

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Ailanthone from *Ailanthus altissima* (Mill.) Swingle as potential natural herbicide

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1713019> since 2019-10-07T00:33:40Z

Published version:

DOI:10.1016/j.scienta.2019.108702

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

1 **Ailanthone from *Ailanthus altissima* (Mill.) Swingle as potential natural herbicide**

2

3 Sonia Demasi^{1a}, Matteo Caser^{1a}, Francesca Vanara¹, Silvia Fogliatto¹, Francesco Vidotto¹, Michéle
4 Negre¹, Francesco Trotta², Valentina Scariot^{1*}

5 ¹Department of Agricultural, Forest and Food Sciences, University of Torino, Largo Paolo Braccini
6 2, 10095, Grugliasco (TO), Italy. E-mail: sonia.demasi@unito.it; matteo.caser@unito.it;
7 francesca.vanara@unito.it; silvia.fogliatto@unito.it; francesco.vidotto@unito.it;
8 michele.negre@unito.it; valentina.scariot@unito.it

9 ²Department of Chemistry, University of Torino, Via Pietro Giuria, 7, 10125, Torino, Italy. E-mail:
10 francesco.trotta@unito.it

11 ^aAuthors contributed equally to this work

12 *Corresponding author: Valentina Scariot, Department of Agricultural, Forest and Food Sciences,
13 University of Torino, Largo Paolo Braccini 2, 10095, Grugliasco (TO), Italy, tel. +390116708932;
14 fax +390116708798; e-mail: valentina.scariot@unito.it

15

16 **Abstract**

17

18 Ailanthone (Ail) is the most phytotoxic quassinoid in plant extracts of *Ailanthus altissima*
19 (Mill.) Swingle, an invasive tree of Simaroubaceae with allelopathic activity. Ail has raised
20 attention as a potential biological herbicide in weed management to reduce the impact on the
21 environment and human health. However, high costs for its extraction and purification, and low
22 persistence in the soil have been considered so far limits for its development as herbicide for open
23 field applications. In this study we explored its phytotoxic activity and persistence, through five
24 experiments, to evaluate its potential for the weed management in the horticulture sector and in
25 urban green areas, where lower herbicide amounts are needed. Ail inhibition activity on

26 germination and growth was evaluated on two model species (garden cress - *Lepidium sativum* L. -
27 and radish - *Raphanus sativus* L.). Firstly, the dose-response curve between Ail concentration and
28 index of germination was calculated; Ail persistence along 30 days was also assessed. Afterwards,
29 Ail bioactivity and persistence were evaluated in a non-sterile urban soil and a horticultural
30 substrate. Ail inhibited by 80 to 90 % the plant growth already at low doses (7.5 mg L^{-1}) in paper
31 and soil, while higher concentrations ($\geq 30 \text{ mg L}^{-1}$) were necessary in the cultivation substrate to
32 obtain similar results. Regarding the phytotoxic persistence, the two species were similarly inhibited
33 at the first evaluation (10 days after treatment) both in paper and cultivation substrate, whereas on
34 the longer period (20 and 30 days after treatment), radish was more affected, with growth inhibition
35 higher than 45 % until 30 days. Results of these experiments implement the knowledge on Ail
36 phytotoxic activity, envisioning its potential use as a biological solution for weed management in
37 urban areas and protected cultivation environments.

38

39 **Keywords:** allelopathy; degradation kinetics; phytotoxicity; quassinoids; seed germination; weed
40 management

41

42 **Abbreviations**

43 Ail: ailanthone

44 DAT: days after treatment

45 IGe: index of germination

46 IGr: index of growth

47 **1. Introduction**

48 Researches to improve the control of weeds are mainly related to agroecosystems (Duke et al.,
49 2000; Kohli et al., 1997; Narwal, 1994; Westwood et al., 2018). This issue, however, is a
50 particularly serious problem in many other productive sectors, such as in horticultural nurseries for
51 the production of vegetable crops or ornamental plants (Altland et al., 2003; Case et al., 2005;
52 Stewart et al., 2017). In this industry, weeds can negatively affect the growth of cultivated species,
53 but also the marketability of the final product, which must be weed-free (Altland et al., 2003; Case
54 et al., 2005; Stewart et al., 2017). Weed control is a challenge in urban environment as well, albeit
55 the undesirability of these plants is attributable to factors not related to plant production, for
56 instance the negative aesthetic effect, the damage to walls and hard surfaces, the reduced visibility
57 on the streets or the diffusion of allergenic pollen. The combination of different control techniques
58 is necessarily required in urban areas, where chemical measures may represent a risk for the
59 population and should be replaced with alternative methods (Benvenuti, 2004; Monaco et al., 2002;
60 Rask and Kristoffersen, 2007), as required also by current legislation in the EU (e.g. Regulation
61 (EU) No 1107/2009 and Directive 2009/128/CE). Studies on weed control in the abovementioned
62 two sectors, i.e. urban areas and horticulture, are thus topical and mandatory in the attempt to
63 answer legislation and both horticulturists and citizen needs.

64 A promising perspective on weed management is the development of herbicides based on natural
65 compounds to cope with the dangerous exposure to humans and the environmental concern on
66 synthetic products (Kohli et al., 1997; Westwood et al., 2018). Among natural compounds, plant
67 by-products have gained increasing attention for their benefits over synthetic compounds for an
68 eco-friendly control of weeds (Benvenuti, 2004; Bhowmik and Inderjit, 2003; Kohli et al., 1997;
69 Narwal, 1994), since they are perceived as safer for the environment and human health (Abbas and
70 Duke, 1995). However, few of them have been developed into commercial herbicides, namely
71 triketones, cinmethylin, bialaphos and glufosinate (Cutler and Cutler, 1999; Duke et al., 2000).

