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## Allelopathic effects of *Ambrosia artemisiifolia* L. in the invasive process

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(Article begins on next page)



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1 Short title: Allelopathy of *Ambrosia artemisiifolia*

2 **Potential allelopathic effects of Common ragweed (*Ambrosia***  
3 ***artemisiifolia* L.)**

4 Francesco Vidotto, Franco Tesio and Aldo Ferrero

5  
6 **Abstract**

7 *Ambrosia artemisiifolia* (common ragweed), an annual native to North America, is now  
8 present in many European countries where it causes summer hayfever and disturbs  
9 several important crops. We investigated if common ragweed invasiveness could be  
10 explained by its leaf tissue and root exudate allelopathic potential on indicator crops  
11 (alfalfa, barley, corn, lettuce, tomato, and wheat), weeds (barnyardgrass, black  
12 nightshade, common purslane, large crabgrass), and common ragweed itself. Different  
13 residue substrates were prepared for soil incorporation and trials were conducted under  
14 both laboratory (1, 2, and 3 g residues /Parker dish) and greenhouse conditions (1.28 g  
15 residues / pot). The effect of the preparations on the germination and growth of the  
16 indicator crops and weeds were evaluated relative to soil previously used to cultivate  
17 common ragweed.

18 Results showed tomato was the most sensitive indicator crop species as growth was  
19 reduced by more than 50% in both laboratory and greenhouse experiments. Lettuce crop  
20 root and shoot growth were also inhibited, but only when common ragweed residues, and  
21 not root exudates, were added to the substrate. Among the weeds, *E. crus-galli* was not  
22 affected by common ragweed while *D. sanguinalis* suffered a large germination reduction  
23 (90%) after incorporation of 3 g of residues.

24

25 **Nomenclature:** alfalfa, *Medicago sativa* L.; barley, *Hordeum vulgare* L.; barnyardgrass,  
26 *Echinochloa crus-galli* (L.) Beauv. ECHCG; black nightshade, *Solanum nigrum* L. SOLNI;  
27 common purslane, *Portulaca oleracea* POROL; corn, *Zea mays* L.; Common ragweed,  
28 *Ambrosia artemisiifolia* L.; large crabgrass, *Digitaria sanguinalis* (L.) Scop. DIGSA; lettuce,  
29 *Lactuca sativa* L.; pea, *Pisum sativum* L.; redroot pigweed,; tomato, *Lycopersicon*  
30 *esculentum* Mill.; wheat, *Triticum aestivum* L.;

31

32 **Key words:** Phitotoxicity; residue degradation; crop rotation, plant invasion, root exudates.

33

## 34 **Introduction**

35 Common ragweed (*Ambrosia artemisiifolia* L.) is a herbaceous, annual plant that is native  
36 to North America and a pioneer dominant in abandoned croplands in several areas of the  
37 United States. This species is often present as a weed in field crops such as maize,  
38 soybean, and wheat (Bassett and Crompton 1979; Fumanal et al. 2008) and may grow  
39 exceptionally dense in fields plowed during the spring and subsequently abandoned  
40 (Raynal and Bazzaz 1975). It is also a common weed found along roadsides, in urban  
41 empty lots, and in other disturbed habitats (Lavoie et al. 2007). First reported in botanical  
42 gardens in Europe during the latter half of the 1800s, common ragweed spread to several  
43 European countries by the beginning of the 1900s (Chauvel et al. 2006; Vogl et al. 2008).  
44 Many current objections to *A. artemisiifolia* center on its powerful provocation of pollen  
45 allergies (Wayne et al. 2002). The plant flowers in the northern hemisphere from mid-  
46 August until cooler weather arrives during with each plant can produce a great number of  
47 pollen grains (Rogers et al. 2006). The high allergenic potential coupled with its broad  
48 spread has caused health organizations to name common ragweed one of the most  
49 problematic invasive plants (Wayne et al. 2002).

50 Even though *Ambrosia artemisiifolia* is non-indigenous to several European plant  
51 communities, little is known about the factors that characterize its invasive processes:  
52 (Rabitsch and Essl 2006) its abiotic-limiting factors, the potential activity of its competitors,  
53 and its natural enemies. One such theory, the “enemy release hypothesis” (Keane and  
54 Crawley 2002), postulates that invasiveness can result from a loss of natural enemies  
55 during the invasion process while another, known as the “novel weapon allelopathy” theory  
56 (Callaway and Ashehoug 2000), suggests that plant community evolution is speeded when  
57 subjected to allelo-chemicals produced by species that have not co-evolved (Warwick  
58 1991). Fortunately, the allelopathic potential, defined as the suppressive activity of one  
59 plant species on its neighbor plant(s) by toxic compound release, of the Asteraceae family  
60 has been widely studied. In particular, the sensitivity of cereals seeded after crop species  
61 belonging to this family (common sunflower) has largely been documented (Leather 1983).  
62 Agricultural management programs that incorporate allelopathic plant residues through  
63 rotational or cover crops is reported to have a positive environmental effect and is often  
64 practiced by producers interested in reducing chemical usage (Tesio and Ferrero 2010).  
65 A potential negative consequence of allelopathy is the production of toxic compounds by  
66 non-native invasive species that unfavorably affect native communities (Vivanco et al.  
67 2004). Noxious weeds may have an effect on other species through competition or the  
68 release of growth inhibitors. In this situation, native plants are unable to tolerate  
69 compounds released by a non-native plant that has not co-evolved in the same  
70 environment (Hua et al. 2005). This process of environmental modification causes  
71 continual change to the relationship and composition among the species in an area until a  
72 new equilibrium and a stable community is established.

