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Plant-mediated interactions: considerations for agent selection in weed biological control programs

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1 **Abstract**

2 Plant-mediated indirect interactions among herbivores (arthropods and pathogens) are common
3 and extensively reported in the ecological literature. However, they are not well-documented
4 with respect to weed biological control. Such interactions between biological control agents can
5 have net positive or negative impacts on total weed suppression depending on the strength of the
6 interaction(s), the relative importance of the agent indirectly impacted, and the combined weed
7 suppression that results. A better understanding of plant-mediated interactions may improve
8 decision-making about which agents to introduce in classical biological control programs for
9 greatest impact on invasive weeds. This paper reviews the subject, including examples from the
10 biological control literature; outlines the need for research on indirect effects of herbivores on
11 other herbivores; discusses how such knowledge may strengthen classical biological control
12 programs for invasive weeds; and provides recommendations for the kind of studies that should
13 be done and how information about plant-mediated interactions could be integrated into agent
14 evaluation protocols, to assist in decision-making about agents for importation and release.

15

16 *Keywords:* Indirect interactions; Weed biological control; Pre-release evaluation; Agent
17 selection

18 **1. Introduction**

19 During the past two decades there has been considerable discussion and debate about how to
20 evaluate candidate agents for classical biological control of weeds so that decisions to release
21 agents will pose a minimal risk to non-target organisms (e.g., Briese and Walker, 2008; Follet
22 and Duan, 2000; Heard, 2000; Louda et al., 2003, 2005; Pemberton, 2000; Sheppard et al., 2005;
23 Wajnberg et al, 2001; Withers et al., 2000). An outcome of this discussion has been a more
24 detailed review of candidate agents which, likely, has reduced the rate of agents released.
25 Adopting a more deliberate and parsimonious approach for releasing biological control agents
26 also increases the need for making better predictions about which agents are most likely to
27 become established, and to have the greatest impact on invasive weeds. In that regard, recent
28 reviews have called for more extensive research on the biology and impact of candidate agents,
29 weed life histories and population dynamics, and environmental conditions affecting weed-
30 herbivore interactions (Mills and Kean, 2010; Morin et al. 2009; Müller-Schärer and Schaffner
31 2008).

32 A potentially important, but neglected, category of weed-herbivore interactions in research
33 and in pre- and post-release evaluations of prospective biological control agents concerns
34 indirect effects of herbivorous agents on other agents resulting from induced changes in host
35 plant quality, or in the quantity of resources available. Because the effects of these plant-
36 mediated interactions (hereafter, P-MIs) on agents may be positive or negative (or not occur at
37 all), they have the potential for increasing or decreasing the level of biological control and, thus,
38 the impact on weed populations. To date, experimental evidence for P-MIs in the weed
39 biological control literature is limited (see references in Table 1). However, extensive
40 documentation of a wide range of plant-mediated effects involving herbivores and pathogens in

41 the ecological literature (see reviews by Agrawal et al., 1999; Damman, 1993; Denno et al.,
42 1995; Hatcher, 1995; Hatcher and Paul, 2001; Karban and Baldwin, 1997; Masters and Brown,
43 1997; Ohgushi et al., 2007; Wootton, 1994) suggests that these indirect interactions are common
44 among organisms used to control invasive weeds. Further, the intensity of indirect interactions
45 can increase with potentially higher densities of introduced herbivores and pathogens due to
46 decreased natural enemy loads (Denno et al., 1995). How frequently P-MIs occur, what impact
47 they have on agents, and ultimately how this effects weed populations, is unknown. Research is
48 needed to provide answers to these questions and, in so doing, will help to determine whether
49 studies of plant-mediated effects should be incorporated into evaluation protocols for biological
50 control programs.

51 In addition to providing practical information, investigations of P-MIs would also benefit
52 theory. For example, the Multiple Stress Hypothesis, which has been used widely in biological
53 control programs, assumes that most weeds require more than one stressor for effective
54 suppression, such as multiple species of biological control agents targeting different locations
55 and/or plant functions (Harris, 1981, 1991). A second, related assumption is that agents that feed
56 in a complementary manner, i.e., on different organs or in ways that partition the weed resource
57 (e.g., James et al., 1992), will not compete because they do not interact directly. Based on what is
58 now known about P-MIs, the second assumption needs to be examined more rigorously.
59 Therefore, even if research suggests that multiple agents may improve weed suppression, a pre-
60 release assessment of agents for possible negative P-MIs should lead to a better introduction
61 strategy and greater overall success (see 4. 'Research needs and recommendations'). We also
62 expect that research on P-MIs targeting weeds will add to our fundamental knowledge by
63 revealing new mechanisms underlying indirect interactions, and by providing a more complete

64 understanding of the complexity of existing interactions. Furthermore, as the list of empirical
65 examples of P-MIs accumulates, new patterns, predictions and hypotheses will result, thus
66 contributing to ecological theory.

67 The scope of this paper encompasses P-MIs between herbivorous agents, which we define
68 broadly to include both arthropods and plant pathogens. Discussion and examples of effects of
69 indirect interactions involving weeds and different species of herbivores are given. The focus is
70 on single season interactions; but we include cases where P-MIs carry over to the next growing
71 season (e.g., winter annual, biennial, and perennial weeds). We also consider situations where
72 life cycles of agents span more than one season (e.g., species that have an intervening period of
73 dormancy). Most of the examples are for agents that have one generation per year (univoltinism);
74 however, P-MIs can occur when one or both species has two or more generations. Because plant-
75 mediated effects can be unidirectional or bidirectional, we discuss cases where one or both
76 agents is responsible for inducing plant-mediated effects. And as P-MIs may occur in agents that
77 are spatially or temporally isolated from one another, we cover both and include a related
78 discussion about persistence (duration) of effects and pervasiveness (local vs. systemic) within
79 plants. We exclude all direct interactions among agents (e.g., direct forms of competition,
80 intraguild predation, etc.), indirect exploitative competition involving agents that are present at
81 the same time and place (e.g., Berube, 1980), and cases involving systemic pathogens (e.g.,
82 Kruess, 2002; see section 3.1). With respect to introduction strategies in classical biological
83 control programs, discussion and recommendations about P-MIs apply to all situations,
84 including: (1) multiple agents being considered for importation (Gerber et al., 2007); (2)
85 additional agents considered to supplement previously imported species (Swope and Parker,
86 2010); and (3) importation and release of agents in environments where resident herbivores

87 (either indigenous or fortuitously introduced) are present (e.g., Caesar, 2003; Hatcher, 1995;
88 Moran, 2005).

89 The primary purpose of our paper is to highlight the role that P-MIs may play in weed
90 biological control programs. In the following sections we review types of P-MIs, summarize
91 known examples of P-MIs in weed biological control programs, discuss research needs to better
92 utilize knowledge about P-MIs for enhancing biological control, and provide recommendations
93 for evaluating outcomes of different categories of P-MIs in relation to agent selection.