72 Higher plants produce several secondary metabolites to compete with or defend from surrounding
73 species, namely terpenoids, tannins, saponins, flavonoids, and lactones (Ferreira and Aquila, 2000).
74 In particular, the release of chemicals into the environment by one plant to affect another plant is
75 called allelopathy and often results in the germination and growth inhibition of the target plant
76 (Rice, 1984). Mergen (1959) firstly observed that extracts from *Ailanthus altissima* (Mill.) Swingle,
77 a medium-sized tree of the Simaroubaceae family, caused phytotoxicity to other species. This plant
78 is native to China, Japan, Vietnam, and Taiwan and was introduced in Europe as ornamental plant
79 (Sladonja et al., 2015). It has become invasive in all continents, colonising a wide range of habitats,
80 with allelopathy having contributed to its competitiveness and invasiveness, together with its
81 abundant production of seeds and root suckers, a fast growth and high tolerance to pollutants
82 (Benvenuti, 2004; Gómez-Aparicio and Canham, 2008; Kowarik and Säumel, 2007; Sladonja et al.,
83 2015). Plant extracts and essential oils from different organs of *A. altissima* contain alkaloids,
84 terpenoids, steroids, flavonoids, phenolic derivatives, and quassinoids (Albouchi et al., 2013; El
85 Ayeb-Zakhama et al., 2014; Ni et al., 2019), that are sesquiterpene lactones abundantly present in
86 all organs of Simaroubaceae plants. The first quassinoid identified in *A. altissima* was ailanthone
87 (Ail) (Heisey, 1996; Kowarik and Säumel, 2007; Sladonja et al., 2015) that is used to treat
88 ascariasis, diarrhea, spermatorrhea, bleeding and gastrointestinal diseases and has also recently
89 showed antiproliferative activity (Daga et al., 2019). As far as concerns herbicidal activity, Ail
90 showed phytotoxic activity on monocots and dicots both in pre- and post-emergence, causing a
91 strong germination and growth inhibition (De Feo et al., 2003; Heisey, 1996, 1999). Despite
92 showing the potential for the development as a natural-product herbicide, Ail is not commercially
93 used (Bhowmik and Inderjit, 2003), since its application in large agrosystems appears limited by
94 various constraints. In particular, Ail separation and purification costs are remarkably high and the
95 molecule seems not stable and persistent in soil, even at high concentrations (Heisey, 1996, 1999).
96 However, the development of Ail as a natural herbicide could be addressed to protected or limited

97 environment applications rather than to the open field, where large amount of herbicide are often
98 required and multiple environmental factors can lead to a quick breakdown of the product (Ashton,
99 1982).

100 This study aimed to provide new insights in Ail herbicidal activity, persistence and kinetics
101 and to assess its application perspectives for weed control in urban green areas and horticulture. To
102 this aim, the phytotoxic activity was, evaluated on two indicator species, i.e. garden cress
103 (*Lepidium sativum* L.) and radish (*Raphanus sativus* L.), chosen for their different sensitiveness to
104 toxins and their rapid growth (De Feo et al., 2003; Heisey, 1990, 1996, 1999; Heisey and Heisey,
105 2003; Molinaro et al., 2016). Trials, performed at first on filter paper, were then carried out both on
106 soil from urban environment and on a horticulture cultivation substrate in the attempt of
107 reproducing the substrate conditions of two application sectors of interest. The duration of
108 phytotoxic activity in these substrates and Ail degradation kinetics were also examined to
109 understand stability and persistence of this natural herbicide.

110

111 **2. Material and methods**

112 Four experiments, summarised in Table 1, were conducted in the DISAFA facilities
113 (45°03'58.5" Lat. N; 7°35'29.1" Long. E) during 2016-2017. Ailanthone was purchased from
114 Herbest (Baoji Herbest Bio-Tech Co., Ltd. Baoji, China). The phytotoxic activity of Ail
115 (*Experiments 1 to 4*) was assessed through bioassays in controlled laboratory conditions on garden
116 cress (*Lepidium sativum* L. 'Inglese') and radish (*Raphanus sativus* L. 'Tondo Rosso BIO'),
117 purchased from Fratelli Ingegnoli Spa (Milano, Italy). Treatments were provided by means of
118 aqueous solutions and deionised water was used as control.

119

120 **2.1. Experiment 1. Ailanthone dose-response curve in filter paper**

121 The phytotoxicity of Ail was evaluated by assessing its influence on germination and on root
122 length of garden cress and radish. These parameters were determined in 9 cm diameter Petri dishes
123 (Supplementary Figure S1) by placing randomly ten seeds on filter paper disk (Whatman No. 1,
124 Maidstone, UK). Then, 5 mL of seven aqueous solutions of Ail (1, 1.5, 2, 2.5, 5, 7.5 and 10 mg L⁻¹)
125 were added. Dishes were maintained in a growth chamber, at 25°C, in dark conditions. The number
126 of germinated seeds (n) in each dish and their root (r) length (mm) were measured 96 hours after the
127 treatment (ISTA, 2011) in treated (t) and control (c) seeds. These data were used to calculate the
128 Index of Germination (IGe) with Eq. (1) (Molinaro et al. 2016):

$$129 \quad IGe\% = \frac{n_{(t)} \times r_{(t)}}{n_{(c)} \times r_{(c)}} \times 100 \quad (1)$$