73 Common ragweed is present throughout the northern part of Italy, where it is considered  
74 an annual summer crop weed after existing for decades in urban or disturbed areas and at  
75 field edges (Patracchini et al. 2011). Recently, this species has been reported to occur

76 after winter cereal field harvest, even if common ragweed seedlings have already emerged  
77 into the cereal crop. After crop harvest, when light conditions and resource availability are  
78 favorable for growth, plants are able to effect large amount of canopy, flower, and produce  
79 seeds (Patracchini et al. 2011).

80 This study was designed to evaluate the potential phytotoxic effects of common ragweed  
81 upon succeeding crops which are common in Italian field rotations. Experiments were  
82 performed in both laboratory and greenhouse studies conducted in Italy. Using controlled  
83 laboratory and greenhouse conditions, we assessed the impact of *A. artemisiifolia* upon  
84 the germination and growth behavior of several crop and weed species after incorporating  
85 common ragweed dried residues into the soil. We also attempted to simulate common field  
86 conditions by performing greenhouse studies in a controlled environment to elucidate the  
87 effects of root exudates upon plant growth.

88

## 89 **Materials and Methods**

### 90 **Plant material**

91 The study was conducted during 2007 – 2008 in the laboratory and greenhouse of the  
92 Dipartimento di Agronomia Selvicoltura e Gestione del Territorio at Grugliasco (Turin, Italy  
93 – 45°03'53"N 7°35'38"E). *Ambrosia artemisiifolia* shoots and seeds were harvested during  
94 2007 from a field heavily infested by the weed within the experimental site. Aboveground  
95 tissues were collected in June 2007 from healthy individuals by cutting plants 10 cm above  
96 the soil surface. The leaves were immediately separated from the stalks and dried in open  
97 trays in the laboratory oven at 60°C. The resulting dried material was stored in tightly  
98 closed plastic bags until needed in the experiment. Seeds were harvested by hand the  
99 during the subsequent September and dried in open trays in the laboratory at room  
100 temperature (22-25°C). Dried seeds were stored until needed in the refrigerator at +4°C.

101 The indicator crops included in the experiment were alfalfa (*Medicago sativa* L.), barley  
102 (*Ordeum vulgare* L.), lettuce (*Lactuca sativa* L.), maize (*Zea mays* L.), tomato  
103 (*Lycopersicon esculentum* Mill.), and winter wheat (*Triticum aestivum* L.). The weeds  
104 considered as indicator species in this study were common ragweed (*A. artemisiifolia* L.),  
105 large crabgrass (*Digitaria sanguinalis* (L.) Scop.), barnyardgrass (*Echinochloa crus-galli*  
106 (L.) Beauv.), common purslane (*Portulaca oleracea* L.) and black nightshade (*Solanum*  
107 *nigrum* L.). With the exception of *A. artemisiifolia*, all weed seeds were purchased from  
108 Herbiseed<sup>1</sup>.

109

## 110 **Laboratory Experiment**

### 111 *Experiment 1*

112 The potential to inhibit potential of dried common ragweed leaf tissue was studied by  
113 assessing its impact on indicator species seed germination and radical and hypocotyl  
114 elongation. The experiment was conducted using square plastic “Petri” dishes that  
115 contained soil and plant residue mixtures as well as indicator seedlings in a modified  
116 Parker bioassay (Weston 2005). Preparation of the experimental substrate began with  
117 collection of sandy-loam, textured alluvium soil (Typic Udifluvents) from the Tetto Frati  
118 research station located in Carmagnola, Italy. Next, the soil was air dried, sifted, and  
119 combined with fine silica sand (1:1 v/v) to allow increased water permeability during  
120 bioassay preparation. Initially, 100 g of the soil mixture was placed in 100 x 100 x 15 mm  
121 Parker dishes. The soil was then topped with varying amounts of chopped common  
122 ragweed plant residues (1, 2, or 3 g), after which an additional 50 g of the soil mixture was  
123 layered over the residues. Dishes were moistened with 35 ml of deionized water and a  
124 square piece of filter paper<sup>3</sup> was placed on the soil surface of each dish. The control

125 treatment consisted of an inert, prewashed paper towel (1.0 g) cut into about 1.5 mm<sup>2</sup>  
126 pieces combined to the soil mixture.