94

95 **2. Overview of plant-mediated interactions**

96 Plant-mediated interactions among herbivores pertain to a particular category of indirect
97 interactions in which feeding or other activities by an arthropod or a plant pathogen induces
98 changes in a plant which then affect other species feeding on the same plant (see reviews by
99 Ohgushi, 2005; Ohgushi et al., 2007; Wootton, 1994). Interspecific interactions among plant-
100 feeding organisms can occur within and between feeding guilds, and with taxonomically-related
101 or unrelated species. Effects on an herbivore may be positive, negative, or neutral depending on
102 how the herbivore responds to the altered plant. Denno and Kaplan (2007) describe herbivore-
103 induced effects that result in positive or negative outcomes for other herbivores as induced
104 susceptibility (facilitation) or induced resistance, respectively.

105 Plant responses to herbivory that result in indirect plant-mediated effects are diverse and
106 include changes in structure (e.g., texture, tissue hardness, growth form, etc.), physiology (e.g.,
107 growth rate and productivity), and/or chemistry. The latter may involve primary products such as
108 nutrients or secondary chemicals, especially those used for plant defense against herbivores
109 (Karban and Baldwin, 1997). In turn, induced effects on plants influence herbivores by a range
110 of mechanisms which include ecological (e.g., food resources, altered risk of natural enemy

111 attack, shelter [Damman, 1993; Marquis and Lill, 2007]), behavioral (Milbrath and Nechols,
112 2004a), phenological (synchrony between herbivores and plant resources [Milbrath and Nechols,
113 2004a]), and physiological (e.g., herbivore growth and development, survival, reproduction
114 [Hunt-Joshi and Blossey, 2005; Simelane, 2006]).

115 Indirect P-MIs occur in plant-feeding species that are separated spatially (Blossey and Hunt-
116 Joshi, 2003; Buccellato et al., 2012; Campanella et al., 2009; Hunt-Joshi and Blossey, 2005;
117 Masters and Brown, 1997; Raghu et al., 2012; Simelane, 2006), temporally (Damman, 1989;
118 Wold and Marquis, 1997), or both (Gerber et al., 2007; Milbrath and Nechols, 2004a; Swope and
119 Parker, 2010; Swope and Stein, 2012; Van Hezewijk and Bouchier, 2012). Thus, species that
120 interact indirectly fall into one of three categories: spatial overlap but temporal separation;
121 spatial separation but temporal overlap; spatial and temporal separation (Denno and Kaplan,
122 2007; Denno et al., 1995; Ohgushi, 2005). The extent to which plant-mediated effects impact an
123 herbivore population may depend on several factors, including (a) how much temporal overlap
124 exists between its population and the one that induces the plant effects, (b) the amount of time
125 separation of each species on the host plant, (c) the amount of time the life stage(s) of the
126 inducing herbivore is present, (d) the persistence of the herbivore-induced plant effect(s) in
127 relation to the plant and/or herbivores' life histories, (e) the strength/magnitude of the effect(s),
128 and (f) the type and distance of spatial separation (see below; Damman, 1993).

129 Another consideration as to what constitutes spatial isolation depends on whether herbivore-
130 induced effects are local or systemic (i.e., influencing the entire plant, affecting source-sink
131 dynamics, etc.) and also on whether herbivores share a feeding guild. For example, although
132 whiteflies and leafminers both feed on leaves and may occur in close physical proximity to one
133 another, the fact that one is a phloem-feeder whereas the other chews through the mesophyll may

134 allow spatial separation, at least in terms of P-MIs. The fact that negative indirect interactions
135 commonly occur refutes the outdated notion that partitioning plant resources spatially or
136 temporally is sufficient for herbivores to avoid competition. Thus, indirect P-MIs have a bearing
137 on how niche separation is defined.

138 Herbivore-induced indirect interactions appear to be quite common in nature. For example,
139 Denno et al. (1995) noted that 53% of the interspecific interactions surveyed in their study
140 involved plant-mediation. They also found that a majority of the indirect interactions was
141 asymmetrical, suggesting that P-MIs are largely unidirectional or, if bi-directional, one herbivore
142 has greater influence than the other.

143 Plant-mediated effects on herbivores are not fixed but, rather, are condition-dependent. Some
144 of the factors known to influence the elicitation, magnitude, and/or persistence of plant-mediated
145 responses are 1) the manner in which herbivores feed, and the feeding sites; 2) the sequence of
146 attack (i.e., ‘priority effects’ in Erb et al. 2011); 3) other plant stressors (e.g., abiotic); 4)
147 herbivore densities (e.g., threshold or graded responses) (Crowe and Bouchier, 2006 vs. Smith
148 and Mayer, 2005); and 5) the species of herbivores involved in the interaction (Agrawal et al.,
149 1999 [various chapters]; Sabelis et al., 2007). Although all of these factors are of potential
150 importance, we view herbivore densities (or pathogen titers) as a key consideration in assessing
151 and predicting P-MIs in weed biological control (see Section 3).

152

153 **3. Plant-mediated interactions in weed biological control**

154 We examined the primary literature and review articles for examples of P-MIs in weed
155 biological control programs in which agents were separated in space and/or time. A general
156 overview of these papers is shown in section 3.1, and Table 1 summarizes the key features of

157 each study in relation to the others. Section 3.2 and Table 2 provide an in-depth analysis of a
158 case study we did on weevils introduced to control musk thistle, which relates P-MIs to weed
159 impact. Section 3.3 discusses problems in predicting P-MIs, and how this relates to weed impact.
160 The relevance and applicability of investigating P-MIs in weed biological control are covered in
161 sections 3.4 and 3.5.

162

163 *3.1. Examples of P-MIs in weed biological control (Table 1)*

164 Until recently relatively few published papers have dealt explicitly with P-MIs between
165 biological control agents. Furthermore, as noted by Hatcher and Paul (2001), evaluations of weed
166 biological control programs have generally considered either the impacts of
167 herbivores/pathogens on weeds (but not necessarily all possible combinations of agents), or
168 interactions among herbivores/pathogens, but not both. Our search found fourteen papers
169 representing ten weeds that examined P-MIs. Six weed examples (seven papers) involved agents
170 separated spatially but present at the same time, and four weed examples (seven papers) involved
171 agents separated in space and time. These are listed in Table 1, which includes the target weed
172 name, the interacting biological control agents, the type of interaction observed, a summary of
173 the combined agent impact to the plant relative to individual agent impact (only six of the ten
174 weed examples included a plant impact assessment), and the experimental venue. Three of the
175 examples involved an insect-pathogen interaction. We could not find appropriate examples
176 involving agents that feed on the same plant parts and are only separated temporally from each
177 other.