130

131 **2.2. Experiment 2. Phytotoxic activity persistence in filter paper**

132 Phytotoxicity dynamics of 1.5 and 7.5 mg L⁻¹ of Ail on germination, root length and
133 hypocotyl length of garden cress and radish were evaluated on filter paper in 100 mL plastic flasks
134 to allow seedling growth. Five seeds were placed randomly on the filter paper and 1.7 mL of
135 treatment or deionised water were added. Flasks were maintained in a growth chamber at 25°C,
136 with a 12h-light photoperiod. In order to evaluate the persistence of Ail effects, the seeds and the
137 obtained seedlings were removed and new seeds were positioned every 10 days after treatment
138 (DAT), till 30 DAT. Deionised water only (without Ail) was added to all flasks to prevent dryness.
139 The number of germinated seeds (n), their root (r) and hypocotyl (h) length (mm) were measured in
140 each evaluation (10, 20, and 30 DAT) (ISTA, 2011) in treated (t) and control (c) plants. These data
141 were used to calculate the Index of Growth (IGr%) with Eq. (2):

$$142 \quad IGr\% = \frac{n_{(t)} \times r_{(t)} \times h_{(t)}}{n_{(c)} \times r_{(c)} \times h_{(c)}} \times 100 \quad (2)$$

143

144 **2.3. Experiment 3. Phytotoxic activity of ailanthone in cultivation substrate and non-sterile soil**

145 The response of garden cress and radish to Ail was also determined in a cultivation substrate
146 suitable for containerized production of ornamentals (Floradur[®] B Seed, Floragard Vertriebs-
147 GmbH) and in non-sterile soil (Table 2) sampled in the DISAFA Campus (Grugliasco, TO, Italy).
148 One-hundred millilitres plastic flasks (Supplementary Figure S2) were filled with 20 g of substrate
149 or soil and moistened with 5 mL of deionised water. Five seeds were then placed randomly in each
150 flask and 1.7 mL of treatment (7.5, 30, 60, and 90 mg L⁻¹ of Ail) were added. Flasks were
151 maintained in a growth chamber, at 25°C, in dark conditions and the parameters to calculate Eq. (1)
152 were recorded 96 hours after the treatment.

153

154 **2.4. Experiment 4. Phytotoxic activity persistence in cultivation substrate and non-sterile soil**

155 Phytotoxicity dynamics of 30, 60 and 90 mg L⁻¹ of Ail was assessed in cultivation substrate
156 and non-sterile soil (Table 2), with the same experimental conditions used with filter paper
157 (*Experiment 2*). Data were collected at 10, 20 and 30 DAT to calculate Eq. (2).

158

159 **2.5. Experiment 5. Degradation kinetics of ailanthonone in cultivation substrate and non-sterile** 160 **soil**

161 **2.5.1. Extraction method**

162 The method was developed by spiking non-sterile soil and cultivation substrate samples with
163 known amounts of an Ail aqueous solution. Different extraction solvents were tested, the best one
164 being methanol/water (10:90, v/v) in the following conditions: suspensions of 20 g soil or substrate
165 in 50 mL methanol/water (10:90, v/v) were stirred on a mechanical shaker (shaker mod. M102-OS,
166 MPM Instruments, Milan, Italy) at 100 rpm for 30 min. The extraction was repeated twice with 25
167 mL of the same solution. The reunited extracts were filtered through a Whatman no. 1 filters
168 (Whatman, Maidstone, UK). A 10 mL aliquot of the extract was eluted on a BAKERBOND[™] SPE
169 C18 (1 g, 6 mL) column (J.T.Baker[®] Avantor Performance Materials, Center Valley, PA, USA) at a

170 rate of about 1–2 drops/second. At last, the analyte was eluted with 5 mL of liquid
171 chromatography–mass spectrometry (LC/MS) grade methanol, filtered with a syringe filter in PP
172 (0.45 µm), then diluted (1:5, V/V) with LC/MS grade water and analysed by LC-MS/MS, according
173 to the method described below.

174 **2.5.2. LC-MS/MS determination**

175 LC-MS/MS analysis was carried out on a Varian 310 triple quadrupole mass spectrometer
176 (Agilent, Milan, Italy) equipped with an electrospray ionization ESI source, a 212 LC pump, a
177 ProStar 410 AutoSampler and dedicated software. LC separation was performed on a Pursuit 5 C18
178 column, 5 µm particle size, 50 × 2.1 mm (Agilent, Milan, Italy). The mobile phase consisted of
179 water (A) and methanol (B), both containing 0.1% (v/v) acetic acid. The gradient was 90 to 10 % A
180 in 3 min with a flow rate of 0.2 mL/min. ESI conditions used in negative polarization were: needle
181 potential – 4000 V, shield -450 V, capillary – 54 V. Gas conditions were set with 25 psi of N₂ as
182 nebulizing gas and 25 psi at 250°C N₂ as drying gas. The respective ion transitions were m/z 375 →
183 300.7 (collision energy 13 V) and 375 → 150.6 (collision energy 24 V). The m/z 150.6 was used for
184 quantification.

185 **2.5.3. Degradation studies**

186 Sub samples of freshly collected soil and substrate were supplemented with 1.7 mL of an
187 aqueous solution of Ail (30 mg L⁻¹) in order to obtain a 2.5 mg kg⁻¹ soil concentration. The flasks
188 were incubated in the same conditions described in *Experiment 3*. Evaporation of water was
189 periodically (4-5 days) compensated by addition of deionized water. The quantification of Ail was
190 performed 0, 1, 2, 3, 7, 11 and 18 DAT by determination of its concentration in three independent
191 flasks.