127 Common ragweed, large crabgrass, barnyardgrass, common purslane, and black  
128 nightshade were all used as weed indicator species while alfalfa (cv. Prosementi), lettuce  
129 (cv. Meraviglia d'inverno), tomato (cv. San marzano) and winter wheat (cv. Isengrain) were  
130 used as crop indicator species. All weed and crop species were exposed to all treatments.  
131 Ten seeds of each indicator species were placed uniformly in two separate but parallel  
132 rows on the filter paper surface. The dishes were taped shut to maintain moisture and  
133 encourage seed residue contact and were stacked and stored at an ambient temperature  
134 of 26°C for 6 days in a germination box to promote downward root growth.

135 Total germination was assessed in two ways: a daily count of germinated seeds  
136 throughout the experiment and hypocotyl and radical length measurement after six days.  
137 The experiment was arranged as a completely randomized design with four replicates, and  
138 the study was replicated twice. While experimental results were analyzed separately for  
139 each species, results were combined over runs.

140

## 141 **Greenhouse Experiment**

### 142 *Experiment 2*

143 Greenhouse experiments were conducted from June through September 2008 in the  
144 department experimental greenhouse at temperatures varying between 15 and 25°C. Pots  
145 (8 x 8 cm, 8 cm height) were filled with the same soil used for Experiment 1 that was  
146 collected at experimental research station Tetto Frati located at Carmagnola, Italy. To  
147 each pot was added 1.28 g (equivalent to 2 t/ha) of powdered common ragweed dried  
148 tissue residue and then it was thoroughly mixed. The amount used is similar to that used in  
149 other allelopathic species experiments reported in the literature for Asteraceae (DongZhi

150 et al. 2004a; DongZhi et al. 2004b; Hong et al. 2004; Khanh et al. 2005; Tesio et al. 2010;  
151 Vidotto et al. 2008). The controls were pots filled with soil only.  
152 The impact of common ragweed residues was evaluated based on the germination and  
153 growth of several crops: alfalfa, lettuce, tomato, and winter wheat. The seeds were planted  
154 in pots devoted to a single indicator species at a rate of 9 seeds for alfalfa and winter  
155 wheat and 12 seeds for lettuce and tomato. Immediately after seeding, the pots were  
156 watered with a soluble fertilizer (NPK 21-10-20) solution. There after, the pots were  
157 watered daily with deionized water to maintain field capacity. The pots were supported by  
158 individual flower pot saucers beneath them to prevent contamination from the leaching of  
159 other treatments, and arranged on greenhouse benches in a completely randomized  
160 design, with four replicates. They were rotated weekly to minimize spatial variation. The  
161 experimental unit was the pot, and the experiment was repeated twice. Metal halide lamps  
162 supplemented natural light to produce a 14 h day length, which delivered about 55  $\mu\text{mol/s}$   
163  $\text{m}^2$ . Germination percentage, seedling height, and shoot dry weight were determined 20  
164 days after seeding.

### 165 *Experiment 3*

166 In early April 2009, pre-germinated seedlings of *A. artemisiifolia* were transplanted in  
167 plastic pots (30 cm height and 27 cm diameter) with a total capacity of about 12 L. Before  
168 transplanting, pots were filled with the same soil used in the previous greenhouse  
169 experiment. When a 3-leaf stge was reached, the common ragweed pots were thinned to  
170 obtain three healthy and uniform plants per pot. Pots were left in an open field in the area  
171 of the Dipartimento di Agronomia Selvicoltura e Gestione del Territorio – Grugliasco where  
172 they were irrigated twice each week by adding water to the post saucer and weeded  
173 weekly by hand. After five months, the common ragweed plants had reached an average  
174 fresh weight of 80 g plant<sup>-1</sup> (aboveground part) and were carefully removed from the pots  
175 with close attention paid to maintain intact roots. Pot soil was then mixed and used as

176 substrate for a greenhouse assay, prepared as described below. This experiment was  
177 conducted during September 2009 in the experimental glasshouse, under temperatures  
178 that varied between 18 and 28°C. Containers (8 x 8 cm, 8 cm height) were filled with soil  
179 from Experiment 3 pots used to grow *A. artemisifolia* during the summer. Control pots  
180 were filled with soil maintained under identical conditions as those that contained common  
181 ragweed, but where no plants were grown.

182 The potential impact of common ragweed root tissue and exudate was investigated by  
183 evaluating the germination and growth of several indicator crop and weed species. The  
184 indicator crop species were alfalfa, barley, corn, lettuce, tomato, and winter wheat while  
185 the weeds included large crabgrass and barnyardgrass. The number of seeds per pot  
186 varied between 4 (maize), 6 (alfalfa, barley, and winter wheat), 9 (barnyardgarss), and 12  
187 (large crabgrass, lettuce, and tomato). Only a single indicator species was seeded per pot.  
188 Seeded pots were maintained as indicated in Experiment 2. Pots were arranged on  
189 greenhouse benches in a completely randomized design with seven replicates, and  
190 rotated weekly to reduce spatial variation. The experimental unit was the pot and the  
191 experiment was repeated twice. Germination percentage and shoot dry weight were  
192 determined 30 days after seeding.

