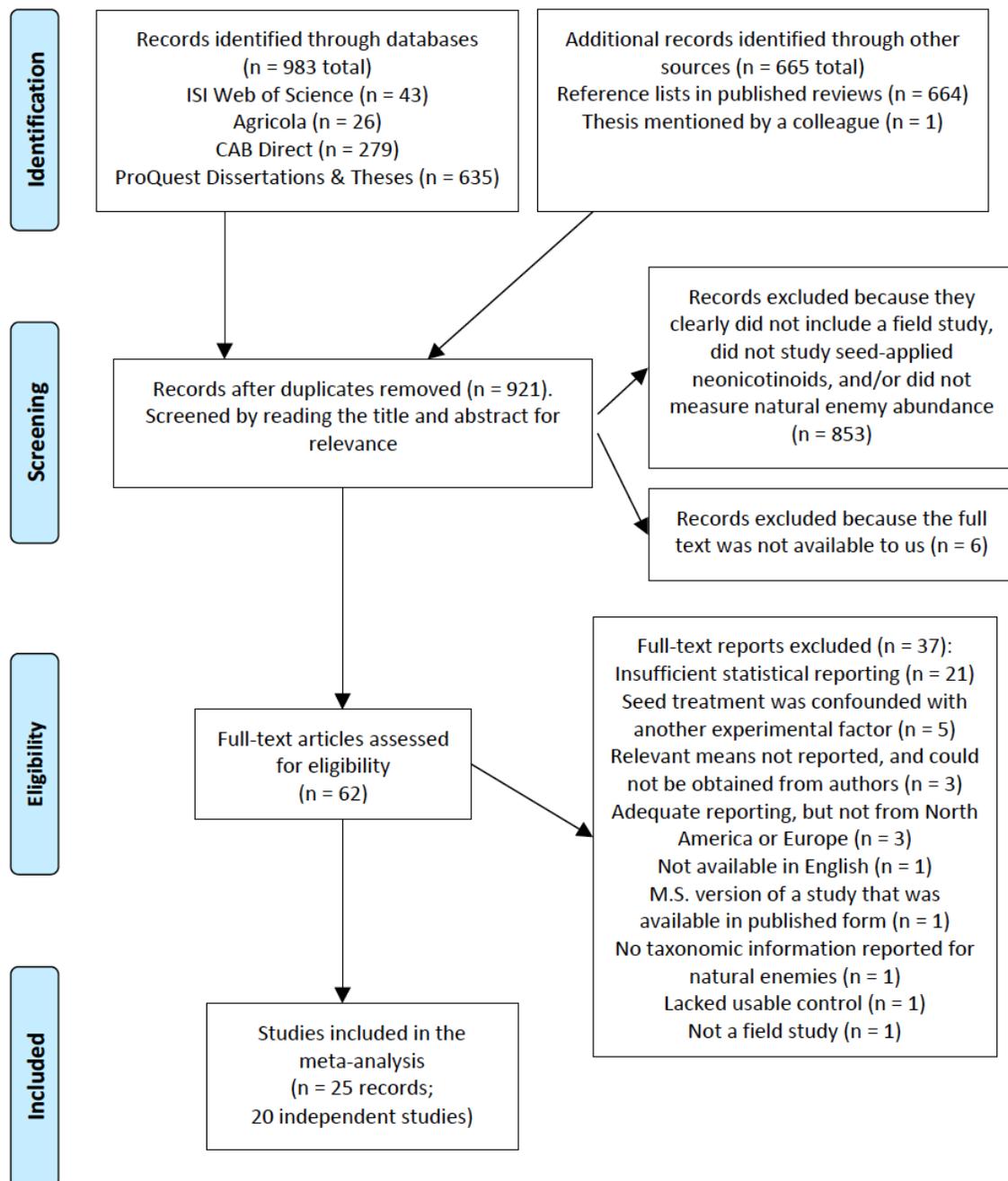
645 **Table 3.** Estimates and tests of significance for moderators in a meta-regression model testing the effect of seed-applied
 646 neonicotinoids on natural enemies, compared to controls treated with no insecticides (n = 607 observations from 56 site-years and 20
 647 studies).