192

193 **2.6. Statistical analyses**

194 Dishes in *Experiment 1* had three replicates (30 seeds in total), while flasks of *Experiment 2*, 3
195 and 4 had six replicates (30 seeds in total) per treatment, arranged in a completely randomized
196 design. All the studies were conducted in triplicate. Arcsine transformation was performed on
197 percentage data prior to analysis. All presented values are means of untransformed data. Data were
198 tested for the homogeneity of variance (Levene test), then mean comparison and one-way ANOVA
199 were performed to analyse the phytotoxic effect of Ail on model species and means were separated
200 according to Tukey post-hoc test ($p < 0.05$). The analyses were performed with SPSS software
201 (SPSS Inc., version 25, Chicago, Illinois). To define the dose-response curve, for each species a
202 separate regression analysis was performed between Ail concentration (independent variable) and
203 the index of germination IGe (dependent variable) fitting the following three parameters log-logistic
204 model, Eq. (3):

$$205 \text{ IGe} = \frac{d}{1 + \exp[b(\log(x) - \log(e))]} \quad (3)$$

206 where *IGe* is the index of germination expressed as a percentage of the control, *b* and *d* are the
207 curve parameters, with *b* being the relative slope at the point of inflection *e* and *d* the upper limit,
208 while *x* is the Ail concentration (in mg L⁻¹). The regression analysis was performed using the
209 function *drm* of the add-on package *drc* of the R software (RCoreTeam, 2017). The effective
210 concentration required to reduce by 10%, 50% and 90% the IGe index (ED₁₀, ED₅₀, ED₉₀) was
211 calculated using the function *ED* of the package *drc*. The function *EDcomp* of the package *drc* was
212 used to calculate the ratio between the ED values of the two species (ED ratio) and to test the
213 significance of differences of ED₁₀, ED₅₀, and ED₉₀ between the two species. Differences were
214 considered significant when the confidence interval of ED ratio at $p \leq 0.05$ did not included one.

215

216 **3. Results**

217 **3.1. Experiment 1**

218 The dose-response curve showed that at increasing rates of Ail the Index of germination
219 (IGe%) gradually reduced (Figure 1). In particular, at lower Ail concentrations, garden cress was
220 more sensitive compared to radish; for example, at 1.5 mg L⁻¹ of Ail the average IGe values were 32
221 and 45 for garden cress and radish, respectively (Figure 1). The higher sensitiveness of garden cress
222 was also demonstrated by its ED₁₀ (Ail concentration able to reduce the index of germination of
223 10%), which was significantly lower compared to that of radish (Table 3). However, at higher rates
224 the behaviour was opposite as radish showed a strong reduction of IGe, with values of about 1 or
225 less at concentrations above 7.5 mg L⁻¹, while for garden cress the recorded values were about 10 or
226 higher at the same concentrations. The ED₅₀ and ED₉₀ for index of germination were not
227 significantly different between the two tested species due to data variability.

228

229 **3.2. Experiment 2**

230 Generally, Ail was more effective at the highest concentration (7.5 mg L⁻¹) in both species
231 (Figure 2), because significant differences in their IGr were recorded in each evaluation. The
232 concentration of 1.5 mg L⁻¹ reduced the IGr of both species by 80% compared with control at 10
233 DAT, but its efficacy was gradually lost in the following evaluations. Conversely, the herbicidal
234 effect of the highest concentration strongly persisted until 20 DAT in garden cress (Figure 2a), and
235 30 DAT in radish (Figure 2b), with a growth reduction of about 95% compared with control in both
236 evaluations.

237

238 **3.3. Experiment 3**

239 The lowest concentration tested (7.5 mg L⁻¹) was not markedly effective on both species in
240 cultivation substrate (Figure 3a), while the growth was reduced by more than 95% in soil compared
241 with control (Figure 3b). The herbicidal activity was very strong at higher concentrations (30, 60,
242 and 90 mg L⁻¹) in both substrates, with no significant differences between species.

243

244 **3.4. Experiment 4**

245 Moulds started to develop after the first measurement (10 DAT) in non-sterile soil in growth
246 chamber conditions, therefore data refer solely to the cultivation substrate. Although little
247 stimulation effects were observed in garden cress at 30 DAT, data were not significantly higher than
248 control. A growth inhibition was instead mostly observed and garden cress (Figure 4a) appeared
249 less sensitive than radish (Figure 4b), especially at 20 and 30 DAT. Every Ail treatments
250 significantly affected seedlings compared with control. Moreover, 60 and 90 mg L⁻¹ had stronger
251 herbicidal activity than 30 mg L⁻¹ at 10 and 30 DAT in radish, which displayed an IGr lower than
252 10% (10 DAT) and 60% (30 DAT), respectively.

253

254 **3.5. Experiment 5**

255 Notwithstanding different polarity extraction solutions were tested at different pH, the highest
256 recovery from the spiked non-sterile soil, obtained with the procedure described above, was 65.6 ±
257 5.9 %. In the case of the spiked cultivation substrate, the recovery dropped to 46.0 ± 5.8 %. The
258 degradation rate of Ail, expressed in µg of Ail recovered at each sampling time, is illustrated in
259 Figure 5. The degradation curves were fitted by an exponential equation ($R^2 > 0.97$), indicating that
260 the degradation followed a first order kinetics. The calculated half-life times (DT₅₀) were 1.5 and
261 1.4 days for soil and substrate respectively, while the DT₉₀ values were 7.0 days in the soil and 6.1
262 days in the substrate.