### 193 **Statistical Analysis**

194 Both laboratory and greenhouse experiments were analyzed. In the case of the laboratory  
195 experiement data, an analysis of variance (ANOVA) was performed separately by species  
196 using statistical software SPSS (version 16). In the few cases lacking variance  
197 homogeneity, a paired samples *t*-test was conducted to detect differences from the control  
198 treatment. After an ANOVA analysis, means were separated using the post-hoc Tukey – b  
199 test ( $p \geq 0.05$ ). In the greenhouse studies (Experiments 2 and 3), differences to the  
200 control were identified with the independent sample *t*-test (SPSS software, version 16).

201 **Results**

202 **Laboratory Experiment.**

203 *Experiment 1*

204 The effect of common ragweed residues on indicator species seed germination and first  
205 seedling growth varied according to indicator species. Results ranged from stimulation to  
206 inhibition, especially when the highest rates of dried residue were utilized.

207 Among the weed species, *D. sanguinalis* seed germination was reduced when common  
208 ragweed residues were added at the highest rate (3 g/plate); a 90% reduction in total  
209 germination (Table 1) resulted compared to the control. By contrast, the germination  
210 percentage of *S. nigrum* was more than 3 times that of the control when high levels (3  
211 g/plate) of residue was used. The root growth of *S. nigrum* germinated seedlings was  
212 reduced by 75% with even the lower amount of residues. Residue addition had neither a  
213 simulation nor inhibition effect on weed species shoot growth. The root growth of both *A.*  
214 *artemisiifolia* and *P. oleracea* were inhibited about 50% at the highest residue quantities.

215 Among the crops, tomato behaved similar to *S. nigrum* and showed a germination  
216 percentage increase compared to the control (Table 2), even when both shoot and root  
217 elongations were depressed by more than 70%. Lettuce suffered growth depression of  
218 more than 50% when the highest residue quantities were added to the plate.

219

220

221 Table 1. Effect of different dried leaf tissue concentrations of *A. artemisiifolia* on total  
 222 germination (GT), shoot and root length of the weeds: *A. artemisiifolia* (AMBAR), *D.*  
 223 *sanguinalis* (DIGSA), *E. crus-galli* (ECHCG), *P. oleracea* (POROL) and *S. nigrum*  
 224 (SOLNI). Mean  $\pm$  standard error. Values sharing the same letter are not significantly different  
 225 (Tukey – b test,  $P \leq 0.05$ ).  
 226

	Indicator species	Control	Dried leaf tissue amounts (g Parker dish <sup>-1</sup> )		
			1.0	2.0	3.0
<b>GT</b> (%)	AMBAR	50.0 $\pm$ 7.07a	40.0 $\pm$ 6.41a	42.5 $\pm$ 6.29a	32.5 $\pm$ 2.50a
	DIGSA	48.8 $\pm$ 0.00a	42.5 $\pm$ 0.15a	30.0 $\pm$ 0.37ab	5.0 $\pm$ 1.34b
	ECHCG	42.5 $\pm$ 0.00a	62.5 $\pm$ 0.13a	45.0 $\pm$ 0.30a	50.0 $\pm$ 0.42a
	POROL	55.0 $\pm$ 0.00a	60.0 $\pm$ 0.13a	77.5 $\pm$ 0.23a	60.0 $\pm$ 0.39a
	SOLNI	12.5 $\pm$ 0.00a	10.0 $\pm$ 0.32a	30.0 $\pm$ 0.37b	45.0 $\pm$ 0.45b
<b>Shoot</b> (mm)	AMBAR	17.7 $\pm$ 0.26a	16.9 $\pm$ 0.67a	17.9 $\pm$ 1.19a	12.5 $\pm$ 1.04a
	DIGSA	16.8 $\pm$ 0.42a	15.8 $\pm$ 0.75a	17.1 $\pm$ 0.35a	19.5 $\pm$ 0.02a
	ECHCG	26.5 $\pm$ 1.14a	16.7 $\pm$ 0.59a	27.2 $\pm$ 1.13a	19.9 $\pm$ 0.54a
	POROL	6.7 $\pm$ 0.47a	9.9 $\pm$ 0.25a	8.45 $\pm$ 0.39a	6.8 $\pm$ 0.26a
	SOLNI	10.0 $\pm$ 0.83a	6.5 $\pm$ 1.99a	5.9 $\pm$ 0.53a	10.8 $\pm$ 0.63a
<b>Root</b> (mm)	AMBAR	40.3 $\pm$ 1.10a	37.6 $\pm$ 2.77a	32.6 $\pm$ 5.02ab	21.9 $\pm$ 3.67b
	DIGSA	16.4 $\pm$ 1.73a	17.2 $\pm$ 2.97a	16.3 $\pm$ 1.44a	20.5 $\pm$ 0.02a
	ECHCG	21.3 $\pm$ 5.85a	13.7 $\pm$ 2.43a	21.1 $\pm$ 5.88a	13.8 $\pm$ 2.39a
	POROL	9.2 $\pm$ 1.23a	8.1 $\pm$ 0.78ab	7.3 $\pm$ 1.14ab	4.34 $\pm$ 0.68b
	SOLNI	35.4 $\pm$ 2.62a	8.2 $\pm$ 5.08b	5.5 $\pm$ 1.29b	13.0 $\pm$ 2.08b