178 The studies were a mixture of greenhouse and field (including common garden) experiments,
179 and most were retrospective (i.e., they involved agents that had already been released in the

180 field), with the exception of Gerber et al. (2007) and Raghu et al. (2012) which were done
181 prospectively (i.e., prior to agent releases). Only three of the studies were conducted in the native
182 range (see Table 1). Interactions between spatially-separated agents, in which a two-way
183 interaction is possible, were variable although two papers did not examine both possible
184 interactions. The paper by Raghu et al. (2012) considered P-MIs among three herbivore species,
185 making for two sets of a two-way interaction. As a result, out of seven pairs of herbivores (14
186 possible interactions) in this category, P-MIs were fully characterized for only five pairs of
187 herbivores. One of the agents usually had no apparent effect on the other species (four of the five
188 herbivore pairs that were fully examined, Table 1). In contrast, the effect of the second species
189 on the first agent could vary depending on the parameter measured or the type of experiment
190 (e.g., Hunt-Joshi and Blossey, 2005). The P-MIs in this case were primarily negative (five of
191 seven herbivore pairs) and ranged from strong to weak, i.e., only one of the measured parameters
192 was affected (Table 1). Only Campanella et al. (2009) and Raghu et al. (2012) noted potential
193 positive (facilitative) interactions among spatially-separated herbivores (see Table 1), although
194 the specific mechanisms were unknown. Interactions between agents separated in space and
195 time, in which a one-way interaction occurred, were neutral (one weed example), negative (two),
196 or mixed (studies of yellow starthistle, *Centaurea solstitialis* L., Table 1). Low densities of some
197 agent species may not have allowed for the full range of P-MIs to be characterized. For example,
198 Hunt-Joshi et al. (2004) noted that the experimental densities achieved for the weevil *Hylobius*
199 *transversovittatus* Goeze were much lower than field infestations in Europe, which could partly
200 explain the relative lack of impact of the weevil on the leaf beetle *Galerucella californiensis* L. or
201 their shared host plant.

202 The combined agent impact on the plant that was reported tended to be equal to that of a
203 single agent, but could vary according to the plant parameter measured or environmental
204 condition such as soil type (Table 1). For example, the combination of the rust fungus *Puccinia*
205 *jaceae* Otth var. *solstitialis* and seed-head insects caused a greater reduction in seed of yellow
206 starthistle than either type of herbivore alone for plants growing on serpentine soils but not on
207 non-serpentine soils (Swope and Stein, 2012). Plants growing on non-serpentine soils were much
208 larger and fecund than those on serpentine soils. An unanticipated effect reported by Swope and
209 Parker (2010) and Swope and Stein (2012) was that the presence of the rust fungus reduced seed
210 feeding by various insect larvae via an unknown mechanism without generally altering survival
211 rates of the larvae. This was hypothesized to be due to either a decrease in seed quality from an
212 induced defensive response (negative effect) or an increase in seed nutrient quality (positive
213 effect). As a result, the negative effect of the fungus on seed production of yellow starthistle
214 growing on non-serpentine soils was cancelled out by the reduced seed predation. Interference
215 (of the fungus with the insect agents) can occur despite the lack of a strong signal of competition.

216 In one case it was difficult to distinguish whether a response was indirect and plant-mediated
217 or a direct response to the agent itself. In the study by Simelane (2006) there were clear-cut P-MI
218 effects of leaf-feeding lace bugs that negatively influenced root quality for larvae of the flea
219 beetle *Longitarsus bethae* Savini & Escalona. However, Simelane (2006) also showed that adult
220 flea beetles avoided lantana plants that were infested with high densities of lace bug nymphs.
221 This may represent a plant-mediated behavioral response, but alternatively may reflect a direct
222 response to the presence of the other herbivore. Similarly, a study by Kruess (2002), which we
223 do not cite as a P-MI example, found that when thistles were systemically infected by the fungal
224 pathogen *Phoma destructiva* (Plowr.), this resulted in negative effects on both adult feeding and

225 oviposition behavior, as well as preimaginal development, survival and body weight of the leaf
226 beetle *Cassida rubiginosa* Müller. However, because the fungal pathogen is distributed
227 throughout the plant, it is unclear whether the leaf beetle responded indirectly to changes in plant
228 quality, directly to the fungal pathogen, or a combination of both (see similar study by Kluth et
229 al. [2001] involving a different systemic fungal pathogen of *Cirsium arvense* (L.) Scop. whose
230 effects on herbivores cannot be distinguished). While we have excluded direct interactions from
231 consideration in this paper, it is clearly important to know if/how the influence of one herbivore
232 on another, whether from direct or indirect interactions, influences agent populations and
233 performance and, thus, the impact on target weeds.

234

235 3.2. Musk thistle case study (Table 2)

236 The interaction between musk, or nodding, thistle, *Carduus nutans* L., and its two primary
237 biological control agents - the weevils *Rhinocyllus conicus* Froelich (head weevil) and
238 *Trichosirocalus horridus* (Panzer) (rosette weevil) - represents perhaps the best known example
239 of plant-mediated indirect effects for weed biological control agents (see Milbrath and Nechols,
240 2004a,b for full details). Musk thistle is a Eurasian/North African plant introduced into North
241 America in the mid-1800s, where it became a noxious weed in pasture and rangeland (Dunn,
242 1976; Stuckey and Forsyth, 1971). It grows primarily as a biennial or winter annual and is
243 propagated only through seed (McCarty and Scifres, 1969). *Trichosirocalus horridus* adults
244 oviposit into vegetative rosettes from late fall through spring. Larvae feed in the root crown and
245 destroy the apical meristem, which may alter subsequent plant architecture and flowering and
246 therefore indirectly seed set (Cartwright and Kok, 1985; Woodburn, 1997). *Rhinocyllus conicus*
247 adults oviposit onto flower heads (capitula) produced in the spring and early summer, and the

248 larvae feed on the receptacle tissue, reducing seed production (Kok, 2001; McCarty and Lamp,
249 1982; Shorthouse and Lalonde, 1984; Surlles and Kok, 1976). Thus, the two species of herbivores
250 attack different plant parts at different times of the year, leading to a one-way (asymmetric)
251 interaction of *T. horridus* on *R. conicus*.

252 A series of field and greenhouse experiments were conducted to retrospectively address two
253 questions in parallel: does early vegetative feeding by *T. horridus* alter musk thistle as a resource
254 for the later-arriving *R. conicus*, and how might this influence the individual and combined
255 effects of *R. conicus* and *T. horridus* on musk thistle seed production? The P-MI was
256 hypothesized to be negative and occur via four mechanisms (Table 2) in which altered plants
257 would affect: the seasonal synchrony between ovipositing *R. conicus* and musk thistle flower
258 head availability (phenological effect); the acceptability of musk thistle flower heads for
259 oviposition by *R. conicus* (behavioral effect); the quantity of plant tissue available to developing
260 *R. conicus* larvae (resource limitation effect); and the quality of musk thistle heads for
261 developing *R. conicus* larvae (physiological effect).