263

264 **4. Discussion**

265 Data obtained in this study deepen the knowledge on the quassinoid Ail derived from *A.*
266 *altissima*, which already proved to have numerous biological activities, not only herbicidal but also
267 antiproliferative, antiplasmodial, antituberculosis, antimicrobial, insecticidal, antioxidant, anti-

268 inflammatory, and algaecide (Albouchi et al., 2013; De Feo et al., 2005; El Ayeb-Zakhama et al.,
269 2014; Gu et al., 2014; Lü and He, 2010; Meng et al., 2015; Okunade et al., 2003; Rahman et al.,
270 2009). In controlled conditions, Ail was extremely efficient on indicator species in filter paper,
271 suggesting that it is an already active compound and does not need to be modified to reach its active
272 form (Soltys et al., 2013). Very low concentrations (2.5-10 mg L⁻¹) were sufficient to depress by
273 more than 80% the IGe of garden cress and radish in Petri dishes in 96 hours under controlled
274 conditions (Figure 1Figure 1), confirming previous results of Heisey (1996) on garden cress at
275 similar concentrations (2 mg L⁻¹). On the whole, garden cress had a lower ED₅₀ and ED₉₀ compared
276 to radish. At increasing Ail doses, a different behaviour between species was observed. A lower
277 amount of Ail was needed to reduce the growth of radish and this was mainly due to a strong total
278 germination decrease in this species, while in garden cress the germination was only partially
279 affected (Demasi et al., Data in brief). Conversely, the root length was markedly inhibited in both
280 species at increasing Ail concentrations. If seedlings were let to grow in paper, radish displayed a
281 high sensitivity (Figure 2) to a very low amount of Ail (1.5 mg L⁻¹) and for 10 days more than cress.
282 Again, data showed that germination of cress was only partially affected, while root and hypocotyl
283 growth were mostly inhibited (Demasi et al., Data in brief).

284 Most of the information on herbicide activity and degradation derive from field soil trials, and
285 are often assumed to be the same in other substrates. However, horticulture and urban green areas
286 use soilless substrates which consist almost entirely of organic matter (Stewart et al., 2017). The
287 effectiveness of Ail can be deeply influenced by the presence of an organic substrate (Heisey,
288 1996). In this study, the amount of 7.5 mg L⁻¹ of Ail, which was very effective on filter paper (IGe
289 about 2.5%) and soil (IGe about 5%), was almost ineffective in cultivation substrate (IGe about
290 75%). This evidence was fundamental to assess the suitable amount of Ail (i.e. ≥ 30 mg L⁻¹) to
291 obtain herbicidal efficacy in substrate on garden cress (IGe lower than 20%) and radish (IGe lower
292 than 10%). When bioassays were conducted in cultivation substrate, radish confirmed its higher

293 sensitiveness than cress to Ail, even at 20 and 30 DAT (Figure 4), even if both species showed a
294 slight reduction of germination (Demasi et al., Data in brief). These data confirm that radish is more
295 prone to be affected by *A. altissima* allelochemicals compared to other species (De Feo et al., 2003).
296 Natural compounds can inhibit the germination or growth of plants through different modes of
297 action (Macías et al., 2003), thus the diverse responses of one species to toxic compounds can be
298 possibly due to the different mechanisms of inhibition of the active ingredient. However, in this
299 regard little is known about ailanthone (Duke et al., 2000) but, similarly to other quassinoids, can be
300 envisaged that Ail might act as mitosis inhibitor (Dayan et al., 1999).

301 In literature, the only studies on Ail toxicity duration (Heisey, 1996, 1999) reported that the
302 effect was rapidly lost (in 3 days) when incubated at 25°C in presence of non-sterile soil. The
303 breakdown of an herbicide in soil depends on several factors, including degradation acted by
304 microbes, which presence is usually supported by organic matter. This latter, in turns, can give
305 suitable surface for sorption of the herbicide, limiting its bioavailability. Both of the
306 abovementioned mechanisms are plausible explanations for the loss of phytotoxicity displayed in
307 this study in the cultivation substrate. Indeed, when the trial was performed on paper (*Experiment*
308 2), Ail was deeply active at low doses until 30 DAT on radish and 20 DAT on garden cress, while
309 on cultivation substrate lasted 30 DAT on radish, but using eight-times higher concentration (60 mg
310 L⁻¹). Ail remained highly toxic throughout 21 days if the soil was sterilised (Heisey, 1996). This
311 study also provided for the first time information on Ail degradation in soilless substrate, compared
312 to that in non-sterile soil. The half-life times for Ail degradation (DT₅₀) were similar in both
313 substrates, while the DT₉₀ value was higher in the soil, meaning that longer time was needed to
314 degrade Ail. This is probably due to the lower amount of organic material in the soil tested. The
315 short persistence of the active principle could be an advantage for the environment and human
316 safety, but at the same time can be an hindrance, leading to a rapid loss of the herbicidal activity

317 (Bhowmik and Inderjit, 2003; Cutler and Cutler, 1999; Duke et al., 2000; Kohli et al., 1997;
318 Narwal, 1994; Sladonja et al., 2015).

319

320 **Conclusions**

321 Ail showed a high phytotoxic activity, supporting evidence of *A. altissima* allelopathy, which
322 contributes to the high invasiveness of the species. The phytotoxic activity was different based on
323 the tested species, thus its efficacy can be variable and related to the species sensitivity. Further
324 researches in this regard are thus needed to confirm Ail potential use in horticulture and urban green
325 areas as natural herbicide.