227

228

230 Table 2. Effect of different dried leaf tissue concentrations of *A. artemisiifolia* on total  
 231 germination (GT), shoot and root length of the crops: *T. estivum* (WHEAT), *L. sativa*  
 232 (LETTUCE), *M. sativa* (ALFALFA), *L. esculentum* (TOMATO). Mean  $\pm$  standard error.  
 233 Values sharing the same letter are not significantly different (Tukey – b test,  $P \leq 0.05$ ). Values  
 234 marked with \* or \*\* are statistically different from the control with  $p \leq 0.05$  or  $0.01$ .

	Indicator species	Control	Dried leaf tissues amount (g Parker dish <sup>-1</sup> )		
			1.0	2.0	3.0
GT (%)	WHEAT	87.5 $\pm$ 0.00a	97.5 $\pm$ 0.10a	87.5 $\pm$ 0.21a	95.0 $\pm$ 0.00a
	LETTUCE	97.5 $\pm$ 0.00a	93.7 $\pm$ 0.10a	90.0 $\pm$ 0.21a	85.0 $\pm$ 0.33a
	ALFALFA	84.4 $\pm$ 0.00a	92.5 $\pm$ 0.10a	92.5 $\pm$ 0.21a	87.5 $\pm$ 0.32a
	TOMATO	93.7 $\pm$ 0.00	97.5 $\pm$ 0.10	100.0 $\pm$ 0.20*	85.0 $\pm$ 0.33
Shoot (mm)	WHEAT	41.6 $\pm$ 0.33a	35.2 $\pm$ 0.55a	34.7 $\pm$ 0.23a	35.2 $\pm$ 0.55a
	LETTUCE	24.5 $\pm$ 1.12	10.0 $\pm$ 0.30**	12.0 $\pm$ 0.25*	10.2 $\pm$ 0.35**
	ALFALFA	20.2 $\pm$ 0.73a	20.4 $\pm$ 0.51a	17.5 $\pm$ 0.44a	20.2 $\pm$ 0.36a
	TOMATO	22.4 $\pm$ 1.90	15.9 $\pm$ 1.60	13.9 $\pm$ 1.59	6.2 $\pm$ 0.51*
Root (mm)	WHEAT	48.1 $\pm$ 2.12a	38.2 $\pm$ 3.28a	40.4 $\pm$ 1.37a	38.2 $\pm$ 3.27a
	LETTUCE	26.2 $\pm$ 5.55	13.5 $\pm$ 0.95	12.4 $\pm$ 0.87*	9.8 $\pm$ 1.12*
	ALFALFA	28.6 $\pm$ 3.29a	27.1 $\pm$ 2.31a	23.9 $\pm$ 1.82a	24.6 $\pm$ 1.61a
	TOMATO	29.6 $\pm$ 9.00	26.4 $\pm$ 6.40	24.2 $\pm$ 5.92	8.06 $\pm$ 1.27*