262 A negative P-MI was confirmed although it was influenced by the density of *T. horridus*
263 larvae (see Table 2 for summary). Low densities (<20 larvae per plant) generally had no effect
264 on musk thistle phenology or flower and seed production (although it did create multiple-
265 stemmed plants) and no effect on adult *R. conicus* oviposition and subsequent production of new
266 adults compared to thistles not previously infested by *T. horridus* (Milbrath and Nechols
267 2004a,b). In contrast, high larval densities of *T. horridus* (66 per plant) resulted in thistles
268 producing fewer flower heads which were also delayed in their first appearance by one week
269 compared to non-infested thistles. A cascade of plant-mediated effects ensued - there was a
270 reduction in 1) *R. conicus*-musk thistle flower head synchrony, 2) the acceptability of *T.*

271 *horridus*-infested musk thistle to ovipositing *R. conicus*, 3) the quantity and 4) quality of
272 resource available to *R. conicus* larvae. As a result, the production of new *R. conicus* adults was
273 reduced 63% (Milbrath and Nechols, 2004a). This study revealed for the first time multiple and
274 diverse plant-mediated effects between spatially- and temporally-isolated herbivores in a weed
275 biological control system.

276 Despite the large, negative effect of *T. horridus* on the recruitment of new *R. conicus*, the
277 reduction in musk thistle viable seed was still greater when both weevils were present (59%)
278 compared to *R. conicus* alone (45%) (Table 2; Milbrath and Nechols, 2004b). Hence, the
279 outcome for biological control programs may not necessarily be adverse even with plant-
280 mediated competition because of compensatory trade-offs concerning the relative impacts of the
281 two herbivores on the weed. Regional environmental conditions also may alter the relative
282 abundance of the two weevils, thus affecting their interaction and combined impact on musk
283 thistle.

284

285 *3.3 Problems in making predictions of P-MIs and weed impact*

286 Because of the low number of published studies concerning P-MIs for weeds, general
287 predictions about whether a plant-mediated response will occur, and, if so, what kind, are not
288 possible at the present time. Besides including a limited taxonomic range and number of feeding
289 guilds among known examples, most if not all studies are incomplete in one or more respects,
290 including not having evaluated reciprocal effects of interacting herbivores, conducting
291 experiments that were either too short or could not be done under realistic field conditions, and
292 not assessing organisms over a range of densities (threshold effects). Despite these limitations, a
293 comparison of studies in which similar taxonomic groups and feeding guilds of herbivores

294 elicited and were affected by P-MIs, suggest that the types of plant-mediated effects produced
295 also may be similar. For example, in both studies where leaf feeders indirectly affected root
296 feeders (spatial separation: Hunt-Joshi and Blossey, 2005; Simelane, 2006), the authors reported
297 reduced survival of root-feeding larvae. As noted by Blossey and Hunt-Joshi (2003), this is only
298 a partial fulfillment of the predictions of the model of Masters et al. (1993) regarding interactions
299 between above- and below-ground herbivores, and contradictory examples are known. Milbrath
300 and Nechols (2004a) and Van Hezewijk and Bouchier (2012) demonstrated that when crown-
301 and root-feeding weevils altered plant structure and floral productivity, the behavior and
302 resources available to spatially- and temporally-separated seed feeding weevils were negatively
303 impacted. Finally, an evaluation of the impact of a leaf rust pathogen on several later-arriving
304 weevil and tephritid fly species, which attack flower heads of thistles, resulted in reduced feeding
305 in all species (Swope and Parker, 2010; Swope and Stein, 2012).

306 Besides known examples of P-MIs from the weed literature, a review of plant-mediated
307 herbivore responses by Denno and Kaplan (2007) also suggested that certain P-MI responses of
308 herbivores that feed and live in particular ways may be predictable. For example, phloem feeders
309 commonly alter the flow of nutrients by creating localized sinks at feeding sites, and other
310 phloem feeders located distally to the inducing species or at other plant locations may suffer
311 from reduced nutrients (Inbar et al., 1995; Larson and Whitham, 1991). We suggest that knowing
312 how early-colonizing herbivores impact plant structure, chemistry, and growth as well as where
313 and how they feed may lead to predictions about the type of plant-mediated responses that might
314 occur, which can then be tested experimentally.

315

316 *3.4. General relevance to weed biological control*

317 The study of P-MIs could improve decision-making regarding single versus multiple agent
318 introductions, or which agents to consider, especially in cases where a competitive or even
319 neutral interaction may occur without the benefit of additional impact on the target weed
320 (Hatcher and Paul, 2001; Swope and Parker, 2010; Swope and Stein, 2012). Issues of agent
321 complementarity, which may involve plant-mediated indirect effects among agents, are
322 acknowledged in programs in New Zealand (see Barratt et al., 2010). Alternatively, as pointed
323 out by Swope and Parker (2010), it could be beneficial to identify facilitative or synergistic
324 interactions in order to prioritize such combinations of agents for release. However, to do so will
325 require experimental evaluation of single and multiple agents for indirect interactions and weed
326 impact. As already noted, there are few studies of P-MIs for weeds that have experimentally
327 evaluated the impact of each agent, and the two agents together, for the target weed. Likewise,
328 the issue of what threshold densities are required to trigger a plant-mediated response has been
329 tested only rarely (e.g., Hunt-Joshi et al., 2004; Milbrath and Nechols, 2004a). Both prospective
330 and retrospective studies are needed to assess general patterns of P-MIs in weed biological
331 control. Interactions of exotic or indigenous species with previously-released agents or resident
332 species are also amenable to this approach, and therefore can be used in classical, augmentative
333 (including bioherbicides), or conservation biological control programs, including hybrid versions
334 of these approaches.

335

336 *3.5. Relationship to efficacy filter-first approaches*

337 Weed biological control workers have advocated for the assessment of a candidate agent's
338 efficacy before proceeding to host range tests in order to minimize the release of ineffective
339 agents (e.g., Balciunas and Coombs, 2004; McClay and Balciunas, 2005; Raghu et al., 2006).

340 One common method to investigate efficacy involves impact studies on individual plants using
341 live insects or simulated herbivory. Our suggested protocol (outlined below) is an expansion of
342 an impact study approach. A second method involves demographic modeling of target weed
343 populations in order to identify guilds of potentially effective agents. In particular, matrix
344 population models are used to identify plant life stage transitions that have the greatest impact on
345 population growth rates of the target weed and should be targeted for disruption (Caswell, 2001).
346 Researchers typically incorporate known impacts of candidate agents in the models to assess
347 their potential effects on plant population growth (e.g., Davis et al., 2006; Raghu et al., 2006). If
348 multiple agents are being modeled simultaneously, their impacts may not be additive, i.e.,
349 independent, in the case of plant-mediated competition or facilitation. Our expanded impact
350 approach can provide more precise information for the perturbation analyses (although impact
351 studies on all relevant plant life stages will be needed).

