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► To cite this version:

Stéphanie Gibot-Leclerc, Fabrice Dessaint, Manon Connault, Rémi Perronne. Can amino acids be used to inhibit germination or deplete the soil seedbank of *Phelipanche ramosa* (L.) Pomel?. *Journal of Plant Diseases and Protection*, 2023, 10.1007/s41348-023-00797-7 . hal-04229258

HAL Id: hal-04229258

<https://institut-agro-dijon.hal.science/hal-04229258>

Submitted on 11 Dec 2023

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1 **Can amino acids be used to inhibit germination or deplete the soil seedbank of**

2 ***Phelipanche ramosa* (L.) Pomel?**

3

4 **Stéphanie Gibot-Leclerc^{1,*} · Fabrice Dessaint^{1,*} · Manon Connault¹ · Rémi Perronne²**

5 *Both the authors contributed equally in this article

6

7 ¹Agroécologie, AgroSup Dijon, INRAE, Univ. Bourgogne, Univ. Bourgogne Franche-Comté,

8 F-21000 Dijon, France

9 ²IGEPP, INRAE, Institut Agro, Univ Rennes, 35653, Le Rheu, France

10

11 Corresponding author:

12 Rémi Perronne, IGEPP, INRAE, Institut Agro, Univ Rennes, 35653, Le Rheu, France

13 E-mail address: remi.perronne@inrae.fr

14

15 ORCID number:

16 Stéphanie Gibot-Leclerc: <https://orcid.org/0000-0002-3722-5321>

17 Rémi Perronne: <https://orcid.org/0000-0002-5089-0510>

18

19 **Abstract**

20 *Phelipanche ramosa* is a weed holoparasite which became a major agronomic problem for
21 several crops in France. The yield and quality losses may be complete and lead to the
22 abandonment of crops in the most infested fields. Chemical weed control using synthetic
23 herbicides do not have a direct effect on this weed because the first half of its reproductive
24 cycle occurs on the root system of its host. The aim of this study is to contribute to the definition
25 of an integrated weed management program of *P. ramosa*, especially focusing on the use of
26 the possible direct effect of amino acids on seed of broomrapes as a potential biocontrol
27 solution. We studied a set of 14 amino acids in *in vitro* conditions to investigate whether some
28 amino acids can have an inhibitory effect on the germination of the *P. ramosa* seeds, whether
29 this effect may be permanent after the removal of the amino acid, and whether some amino
30 acids can cause a high dormancy or seed mortality rate. Our main results showed (1) a strong
31 inhibitory effect of four amino acids on the seed germination concerning arginine, lysine,
32 phenylalanine and tryptophan, and (2) that this effect appeared temporary for arginine and
33 lysine, but permanent for phenylalanine and tryptophan. Our study thus highlighted the interest
34 of phenylalanine and tryptophan as they caused both high inhibition of germination and high
35 seed mortality of *P. ramosa*. Based on these results, we suggest three options for the use of
36 these amino acids that could provide concrete answers to farmers.

37

38 **Keywords**

39 Biocontrol · Weed management · Parasitic plant · Branched broomrape · Natural product

40

41 **Introduction**

42

43 Broomrapes constitute a large group of root holoparasites, including several major arable
44 weeds damaging crop production and causing substantial economic damage. They remain a
45 serious challenge in agriculture, especially in Mediterranean, Central and Eastern Europe
46 (Parker 2009) because some of these weed broomrapes can infest a broad host range. The
47 branched broomrape, *Phelipanche ramosa* (syn. *Orobanche ramosa* L., Joel 2009), can infest
48 a wide set of major crops, but also many weeds among Brassicaceae, Cannabaceae,
49 Cucurbitaceae, Fabaceae and Solanaceae (Brault et al. 2007; Gibot-Leclerc et al. 2003; Parker
50 2009; Gibot-Leclerc et al. 2016; Pointurier et al. 2019), thus increasing its harmfulness. In
51 Europe, and especially in France, *P. ramosa* represents a major agronomic problem in tobacco
52 fields, leading to yield and quality losses that may be complete, and then to the long-term
53 abandonment of this crop production on highly infested arable fields (Brault et al. 2007; Julio
54 et al. 2020).

55 Currently, crop protection against broomrapes has largely focused on chemical control
56 using synthetic herbicides. This lever is usually combined with others, such as cropping
57 practices preventing seed dispersal, trap crop, biological control and/or varietal resistance, as
58 part of an integrated weed management program (Pérez-De-Luque et al. 2009; Rubiales and
59 Fernández-Aparicio 2012; Fernández-Aparicio et al. 2016). However, agronomic,
60 environmental, public-health and socio-economic issues related to the side effects of chemical
61 control methods question the relevance of this lever to manage broomrapes (van der Werf 1996;
62 Loyce et al. 2012; Bourguet and Guillemaud 2016; van Bruggen et al. 2018). The development
63 of a more sustainable agriculture based on agro-ecological approaches is aimed at reducing the
64 use of synthetic pesticides, while continuing to ensure a high level of production (Duru et al.
65 2015; Cordeau et al. 2016; Petit et al. 2018; Vila-Aiub 2019).