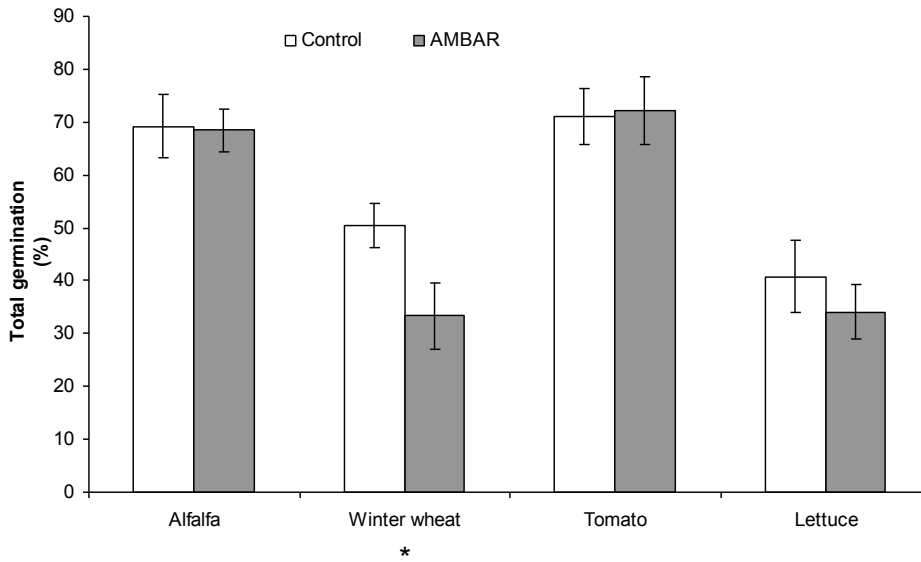
235  
236

## 237 Greenhouse Experiment.

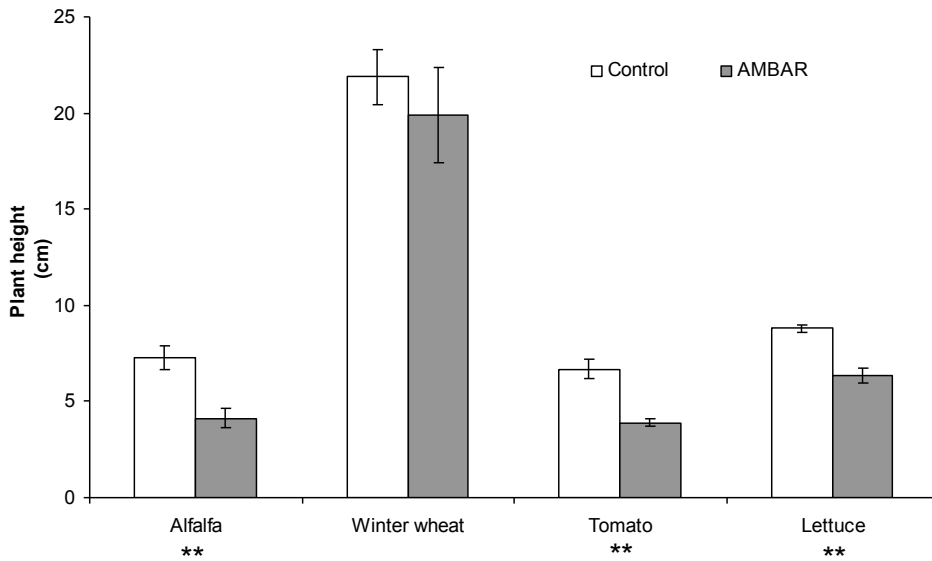
### 238 Experiment 2

239 Across the seeded crops, total germination (GT) differences relative to the control were  
 240 observed only for winter wheat (Figure 1), with a germination inhibition of about 30%. The  
 241 presence of common ragweed residues did not, however, result in a winter wheat plant  
 242 height reduction (Figure 2) as was true for alfalfa, tomato, and lettuce which showed height  
 243 reductions of 43%, 41%, and 26%, respectively. All other species showed sensitivity as  
 244 well. Among the three factors evaluated, total germination, plant height, and plant weight,  
 245 plant weight displayed the least effect from incorporating *A. artemisiifolia* residues into the

246 substrate (Figure 3). In fact, all crops showed plant weight values of roughly half compared  
247 to the control treatment. As in Experiment 1, tomato was reduced the most (58%).  
248



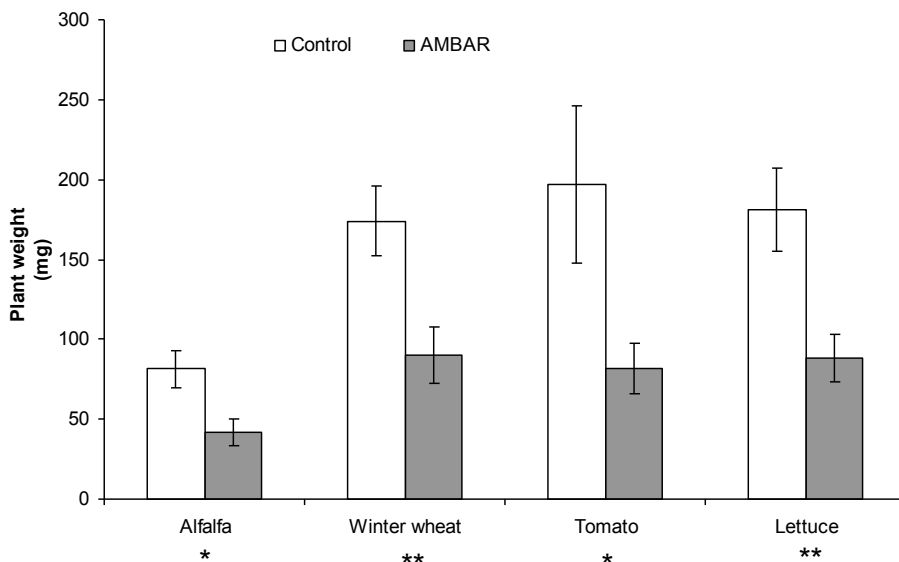
249  
250 Figure 1. Greenhouse experiment: germination response of crop species to the presence  
251 of 2 t/ha of common ragweed (AMBAR) dried residues. Bars indicate standard error.  
252 \* refers to significant differences from the control with  $p \leq 0.05$ .



253

254 Figure 2. Greenhouse experiment: plant height response of crop species to the presence  
 255 of 2 t/ha of common ragweed (AMBAR) dried residues. Bars indicate standard error. \*  
 256 refers to significant differences from the control with  $p \leq 0.05$  or \*\* with  $p \leq 0.01$ .