352

353 **4. Research needs and recommendations**

354 Several questions need to be explicitly addressed in experimental evaluations of P-MIs to
355 generate fundamental information on the frequency and type of P-MIs between biological control
356 agents and the outcome for weed control. Among them are: 1) Do P-MIs occur among specific
357 agents, and if so, are they positive or negative? 2) How strong is the effect? 3) What is the
358 impact, and hence potential contribution to control, of each agent individually on the target
359 weed, and does the level of impact change with a combination of agents? 4) If only one agent
360 elicits a plant-mediated effect and it is negative, does it compensate for any reduced impact by
361 the other agent? 5) If more than one agent is affected simultaneously, are there off-setting
362 positive and negative effects? 6) Are plant responses triggered at critical agent densities?

363 In addition, it will be important to know how climatic and other environmental conditions
364 affect herbivore populations (and therefore the relative abundances of interacting species),
365 resulting plant-mediated responses, and ultimately the level of biological control achieved. One
366 area of research that has received little attention involves plant-mediated effects across growing
367 seasons (Denno and Kaplan, 2007). Given the number of perennial weed targets, this is a crucial
368 gap in our knowledge. It is also of value for short-term studies, especially if conducted in a
369 quarantine-laboratory or greenhouse, to be validated post-release given concerns of how
370 accurately such studies translate to long-term field populations of the agents and the weeds
371 (Hunt-Joshi and Blossey, 2005; Rayamajhi et al., 2010).

372 We conclude that investigating P-MIs will lead to better decisions about which agents to
373 release, at least for some weed biological control programs. In that regard, the recommendations
374 for evaluation and release which follow should provide a useful framework for investigators who
375 wish to incorporate plant-mediated effects into candidate evaluation protocols. In putting forth
376 these protocols, we acknowledge that they do not cover the full scope of possible interactions.
377 For example, the scenarios we developed are for only two candidate agents because including
378 more would make the experimental evaluation much more complex (e.g., see study by Kluth et
379 al. (2001) involving multiple weed-feeding herbivores that exhibited numerous effects ranging
380 from positive to negative to neutral, at least some of which may have been plant-mediated). In
381 addition, our examples cover plant-mediated effects that occur within plant growing seasons, but
382 not all cases of indirect effects that might occur between growing seasons. Specifically, we
383 include one-way interactions whereby an agent feeding in one growing season induces either
384 delayed or persistent plant changes that impact a second agent in a subsequent growing season.
385 Two-way interactions between temporally-separated agents utilizing a perennial host that occur

386 between growing seasons (e.g., Strauss, 1991) are not considered in this paper because of the
387 complexity of developing evaluation protocols. However, carefully-designed long-term studies
388 accompanied by single- vs. dual-agent experimental evaluations could help document whether
389 such interactions impact weed suppression positively or negatively.

390 We do not minimize the challenges of experimental evaluations in the field which, although
391 more realistic, can be subject to uncontrolled variables versus logistical issues regarding the
392 ecological realism of quarantine-based studies and hence the post-release predictability of results
393 (Lonsdale et al., 2001). Ultimately, practitioners should document the outcomes of programs
394 utilizing release decisions that were derived from our recommended protocols, in order to
395 validate the usefulness (predictability) of this approach.

396

397 *4.1. P-MI experimental protocols*

398 The following protocols expand upon the design of plant impact studies widely used in
399 biological control of weeds programs and apply to cases where two agents are separated in space
400 and/or time. Numbered items represent a logical sequence.

401 1. Evaluate impact of candidate species on target weed, and on each other, experimentally.
402 Design should evaluate candidate agents singly and in combination along with controls (no
403 agents). Studies could be done concurrently with host specificity testing if desired, but prior to
404 agent release. Field tests in the native range are preferred (e.g., common garden approach of
405 Gerber et al., 2007), but logistical problems may require that evaluations be done under
406 quarantine conditions.

407 2. Incorporate a range of densities for both agents in the design, including zero (agents not
408 present), as it is difficult to predict field densities that will result following a release (Gassmann,

409 1996; Zalucki and van Klinken, 2006) or those that are needed to elicit a plant-mediated indirect
410 response (Milbrath and Nechols, 2004a). This might require the use of cages, such as in open
411 field experiments in the native range, to ensure high densities are maintained. We expect that, as
412 agent densities increase or decrease, the relative impact of an individual agent, as well as the
413 indirect interaction between agents (and hence the combined impact of the two agents), will
414 change. Threshold effects are likely.

415 3. Use introduced plant genotypes in the experiment, due to known or potential differences
416 between introduced and native populations in genetics (e.g., Treier et al., 2009), defensive
417 chemistry (e.g., Wheeler et al., 2003) and/or tolerance (e.g., Wang et al., 2011). If multiple
418 genotypes exist in the invaded range, consider including this as an experimental variable (e.g.,
419 Campanella et al., 2009).

420 4. Other factors that can alter P-MIs, such as plant size, plant competition, nutrient status,
421 mycorrhizal fungi (Ohgushi et al., 2007), and soil type (Swope and Stein, 2012), should be
422 considered but are at the researchers' discretion and will not be discussed further.

423 5. When conducting experiments, release agents at phenologically appropriate stages of plant
424 development (e.g., Smith and Mayer, 2005 compared to Crowe and Bouchier, 2006).

425 6. If possible, allow agents to make choices, especially the adult stage. Not only does this
426 reflect a realistic scenario in many field situations where some but not all plants have been
427 exposed to an agent, it may also reveal plant-mediated effects on herbivore behavior. For
428 example, in our field experiment with musk thistle, *R. conicus* females could choose between *T.*
429 *horridus*-infested and non-infested thistles. The experimental outcome of reduced recruitment of
430 new *R. conicus* adults was heavily influenced by the initial choice of oviposition sites of female
431 *R. conicus*. Choice experiments with different levels of herbivory, including none, could be done

432 in a pair-wise design or a multiple-choice design (e.g., Simelane, 2006). In addition, no-choice
433 tests could be conducted to determine if plant-mediated effects result in outright rejection
434 (dispersal). However, experiments would need to be carefully designed to avoid restrictions on
435 dispersal resulting from cages. For example, it is possible that the results of Gerber et al. (2007),
436 which involved individual caged plants, would have differed if *Ceutorhynchus alliariae* Brisout
437 had been allowed a choice to leave plants previously infested by *C. scrobicollis* Nerensheimer &
438 Wagner.