326

327 **Declaration of interest:** none.

328 **Funding:** this work was supported by Compagnia San Paolo, Italy - Project GreeNS3P
329 (Torino_call2014_L1_141).

330 **Acknowledgements:** Walter Gaino is thanked for the helpful support in bioassays setup.

331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380

References

- Abbas, H.K., Duke, S.O., 1995. Phytotoxins from plant pathogens as potential herbicides. *Toxin Rev.* 14, 523–543. <https://doi.org/10.3109/15569549509016440>
- Albouchi, F., Hassen, I., Casabianca, H., Hosni, K., 2013. Phytochemicals, antioxidant, antimicrobial and phytotoxic activities of *Ailanthus altissima* (Mill.) Swingle leaves. *South African J. Bot.* 87, 164–174. <https://doi.org/10.1016/j.sajb.2013.04.003>
- Altland, J.E., Gilliam, C.H., Wehtje, G., 2003. Weed control in field nurseries. *Horttechnology* 13, 9–14.
- Ashton, F.M., 1982. Persistence and Biodegradation of Herbicides, in: *Biodegradation of Pesticides*. Springer US, Boston, MA, pp. 117–131. https://doi.org/10.1007/978-1-4684-4088-1_5
- Benvenuti, S., 2004. Weed dynamics in the Mediterranean urban ecosystem: Ecology, biodiversity and management. *Weed Res.* 44, 341–354. <https://doi.org/10.1111/j.1365-3180.2004.00410.x>
- Bhowmik, P.C., Inderjit, 2003. Challenges and opportunities in implementing allelopathy for natural weed management. *Crop Prot.* 22, 661–671. [https://doi.org/10.1016/S0261-2194\(02\)00242-9](https://doi.org/10.1016/S0261-2194(02)00242-9)
- Case, L.T., Mathers, H.M., Senesac, A.F., 2005. A review of weed control practices in container nurseries. *Horttechnology* 15, 535–545.
- Cutler, H.G., Cutler, S.J., 1999. *Biologically Active Natural Products: Agrochemicals*, New York. CRC Press. <https://doi.org/10.1007/BF02987557>
- Daga, M., Pizzimenti, S., Dianzani, C., Cucci, M.A., Cavalli, R., Grattarola, M., Ferrara, B., Scariot, V., Trotta, F., Barrera, G., 2019. Ailanthone inhibits cell growth and migration of cisplatin resistant bladder cancer cells through down-regulation of Nrf2, YAP, and c-Myc expression. *Phytomedicine* 56, 156–164. <https://doi.org/10.1016/j.phymed.2018.10.034>
- Dayan, F.E., Watson, S.B., Galindo, J.C.G., Hernández, A., Dou, J., McChesney, J.D., Duke, S.O., 1999. Phytotoxicity of quassinoids: Physiological responses and structural requirements. *Pestic. Biochem. Physiol.* 65, 15–24. <https://doi.org/10.1006/pest.1999.2432>
- De Feo, V., De Martino, L., Quaranta, E., Pizza, C., 2003. Isolation of phytotoxic compounds from Tree-of-heaven (*Ailanthus altissima* Swingle). *J. Agric. Food Chem.* 51, 1170–1180. <https://doi.org/10.1021/JF020686+>
- De Feo, V., De Martino, L., Santoro, A., Leone, A., Pizza, C., Franceschelli, S., Pascale, M., 2005. Antiproliferative effects of tree-of-heaven (*Ailanthus altissima* Swingle). *Phyther. Res.* 19, 226–230. <https://doi.org/10.1002/ptr.1670>
- Duke, S.O., Dayan, F.E., Romagni, J.G., Rimando, A.M., 2000. Natural products as sources of herbicides: Current status and future trends. *Weed Res.* 40, 99–111. <https://doi.org/10.1046/j.1365-3180.2000.00161.x>
- El Ayeb-Zakhama, A., Ben Salem, S., Sakka-Rouis, L., Flamini, G., Ben Jannet, H., Harzallah-Skhiri, F., 2014. Chemical composition and phytotoxic effects of essential oils obtained from *Ailanthus altissima* (Mill.) Swingle cultivated in Tunisia. *Chem. Biodivers.* 11, 1216–1227. <https://doi.org/10.1002/cbdv.201300409>
- Ferreira, A.G., Aquila, M.E.A., 2000. Allelopathy: an Emerging Topic in Ecophysiology. *Rev. Bras. Fisiol. Veg.* 12, 175–204.
- Gómez-Aparicio, L., Canham, C.D., 2008. Neighbourhood analyses of the allelopathic effects of the invasive tree *Ailanthus altissima* in temperate forests. *J. Ecol.* 96, 447–458. <https://doi.org/10.1111/j.1365-2745.2007.01352.x>
- Gu, X., Fang, C., Yang, G., Xie, Y., Nong, X., Zhu, J., Wang, S., Peng, X., Yan, Q., 2014. Acaricidal properties of an *Ailanthus altissima* bark extract against *Psoroptes cuniculi* and *Sarcoptes scabiei* var. *cuniculi* in vitro. *Exp. Appl. Acarol.* 62, 225–232. <https://doi.org/10.1007/s10493-013-9736-0>
- Heisey, R.M., 1999. Development of an allelopathic compound from tree-of-heaven (*Ailanthus*