257



258

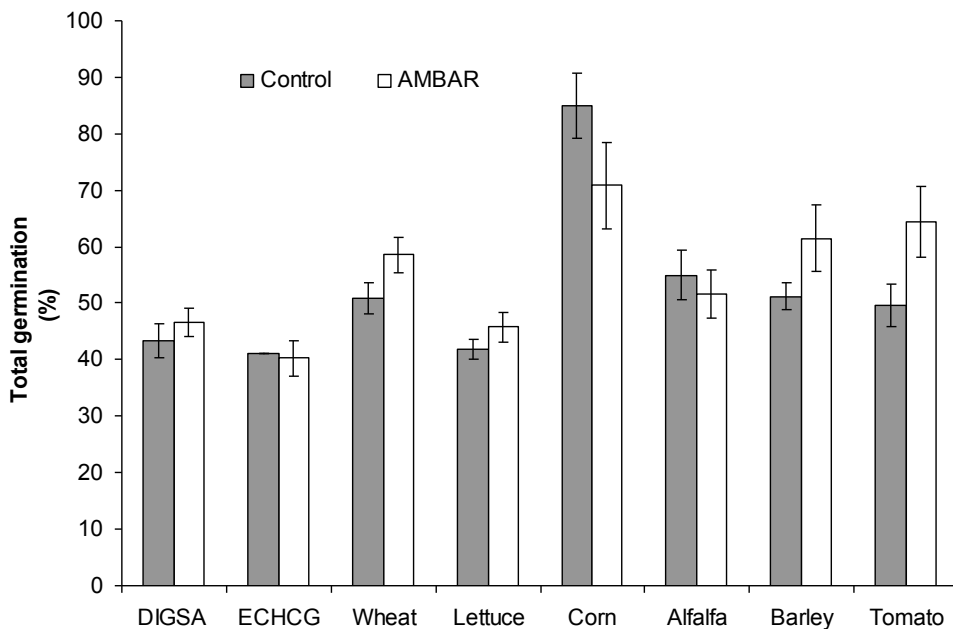
259 Figure 3. Greenhouse experiment: plant weight response of crop species to the presence  
 260 of 2 t/ha of common ragweed (AMBAR) dried residues. Bars indicate standard error.  
 261 \* refers to significant differences from the control with  $p \leq 0.05$  or \*\* with  $p \leq 0.01$ .

262

263 *Experiment 3*

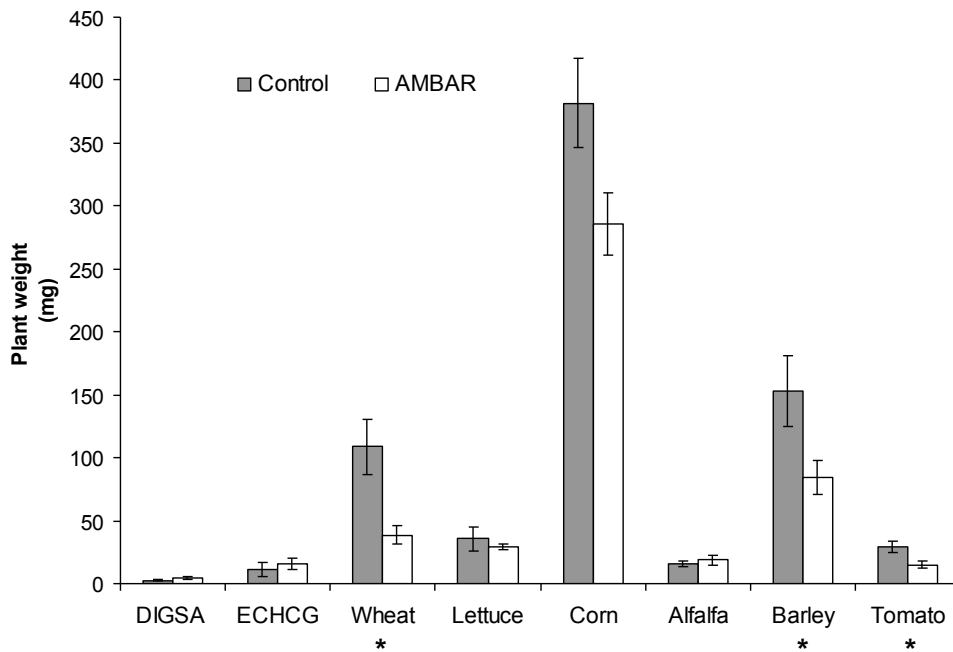
264 Germination was not significantly affected if seeds were placed in pots in which *A.*  
265 *artemisiifolia* was grown (Figure 4). No weed species plant weight was depressed  
266 compared to the control treatment, nor were crops lettuce and alfalfa (Figure 5).  
267 Conversely, the growth of winter wheat and barley was inhibited by about 60% and 45%,  
268 respectively. The high sensitivity of tomato to the allelopathic potential of common  
269 ragweed was also confirmed in this experiment; a growth reduction of about 50% was  
270 assessed on tomato seedling weight *versus* the control.

271



272

273 Figure 4. Greenhouse experiment: germination response to root exudates of common  
274 ragweed (AMBAR). Bars indicate standard error.



275

276 Figure 5. Greenhouse experiment: plant weight response to root exudates of common  
 277 ragweed (AMBAR). Bars indicate standard error. \* refers to significant differences from the  
 278 control with  $p \leq 0.05$ .