439 7. For temporally isolated agents, assess if recruitment of Agent 2 is altered (positively,
440 negatively, or not) based on prior presence of Agent 1. If the agents are separated in space only,
441 then assess if recruitment of each agent is altered by the simultaneous presence of the other
442 species. If multivoltine agents are being tested, recruitment can be assessed at each generation.
443 Recruitment can refer to colonization, feeding and oviposition by adults, as well as larval
444 development and survival, disease incidence, etc. All life stages should be assessed because
445 indirect effects may target different stages of an herbivore's life cycle (Swope and Parker, 2010),
446 including affecting a non-damaging adult stage that in turn could alter the density of a damaging
447 larval stage (Briese, 1991; Milbrath and Nechols, 2004a).

448 8. Quantify plant impact and rate as 'greater', 'same', or 'less' when comparing one vs. two
449 agents (see Tables 3 and 4). Several plant parameters may be measured and assessed separately
450 (e.g., Buccellato et al., 2012). The researcher will need to rank the importance of different
451 parameters for control. With respect to the categories of Hatcher (1995) as modified by Turner et
452 al. (2010), greater = synergistic and additive outcomes, same = equivalent outcome of the highest
453 impacting agent acting alone, and less = all other outcomes including equivalent to the lowest
454 impacting agent when acting alone or inhibitory.

455

456 4.2. Release decisions (Tables 3 and 4)

457 Once experimental data on plant impact and indirect agent interactions are obtained for the
458 various treatment combinations, a sequential action model such as what we propose in Tables 3
459 and 4 could be used to guide release decisions for the two agents. Table 3 was developed for
460 temporally-separated agents (which may or may not be separated in space) and Table 4 is for
461 agents separated in space only. Literature examples from section 3.1 (Table 1) are also noted in
462 Tables 3 and 4 to help illustrate where such studies may fit in our scheme.

463 We illustrate how release decisions could be made using the musk thistle study. As discussed
464 under section 3.2, because the musk thistle system involves plant-mediated effects that are one-
465 way (*T. horridus* negatively impacts *R. conicus* but not the reverse) and because the two agents
466 are both temporally- and spatially-separated, we refer to Table 3.

467 At low densities, *T. horridus* (Agent 1) had no measurable impact on musk thistle, and no P-
468 MI between *T. horridus* and *R. conicus* (Agent 2) was discernible. Thus, the only impact on
469 musk thistle was from *R. conicus*. In cases like this, if no plant impact was observed for any
470 tested density of Agent 1 (Table 3, last scenario), we would either discontinue working with
471 Agent 1 or consider running tests at higher densities. The value of testing agents over a range of
472 densities is seen in the very different experimental results we obtained at higher *T. horridus*
473 densities. Here, the relative impact of *T. horridus* alone was less than that of *R. conicus* alone,
474 and a negative P-MI was evident; but despite that, the combined impact of the two agents was
475 greater than that of *R. conicus* alone (Table 3, second to last scenario). Based on these results,
476 either both species or Agent 2 alone could be recommended for release, although additional
477 study might be recommended in light of the competitive interaction observed. This is the only

478 case in Table 3 where additional assessment may be warranted by including a greater range of
479 Agent 1 densities in a follow-up study. Specifically, in this case Agent 1 (*T. horridus*) would be
480 characterized as a competitively superior species but an inferior agent (similar reasoning applies
481 for two of the recommendations in Table 4). Therefore, higher densities of *T. horridus* (Agent 1)
482 could further interfere with *R. conicus*' (Agent 2) ability to utilize musk thistle, although it is
483 presently unknown if this would lead to an overall increase or decrease in plant impact,
484 depending on gains and losses in impact from *T. horridus* and *R. conicus*, respectively.

485 To our knowledge no studies have been published comparing the individual and combined
486 impacts of the two weed biological control agents, as well as documenting plant-mediated
487 competition, under these conditions. However, Woodburn (1997) reported that under very high
488 *T. horridus* densities in Australia, twice the levels we observed in Kansas, flowerhead production
489 was delayed by two weeks instead of the one week we observed in Kansas. This result, if
490 transferrable to our situation in Kansas, potentially could exacerbate the negative indirect
491 competitive effect of *T. horridus* on *R. conicus*. Interestingly though, in Woodburn's study the
492 relative impact of *T. horridus* alone was greater than the previously reported impact of *R.*
493 *conicus* alone (72% versus 40% seed reduction, respectively). Thus, experimental evaluations
494 must include a wide range of densities in order to reveal potential interference between agents,
495 and net impacts on weed biological control.

496

497 **5. Conclusion**

498 Plant-mediated indirect interactions undoubtedly occur commonly among organisms that
499 attack weeds. However, very little is known about how these interactions change, and what the
500 consequences are for weed biological control, when new assemblages of agents are created via

501 classical biological control efforts. Part of the problem is that, apart from host range tests and
502 basic biological studies of candidate agents, relatively few additional pre-release experimental
503 evaluations are done. Thus, the release of a new agent may or may not result in a net gain in the
504 impact on a weed population. As we have discussed, plant-mediated effects that reduce the
505 efficacy of a newly-introduced or existing agent can be compensated for by the agent that
506 induces a negative indirect interaction. However, only by conducting experimental evaluations
507 that take into consideration impacts of agents on the weed and on each other, will it be possible
508 to understand the complex interactions involving target weeds and herbivorous candidate agents.
509 If these more comprehensive evaluations are done, better predictions about agent selection for
510 specific programs should result, and may also reveal general patterns that can be extrapolated to
511 future programs.

512

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Table 1. Examples of plant-mediated interactions in weed biological control systems (with or without plant impact studies) for agents separated in space and/or time.

Target weed	Herbivore/pathogen	Plant-mediated interaction	Combined agent impact to plant compared to individual impact	Reference (type of study)
Separated in space (two-way interaction)				
Pathogen-Arthropod				
Crofton weed	leaf-spot fungus	<u>Fly on fungus</u>	Equal to fly alone (stem height)	Buccellato et al., 2012
<i>Ageratina adenophora</i> (Sprengel) King & Robinson (Asteraceae)	<i>Passalora ageratinae</i> Crous & A.R. Wood (Mycosphaerellales: Mycosphaerellaceae)	Negative effect (reduced % infected leaves on double-galled stems)	Equal to fungus alone (side shoot production) Additive (% live leaves)	(greenhouse, retrospective)
	stem gall fly	<u>Fungus on fly</u>	Complementary overall	
	<i>Procecidochares utilis</i> Stone (Diptera: Tephritidae)	No effect (gall size, adult fly emergence)		
Rush skeletonweed	leaf rust fungus	<u>Fungus on mite</u>	Equal to fungus alone (shoot biomass, rosette diameter, rate of senescence)	Campanella et al., 2009
<i>Chondrilla juncea</i> L. (Asteraceae)	<i>Puccinia chondrillina</i> Bubak and Sydow (Uredinales: Pucciniaceae)	Negative effect (reduced shoot growth and gall biomass), Slight positive effect (possible increased susceptibility to	Equal to mite alone (fecundity)	(common garden, retrospective)
	leaf gall mite	increased susceptibility to	Additive (shoot length)	