381 *altissima*) as a natural product herbicide, in: Cutler, J.S., Cutler, H.G. (Eds.), Biologically
382 Active Natural Products: Agrochemicals. CRC Press, Boca Raton, pp. 57–68.
383 <https://doi.org/10.1201/9781420048629-8>

384 Heisey, R.M., 1996. Identification of an allelopathic compound from *Ailanthus altissima*
385 (Simaroubaceae) and characterization of its herbicidal activity. *Am. J. Bot.* 83, 192–200.
386 <https://doi.org/10.2307/2445938>

387 Heisey, R.M., 1990. Allelopathic and herbicidal effects of extracts from tree of heaven (*Ailanthus*
388 *altissima*). *Am. J. Bot.* 77, 662–670. <https://doi.org/10.1002/j.1537-2197.1990.tb14451.x>

389 Heisey, R.M., Heisey, T.K., 2003. Herbicidal effects under field conditions of *Ailanthus altissima*
390 bark extract, which contains ailanthone. *Plant Soil* 256, 85–99.
391 <https://doi.org/10.1023/A:1026209614161>

392 ISTA, 2011. International Seed Testing Association.

393 Kohli, R.K., Batish, D., Singh, H.P., 1997. Allelopathy and Its Implications in Agroecosystems. *J.*
394 *Crop Prod.* 1, 169–202. https://doi.org/10.1300/J144v01n01_08

395 Kowarik, I., Säumel, I., 2007. Biological flora of Central Europe: *Ailanthus altissima* (Mill.)
396 Swingle. *Perspect. Plant Ecol. Evol. Syst.* 8, 207–237.
397 <https://doi.org/10.1016/j.ppees.2007.03.002>

398 Lü, J.H., He, Y.Q., 2010. Fumigant toxicity of *Ailanthus altissima* Swingle, *Atractylodes lancea*
399 (Thunb.) DC. and *Elsholtzia stauntonii* Benth extracts on three major stored-grain insects. *Ind.*
400 *Crops Prod.* 32, 681–683. <https://doi.org/10.1016/j.indcrop.2010.06.006>

401 Macías, F.A., Galindo, J.C.G., Molinillo, J.M.G., Cutler, H.G., 2003. Allelopathy : chemistry and
402 mode of action of allelochemicals. CRC Press.

403 Meng, P., Pei, H., Hu, W., Liu, Z., Li, X., Xu, H., 2015. Allelopathic effects of *Ailanthus altissima*
404 extracts on *Microcystis aeruginosa* growth, physiological changes and microcystins release.
405 *Chemosphere* 141, 219–226. <https://doi.org/10.1016/j.chemosphere.2015.07.057>

406 Mergen, F., 1959. A toxic principle in the leaves of *Ailanthus*. *Bot. Gaz.* 121, 32–36.

407 Molinaro, F., Monterumici, C.M., Ferrero, A., Tabasso, S., Negre, M., 2016. Bioherbicidal activity
408 of a germacranolide sesquiterpene dilactone from *Ambrosia artemisiifolia* L. *J. Environ. Sci.*
409 *Heal. - Part B Pestic. Food Contam. Agric. Wastes* 51, 847–852.
410 <https://doi.org/10.1080/03601234.2016.1208466>

411 Monaco, T.J., Weller, S.C., Ashton, F.M., 2002. Weed science: principles and practices. John Wiley
412 & Sons.

413 Narwal, S.S., 1994. Allelopathy in crop production. Scientific Publishers, Jodhpur, India.

414 Ni, J.C., Shi, J.T., Tan, Q.W., Chen, Q.J., 2019. Two new compounds from the fruit of *Ailanthus*
415 *altissima*. *Nat. Prod. Res.* 33, 101–107. <https://doi.org/10.1080/14786419.2018.1437434>

416 Okunade, A.L., Bikoff, R.E., Casper, S.J., Oksman, A., Goldberg, D.E., Lewis, W.H., 2003.
417 Antiplasmodial activity of extracts and quassinoids isolated from seedlings of *Ailanthus*
418 *altissima* (Simaroubaceae). *Phyther. Res.* 17, 675–677. <https://doi.org/10.1002/ptr.1336>

419 Rahman, A., Kim, E.L., Kang, S.C., 2009. Antibacterial and antioxidant properties of *Ailanthus*
420 *altissima* swingle leave extract to reduce foodborne pathogens and spoiling bacteria. *J. Food*
421 *Saf.* 29, 499–510. <https://doi.org/10.1111/j.1745-4565.2009.00172.x>

422 Rask, A.M., Kristoffersen, P., 2007. A review of non-chemical weed control on hard surfaces.
423 *Weed Res.* 47, 370–380. <https://doi.org/10.1111/j.1365-3180.2007.00579.x>

424 Rice, E.L., 1984. Allelopathy. Academic Press, Orlando, FL.

425 Sladonja, B., Sušek, M., Guillermic, J., 2015. Review on invasive tree of heaven (*Ailanthus*
426 *altissima* (Mill.) Swingle) conflicting values: assessment of its ecosystem services and
427 potential biological threat. *Environ. Manage.* 56, 1009–1034. <https://doi.org/10.1007/s00267-015-0546-5>

428

429 Soltys, D., Krasuska, U., Bogatek, R., Gniazdowski, A., 2013. Allelochemicals as Bioherbicides —
430 Present and Perspectives, in: *Herbicides - Current Research and Case Studies in Use*. InTech,

431 pp. 517–542. <https://doi.org/10.5772/56185>
432 Stewart, C.J., Marble, S.C., Pearson, B.J., Wilson, P.C., 2017. Impact of container nursery
433 production practices on weed growth and herbicide performance. *HortScience* 52, 1593–1600.
434 <https://doi.org/10.21273/HORTSCI12241-17>
435 Westwood, J.H., Charudattan, R., Duke, S.O., Fennimore, S.A., Marrone, P., Slaughter, D.C.,
436 Swanton, C., Zollinger, R., 2018. Weed Management in 2050: Perspectives on the Future of
437 Weed Science. *Weed Sci.* 66, 275–285. <https://doi.org/10.1017/wsc.2017.78>
438
439 Data in Brief: Ailanthone inhibition data on seed germination and seedling growth of *Lepidium*
440 *sativum* L. and *Raphanus sativus* L. Demasi S., Caser M., Fogliatto S., Vidotto F., Trotta F.,
441 Scariot V.