Moderator	Level	β	Q_M	df	P value
Intercept	-	-0.23	-	-	-
Taxonomic group	-		8.70	1	0.003
	Insects	-0.11			
	Non-insect arthropods	0.11			
Habitat	-		1.42	1	0.23
	Aboveground	0.057			
	Soil-associated	-0.057			
Functional group	-		5.61	3	0.13
	Omnivore	0.186			
	Mixed	0.049			
	Predator	-0.071			
	Parasitoid	-0.164			
Crop species	-		0.79	2	0.67
	Corn (<i>Zea mays</i>)	0.072			
	Soybean (<i>Glycine max</i>)	0.002			
	Other	-0.074			
Active ingredient	-		0.99	1	0.32
	Imidacloprid	0.043			
	Clothianidin/Thiamethoxam	-0.043			
Publication type	-		0.51	1	0.56
	Peer-review journal	0.062			
	Dissertation/Thesis/Other	-0.062			
ln(Plot size)	-	-0.016	0.34	1	0.56
ln(Early sampling+0.1)	-	-0.076	0.61	1	0.44

648

649

650 FIGURES



651

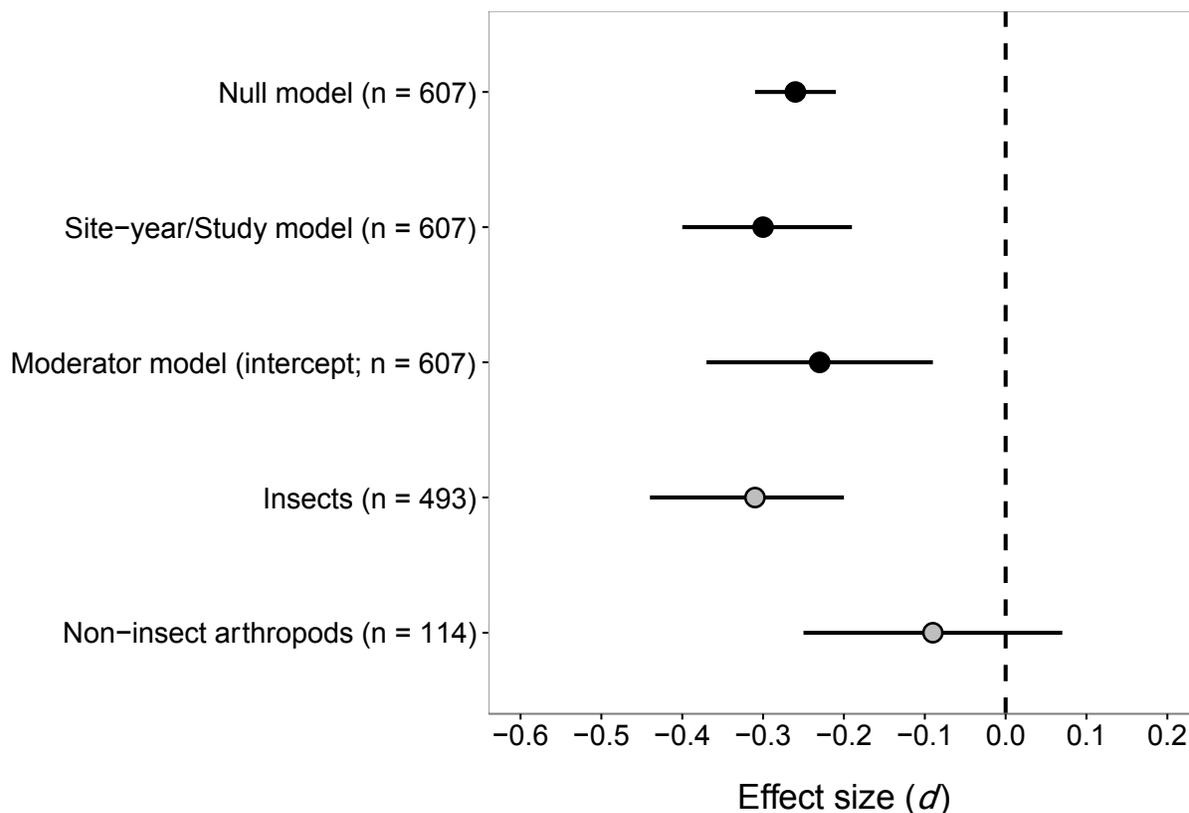
652 **Figure 1.** Flow diagram based on the Preferred Reporting Items for Systemic Reviews and Meta-

653 analyses (PRISMA; Moher et al. 2009), describing the literature search and screen used to

654 identify studies for a meta-analysis on the influence of seed-applied neonicotinoids on natural
655 enemies.