Eriophyes chondrillae (Canestrini) galling in one year
 (Acari: Eriophyidae) Complementary overall

Mite on fungus **not studied**

Fungus applied first but eventual
 temporal overlap; results for rust-
 susceptible plants

Insect-Insect

Lantana	leaf-sucking lace bug	<u>Lace bug on flea beetle</u>	Not assessed	Simelane, 2006
<i>Lantana camara</i> L. (Verbenaceae)	<i>Teleonemia scrupulosa</i> Stål (Heteroptera: Tingidae)	No effect (adult beetle feeding and oviposition, preimaginal development, adult size),		(greenhouse, retrospective)
	root-feeding flea beetle <i>Longitarsus bethae</i> Savini & Escalona (Coleoptera: Chrysomelidae)	Negative effect (reduced larval survival), Also, direct (?) negative effect of nymphs on adult beetle colonization and oviposition		

Beetle on lace bug **not studied**

Purple loosestrife	leaf-feeding beetle	<u>Leaf beetle on weevil</u>	Equal to leaf beetle alone (shoot	Hunt-Joshi and
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<i>Lythrum salicaria</i> L. (Lythraceae)	<i>Galerucella calmariensis</i> L. (Coleoptera: Chrysomelidae)	No effect (weevil oviposition, larval mass, % plants infested, # larvae),	growth and height, flowering, most plant biomass)	Blossey, 2005 (outdoor pot and multi-year field cage, retrospective)
	rootstock -boring weevil	Negative effect (reduced larval survival with reduced resources [pot only])	Equal to weevil alone (biomass of dead stems)	
	<i>Hylobius transversovittatus</i> Goeze (Coleoptera: Curculionidae)		Complementary? (perhaps if densities of weevil are higher)	Hunt-Joshi et al., 2004 (multi-year field cage, retrospective)
<u>Weevil on leaf beetle</u>				
		No effect (larval mass or survival, insect abundance, oviposition; but very low densities of weevil)		
Broad-leaved paperbark <i>Melaleuca quinquenervia</i> (Cav.) S.T. Blake (Myrtaceae)	Early-stage vegetative tips puff-ball gall <i>Sphaerococcus ferrugineus</i> Froggatt (Homoptera: Eriococcidae)	<u>Early galls on late galler</u> No relationship (resource availability and previous abundance of early galls),	Not assessed	Raghu et al., 2012 (native range field surveys, prospective)
	bud gall	Positive relationship (late galler abundance and previous abundance of puff-ball galler		

Fergusonina turneri Taylor (Diptera: Fergusoninidae) only)

Late galler on early galler

Late-stage vegetative tips

blister gall

Lophodiplosis indentata Gagne' (Diptera: Cecidomyiidae)

Negative relationship (resource availability and previous abundance of late galler),

Positive relationship (only puff-ball galler abundance and previous abundance of late galler)

Dalmation toadflax	root-boring moth	<u>Moth on weevil</u>	Not fully reported but stem-	Saner et al., 1994
<i>Linaria dalmatica</i> (L.) Mill. (Scrophulariaceae)	<i>Eteobalea intermediella</i> Riedl (Lepidoptera: Cosmopterigidae)	No effect (weevil oviposition, preimaginal survival; but only one of two generations of moth included in this study)	mining weevil reduced stem biomass	(native range, greenhouse, retrospective)
	stem-mining weevil			
	<i>Mecinus janthinus</i> Germar (Coleoptera: Curculionidae)	<u>Weevil on moth</u>		
		No effect (larval survival)		

Separated in time but not space (one-way interaction)

No examples found

Separated in space and time (one-way interaction of 1st agent on 2nd agent)

Pathogen-Arthropod

Yellow starthistle	1 st to colonize: leaf rust fungus	No effect (number of seed heads attacked by insects)	Not assessed	O'Brien et al., 2010 (field, retrospective)
<i>Centaurea solstitialis</i> L. (Asteraceae)	<i>Puccinia jaceae</i> Otth var. <i>solstitialis</i> (Uredinales: Pucciniaceae)			
	2 nd to colonize: seed-head insects			
	<i>Chaetorellia succinea</i> (Costa) (Diptera: Tephritidae)			
	<i>Eustenopus villosus</i> (Boheman) (Coleoptera: Curculionidae)			
	1 st : leaf rust fungus	No effect (adult feeding or oviposition/plant, larval survival),	Equal to seed-head weevil alone (seed production, partial assessment only)	Swope and Parker, 2010 (field, retrospective)
	<i>Puccinia jaceae</i>			
	2 nd : seed-head weevil	Negative/positive effect (lesser proportion of seeds consumed)		
	<i>Eustenopus villosus</i>			

		by larvae)		
	1 st : leaf rust fungus	<u>Non-serpentine soils</u>	<u>Non-serpentine soils</u>	Swope and Stein, 2012
	<i>Puccinia jaceae</i>	No effect (larval survival),	Equal to seed-head insects alone	(field, retrospective)
		Negative/positive effect (lesser	(seed production, partial	
	2 nd : seed-head insects	proportion of seeds consumed	assessment only)	
	<i>Chaetorellia australis</i> Hering	by larvae)		
	(Diptera: Tephritidae)		<u>Serpentine soils</u>	
	<i>Chaetorellia succinea</i>	<u>Serpentine soils</u>	Greater (seed production, partial	
	<i>Eustenopus villosus</i>	Positive effect (larval survival),	assessment only)	
	<i>Urophora sirunaseva</i> (Hering)	Negative/positive effect (lesser		
	(Diptera: Tephritidae)	proportion of seeds consumed		
		by larvae)		
Insect-Insect				
Garlic mustard	1 st : root-crown weevil	<u>Stem level</u>	Equal to root-crown weevil	Gerber et al., 2007
<i>Alliaria petiolata</i> (M.	<i>Ceutorhynchus scrobicollis</i>	Negative (reduced attack rate	alone (plant survival, biomass,	(native range,
Bieb.) Cavara &	Nerensheimer & Wagner	due to reduced stem height and	seed production)	common garden,
Grande (Brassicaceae)	(Coleoptera: Curculionidae)	diameter)	Additive (stem height)	prospective)
	2 nd : shoot-mining weevil	<u>Plant level (increased stem #</u>		
	<i>Ceutorhynchus alliariae</i> Brisout	<u>canceled out reduced stem</u>		

	(Coleoptera: Curculionidae)	<u>height</u>		
		No effect (attack rate, larval survival despite increased N-content, new adult recruitment)		
Musk thistle <i>Carduus nutans</i> L. (Asteraceae)	1 st : rosette (crown) weevil <i>Trichosiromus horridus</i> (Panzer) (Coleoptera: Curculionidae)	Neutral to negative effect (density-dependent, see Table 2)	Equal to seed-head weevil alone to greater (seed production) (see Table 2)	Milbrath and Nechols, 2004a (greenhouse and common garden, retrospective)
	2 nd : seed-head weevil <i>Rhinocyllus conicus</i> Froelich (Coleoptera: Curculionidae)			Milbrath and Nechols, 2004b (common garden, retrospective)
Diffuse knapweed <i>Centaurea diffusa</i> Lamarck (Asteraceae)	1 st : root-boring weevil <i>Cyphocleonus achates</i> (Fahr.) (Coleoptera: Curculionidae)	Negative effect (proportion of heads attacked)	Not assessed	Van Hezewijk and Bourchier, 2012 (field, retrospective)
	2 nd : seed-head weevil <i>Larinus minutus</i> Gyll. (Coleoptera:			

Curculionidae)

Table 2. Plant-mediated interaction of *Trichosirocallus horridus* on *Rhinocyllus conicus*, two biological control agents of musk thistle (from Milbrath and Nechols, 2004a,b).