442 **Tables**

443 **Table 1.** Summary of the bioassays performed to evaluate the phytotoxic activity of ailanthone
 444 (Ail).

Experiment No.	Ail concentration (mg L ⁻¹)	Container	Substrate ^a	Conditions ^b	Duration	Evaluation
1	1, 1.5, 2, 2.5, 5, 7.5, 10	Petri dish	P	D	96 h	IGe% ^c
2	1.5, 7.5	Plastic flask	P	L	10, 20, 30 DAT ^e	IGr% ^d
3	7.5, 30, 60, 90	Plastic flask	C and S	L	96 h	IGe%
4	30, 60, 90	Plastic flask	C and S	L	10, 20, 30 DAT	IGr%

445 ^aP=paper; C=cultivation substrate; S=soil. ^bD=dark, growth chamber, 25°C; L=12h photoperiod, growth chamber, 25°C. ^cIGe%= Index of Germination. ^dIGr%= Index of Growth. ^eDAT= days after
 446 treatment
 447
 448

449 **Table 2.** Physical and chemical characteristics of the cultivation substrate and non-sterile soil used
 450 in the *Experiments 3 and 4*.

Floradur® B Seed			Non-sterile soil		
pH (CaCl ₂)		5.6	pH ^a		8.0
Salinity	g L ⁻¹	0.8	Carbonates ^b	%	12.02
N (CaCl ₂)	mg L ⁻¹	140	C tot ^c	%	2.257
P (P ₂ O ₅)	mg L ⁻¹	80	N tot ^c	%	0.069
K (K ₂ O)	mg L ⁻¹	190	CEC ^d	meq 100g ⁻¹	4.61
			Exchangeable Ca	mg L ⁻¹	1012
			Exchangeable Mg	mg L ⁻¹	27
			Exchangeable K	mg L ⁻¹	30
			Available P ^e	mg L ⁻¹	9.5
			Clay	%	3.69
			Silt	%	7.60
			Sand	%	88.71

451 ^aISO 10390; ^bISO 10693; ^cISO 10694; ^dcation exchange capacity, ISO 11260; ^eOlsen.

452
 453 **Table 3.** Concentration required to reduce by 10%, 50% and 90% (ED₁₀, ED₅₀ and ED₉₀) the IGe
 454 index and their ratio with lower and upper limits of the confidence interval for garden cress and
 455 radish (*Experiment 1*).

	Garden cress	Radish	ED ratio ^a	ED ratio lower limit	ED ratio upper limit
ED ₁₀	0.16	0.41	0.40*	0.06	0.74
ED ₅₀	1.06	1.19	0.89ns	0.63	1.16
ED ₉₀	6.93	3.46	2.00ns	0.99	3.01

456 ^aED ratio: calculated as EDx garden cress/EDx radish. The statistical relevance is provided (ns,
 457 non-significant; * $p \leq 0.05$).

458 **Figure captions**

459 **Figure 1.** Dose-response curve of index of germination (IGe%) of garden cress (*Lepidium sativum*)
460 and radish (*Raphanus sativus*) in response to different concentrations of ailanthonone on filter paper
461 after 96 hours under controlled conditions (*Experiment 1*).

462 **Figure 2.** Index of growth (IGr%) of a) garden cress (*Lepidium sativum*) and b) radish (*Raphanus*
463 *sativus*) in response to 1.5 (grey) and 7.5 mg L⁻¹ (black) of ailanthonone in filter paper 10, 20, and 30
464 days after treatment (DAT) under controlled conditions (*Experiment 2*). Similar upper case letters
465 along the same treatment and similar lower case letters within the same group denote no significant
466 differences according to Tukey post-hoc test ($p < 0.05$). Bars indicate the standard error.

467 **Figure 3.** Index of germination (IGe%) of garden cress (*Lepidium sativum*) and radish (*Raphanus*
468 *sativus*) in response to different concentrations of ailanthonone on a) cultivation substrate and b) non-
469 sterile soil in controlled conditions (*Experiment 3*). Similar lower case letters in garden cress and
470 similar upper case letters in radish denote no significant differences among concentrations,
471 according to Tukey post-hoc test ($p < 0.05$). Bars indicate the standard error.

472 **Figure 4.** Index of growth (IGr%) of a) garden cress (*Lepidium sativum*) and b) radish (*Raphanus*
473 *sativus*) in response to 30, 60 and 90 mg L⁻¹ of ailanthonone on cultivation substrate after 10, 20, and
474 30 days after treatment (DAT) (*Experiment 4*). Similar upper case letters along the same treatment
475 and similar lower case letters within the same group denote no significant differences according to
476 Tukey post-hoc test ($p < 0.05$). Bars indicate the standard error.

477 **Figure 5.** Degradation rate of ailanthonone in non-sterile soil and cultivation substrate.