279 **Discussion and conclusion**

280 Our findings showed that varying the amount of *A. artemisiifolia* dried residue in soil  
 281 affected the seed germination and seedling growth variation of differing indicator crop and  
 282 weed species. Even though some stimulation effects were observed on germination,  
 283 growth inhibition was observed more frequently, especially in root growth.

284 In allelopathic experiments, many factors play a role in the bioavailability of the toxic  
 285 compounds in the rhizosphere, including organic matter adsorption, chemical inactivation  
 286 and microbial degradation. The Parker dish bioassay used in the laboratory experiment  
 287 was designed to simulate the allelochemical release into soil by tissue degradation over  
 288 time after incorporation. This experiment allowed simulation of more realistic conditions  
 289 than in the natural environment because toxic compound release occurred gradually. Our  
 290 experimental results can, therefore, be compared to those obtained in the greenhouse.

291 On average, among the crops, tomato was the most sensitive indicator species to common  
292 ragweed allelopathic residues; both in the laboratory and greenhouse experiments, more  
293 than 50% growth reduction occurred. The other crops tested showed a different sensitivity  
294 to common ragweed. Root exudates showed an inhibitory effect on winter wheat growth  
295 that was not observed in the residue degradation experiments. This species showed  
296 sensitivity only in the greenhouse experiment. Lettuce displayed root and shoot growth  
297 inhibitory effects only if common ragweed residues were added to the substrate; there  
298 were no depressive effects associated with root exudates.

299 Weed species *E. crus-galli* was never affected by common ragweed, neither in terms of  
300 germination nor first seedling growth. Conversely, *D. sanguinalis* suffered an important  
301 germination reduction. From an overall review of the weed species, *P. oleracea* and *S.*  
302 *nigrum* showed themselves to be the most sensitive species, with more than a 50% root  
303 growth reduction. One explanation of sensitivity to common ragweed of the tested weed  
304 species might be related to water imbibition. Indeed, several studies have pointed out that  
305 the allelopathic activity of plant residue degradation upon a species that follows is strongly  
306 related to seed dimension of the crop (Tesio et al. 2011). The insensitivity observed by *E.*  
307 *crus-galli* might be explained by its very hard seed coat and associated reduced  
308 permeability (Tesio et al. 2008). Even in the case of crops, a similar seed dimension effect  
309 could be ascribed as the inhibition effect roughly followed the same rank order as that of  
310 seed dimension (tomato > lettuce > winter wheat).

311 No important autotoxic effects were observed as only the root growth of *A. artemisiifolia*  
312 was depressed by residue degradation.

313 The costs to limit the rapid expansion of *A. artemisiifolia* to Europe's crops and urban  
314 landscape, combined with the economics of pollinosis relief, demand a management  
315 response. Presently, eradication is impractical as whether in natural or disturbed settings,

316 common ragweed has made clear that it is a widespread and noxious weed of several  
317 crops. It has also made evident, in some cases, its ability to colonize the soil rapidly after  
318 crop harvest (DiTommaso 2004) Several reports have related the consequence of its  
319 presence in the summer after winter wheat or barley cultivations when biomass and root  
320 exudates accumulate in the soil and potentially inhibit growth of the succeeding crop  
321 (DiTommaso 2004). Awareness of the relationships between this species and other crops  
322 and weeds, and knowing that rapid diffusion and expanded presence of the species across  
323 southern Europe might be linked to its allelopathic potential, its rapid growth rate, and its  
324 remarkable ability to recover from mulching interventions to produce, even after cutting,  
325 large amounts of seeds (Patracchini et al. 2011) is useful knowledge for both farmers and  
326 the scientific community.

327 Given the potential of common ragweed to reduce crop growth and yields, as well as its  
328 costly impact on human health, made a strong case for investigatting the role allelopathy  
329 might play in the invasive process. In our studies, germination and initial growth of lettuce  
330 and alfalfa were not affected, but winter wheat and tomato were quite sensitive to residue  
331 presence. Furthermore, our studies highlighted the cricial concern associated with the  
332 sensitivity of the succeeding crop, especially when establishment of a less competitive  
333 crop such as tomato is involved. Given these considerations, this work suggests that the  
334 management of common ragweed infestation, especially its impact on rotational crops,  
335 requires particular attention. In the greenhouse and laboratory experiments we attempted  
336 to recreate a possible field situation in which weed biomass is incorporated into the soil  
337 and a successive crop is planted.

338 Finally, it should be noted that the greenhouse and laboratory studies conducted here, the  
339 effects of residue incorporation and root exudates were evaluated separately. Further  
340 studies to consider the synergistic effects of residues and root exudates in greenhouse

341 and field settings are necessary to fully determine the impact of this invasive species upon  
342 weed and crop establishment in successive cropping systems, as well as the invasion  
343 process in natural plant communities.

344

345 **Source of material:**

346 <sup>1</sup> Herbiseed, Twyford, UK.

347 <sup>3</sup> Whatman No. 1, Wathman International Ltd., Maidstone, UK.

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