Mechanism of plant-mediated interaction	Outcome of interaction due to prior infestation by <i>T. horridus</i>	
	Low <i>T. horridus</i> larval density	High <i>T. horridus</i> larval density
<u>Phenology</u> : synchrony between <i>R. conicus</i> adults and flower heads altered?	No	Yes, 1 week delay
<u>Behavior</u> : host plant selection (adult presence, oviposition) by adult <i>R. conicus</i> altered?	No	Yes, adults/plant reduced 57% and eggs/plant reduced 64%
<u>Resource limitation</u> : change in quantity of food for <i>R. conicus</i> larvae?	No	Yes, 34% less receptacle dry mass/plant
<u>Physiology</u> : change in plant quality for <i>R. conicus</i> larvae?	Not tested	Yes, but only observed when <i>R. conicus</i> larval densities low
<u>Combined effect</u> on recruitment of new <i>R. conicus</i> adults	No effect	63% reduction
	Percentage reduction in musk thistle seed production (compared to control)	
<i>T. horridus</i> alone	0%	Not tested but less than effect of <i>R. conicus</i>

<i>R. conicus</i> alone	45%	Assumed ~45%
Combined	45%	Estimated 59%

Table 3. Recommendations for agent release based on different outcomes of plant-mediated impact studies for agents involved in negative (competitive), neutral, or positive (facilitative) one-way interactions, where the agents are separated in time, or in space and time. Agent 1 attacks plant before agent 2 within the life cycle of the plant (annuals and biennials) or within a growing season (perennials); between-season effects involving perennial plants are not considered.

Experimental outcome				
Agent 1 impact on plant relative to Agent 2	IF plant-mediated interaction ^a	AND combined agent impact to plant compared to highest individual impact	THEN recommendation for agent release is ^b	Literature example
Agent 1 greater than agent 2 (and agent 2 not zero)	+ or 0 or -	Greater (than Agent 1 alone)	Both species ^c	Neutral: Gerber et al., 2007
	-	Same as Agent 1	Agent 1	
		Less	n/a	
Agent 1 has impact; agent 2 has NO impact	+ or 0 or -	Same as Agent 1	Agent 1	

Agent 1 and 2 same	+ or 0 or -	Greater	Both ^c	
	-	Same as Agent 1 or 2	Agent 1 or Agent 2 ^c	
		Less	n/a	
Agent 2 greater than agent 1 (and agent 1 not zero)	+ or 0 or -	Greater (than Agent 2 alone)	Positive or Neutral	
			Both ^c	
			Negative	Milbrath and Nechols, 2004a,
			Both or Agent 2? ^d	b
	-	Same as Agent 2	Agent 2	
	-	Less	Agent 2	
Agent 2 has impact; agent 1 has NO impact	+ or 0 or -	Same as Agent 2 or Less	Agent 2	Milbrath and Nechols, 2004a, b

^a + = positive interaction, 0 = neutral interaction, - = negative interaction

^b Assuming appropriate host-specificity. If no specific plant-mediated interaction noted (positive, neutral, negative), then recommendation applies to all scenarios. n/a = specific combined impact outcome not possible by definition of a one-way interaction.

^c For recommendations of Both, may choose to import only Agent 1 at first. For recommendations of Both or a choice between agents, may modify choice using plant demography model-derived recommendations.

^d Further risk assessment needed.

Table 4. Recommendations for agent release based on different outcomes of plant-mediated impact studies for agents involved in negative (competitive), neutral, or positive (facilitative) two-way interactions. Agents attack plant simultaneously within a growing season but are spatially separated; between-season effects are not considered.

Experimental outcome					
Agent 1 impact on plant relative to agent 2	IF plant-mediated interaction ^a		AND combined agent impact to plant compared to highest individual impact	THEN recommend-ation for agent release is ^b	Literature example
	Agent 1 on 2	Agent 2 on 1			
Agent 1 greater than agent 2 (and agent 2 not zero)	+ or 0 or -	+ or 0 or -	Greater (than Agent 1 alone)	Agent 2 on 1= negative	
				Both or Agent 1? ^d	
				All other cases	
	+ or 0 or -	-	Same as Agent 1 or Less	Agent 1	
	-	+ or 0	Same as Agent 1 (Less n/a)	Agent 1	Buccellatto et al., 2012
Agent 1 has impact; agent 2 has no impact	+ or 0 or -	+ or 0 or -	Same as Agent 1 or Less	Agent 1	

2 has NO impact

Agent 1 and 2 same	+ or 0 or -	+ or 0 or -	Greater	Both ^c	Buccellatto et al., 2012
	-	-	Same as Agent 1 or 2, or Less	Agent 1 or Agent 2 ^c	
	-	+ or 0	Same as Agent 1 or 2 (Less n/a)	Agent 1 or Agent 2 ^c	
	+ or 0	-	Same as Agent 1 or 2 (Less n/a)	Agent 1 or Agent 2 ^c	
Agent 2 greater than agent 1 (and agent 1 not zero)	+ or 0 or -	+ or 0 or -	Greater (than Agent 2 alone)	Agent 1 on 2= negative Both or Agent 2? ^d All other cases Both species ^c	Buccellatto et al., 2012
	-	+ or 0 or -	Same as Agent 2 or Less	Agent 2	
	+ or 0	-	Same as Agent 2 (Less n/a)	Agent 2	

Agent 2 has impact; + or 0 or - + or 0 or - Same as Agent 2 or Less Agent 2
agent 1 has NO impact

^a + = positive interaction, 0 = neutral interaction, - = negative interaction

^b Assuming appropriate host-specificity. If no specific plant-mediated interaction noted (positive, neutral, negative), then recommendation applies to all scenarios. n/a = specific combined impact outcome not considered possible.

^c For recommendations of Both, may choose to import only one agent at first. For recommendations of Both or a choice between agents, may modify choice using plant demography model-derived recommendations.

^d Further risk assessment needed.