656

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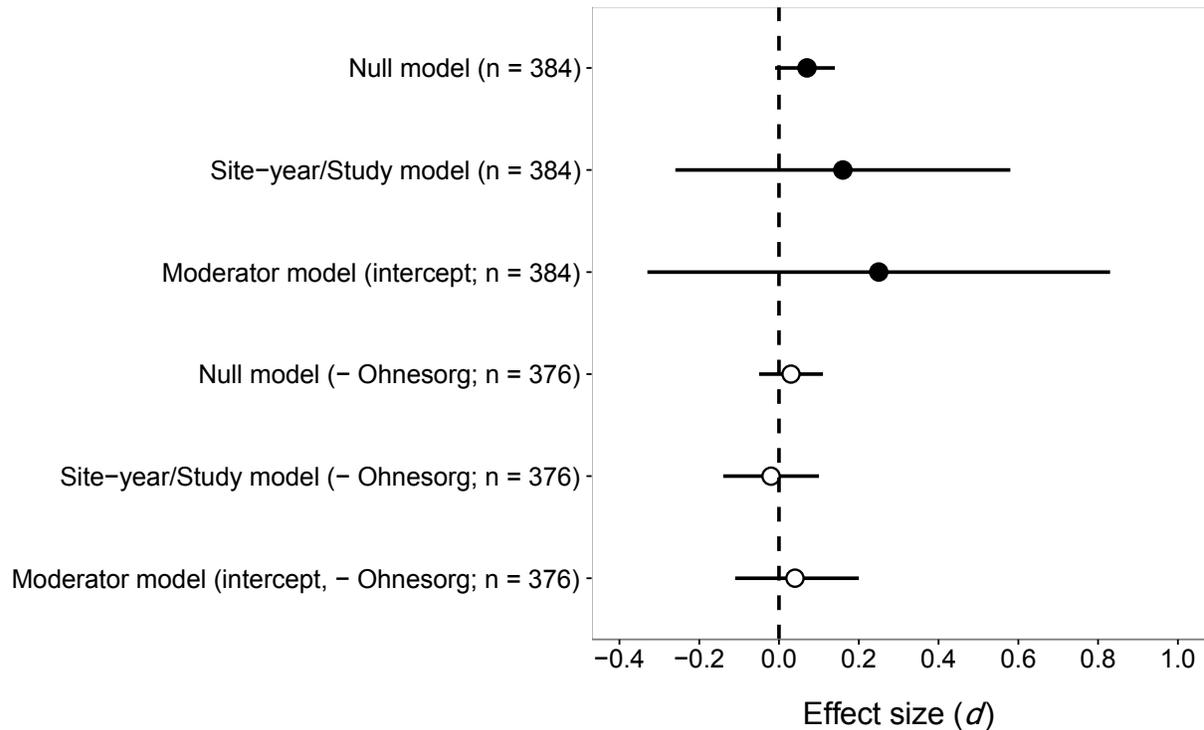


658

659 **Figure 2.** Confidence intervals (95%) for the effect of seed-applied neonicotinoids on natural-
660 enemy abundance, relative to no-insecticide controls. n = the number of observations associated
661 with each estimate; observations were derived from 56 site-years and 20 studies (see Table 2 for
662 further description of the dataset). See text for details on models used to generate these estimates.

663

664



665

666 **Figure 3.** Confidence intervals (95%) for the effect of seed-applied neonicotinoids on natural-
 667 enemy abundance, relative to controls treated with foliar or soil-applied pyrethroids. n = the
 668 number of observations associated with each estimate; observations were derived from 15 site-
 669 years and 8 studies (see Table 2 for further description of the dataset). Results are presented both
 670 with and without data from Ohnesorg et al. 2009, which had effect sizes quite different from the
 671 other studies. See text for details on models used to generate these estimates.

672

673

674

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675

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