66 *P. ramosa* is characterized by a biological cycle consisting of two distinct phases
67 (Gibot-Leclerc et al. 2012). The first phase is underground, including seed germination,
68 attachment to host roots and penetration into host tissues with the development of an absorptive
69 system called haustorium. The second phase is aboveground, with the emergence and growth
70 of floral stems, flowering and fructification (Gibot-Leclerc et al. 2012). The long duration of
71 the underground phase usually leads to a reduced effectiveness of the standard levers identified
72 so far, making their use limited to the end of its biological cycle or/and very expensive (Gibot-
73 Leclerc et al. 2012; Gaba et al. 2017). The end of the second phase leads to the release of
74 several hundred thousand tiny seeds per floral spike, seeds characterized by a longevity in the
75 soil of several decades (Gibot-Leclerc et al. 2012; Fernández-Aparicio et al. 2016). Moreover,
76 the seed germination of *P. ramosa* is a two-step process, including first a conditioning period
77 requiring suitable environmental conditions, and then a period during which seeds become
78 sensitive to germination stimulants exuded by surrounding host roots, such as strigolactones
79 and isothiocyanates (Fernández-Aparicio et al. 2011; Auger et al. 2012). All these traits explain
80 why managing *P. ramosa* is particularly difficult.

81 Among several options based on bioherbicides, such as microbial herbicides,
82 mycoherbicides, allelopathic plants producing herbicidal compounds and biochemical
83 herbicides (Fernández-Aparicio et al. 2020; Gibot-Leclerc et al. 2022), an interesting one
84 would be to use the potential herbicidal effect of natural products like amino acids, especially
85 because this effect can potentially affect the germination rate of *P. ramosa* while depleting the
86 soil seedbank by causing a high seed mortality rate (Vurro et al. 2006; Fernández-Aparicio et
87 al. 2020). Amino acids are organic molecules that play an important role in cell metabolism.
88 In particular, some amino acids can intervene in the regulation of biosynthesis pathways of
89 other amino acids (Galili et al. 2016), or as precursors in pathways related to the storage of
90 mineral elements such as nitrogen and sulphur. Some studies have assessed their effects on

91 parasitic plants (Vurro et al. 2006; Nzioki et al. 2016; Fernández-Aparicio et al. 2017; Kuruma
92 et al. 2021). In particular, Vurro et al. (2006) showed that some amino acids can decrease more
93 or less the germination rate of *P. ramosa* or even inhibit it. This is especially the case of
94 arginine, proline and histidine that appears to strongly inhibit *P. ramosa* germination.
95 Nevertheless, these studies did not assess the persistence of the inhibitory effect of germination
96 after the removal of the amino acid from the medium. Moreover, these studies do not indicate
97 the state of the seed in the absence of germination, even though the seed may be dormant or
98 dead.

99 The successful biocontrol with amino acid lies in distinguishing the different effects of
100 amino acids on seeds. As an annual plant, *P. ramosa* growth and survival depends on the seeds
101 occurring in the soil seedbank. Therefore, the management of the seeds by amino acid could
102 have a curative effect, by reducing or delaying the germination and emergence of *P. ramosa* or
103 a preventive effect, by depleting the soil seedbank. To our knowledge, no study has ever tested
104 these different effects: inhibiting, delaying or killing.

105 This study aims at identifying amino acids characterized by a high effect on the *P.*
106 *ramosa* seeds through in vitro experiments, especially by focusing on *P. ramosa* populations
107 parasitizing tobacco, so as to assess the relevance of using amino acids in field conditions. To
108 do this, fourteen amino acids were chosen and applied on *P. ramosa* seeds in order to assess
109 their potential effect and determine the various causes of this effect. Our main hypotheses are
110 (1) that at least some amino acids can reduce more or less strongly the germination rate of *P.*
111 *ramosa* seeds (inhibitory effect), (2) that this reduction of the germination rate may be
112 transitory and can disappear if the amino acids are removed from the medium (delaying effect),
113 (3) that this reduction effect may be permanent and related to seed death (killing effect).

114

115

116 **Materials and methods**

117

118 **Seed material and conditioning**

119

120 Seeds of *Phelipanche ramosa* were harvested from a natural population within a highly infested
121 field of tobacco located in the Nouvelle-Aquitaine region, a region characterized by the highest
122 levels of infestation of this parasitic weed in tobacco in 2017 (45° 53' 38.908" N, 0° 0' 36.803"
123 E; Aigre). All seeds were kept in watertight glass containers at approximately 20°C until the
124 beginning of the experiment.

125 Before any experiment, *P. ramosa* seeds were conditioned to make them susceptible to
126 germination stimulants. Seeds were surface disinfected under a laminar flow hood by a 5-min
127 immersion in 70% ethanol, followed by a 5-min immersion in a solution of Ca(OCl)₂ at 3%
128 (p/v) and Tween 20 (0.1%) so as to limit fungal spread (Gibot-Leclerc et al. 2012). Seeds were
129 rinsed five times with twice-distilled water and placed onto 1 × 1 cm paper squares
130 (Whatman®, Glass microfiber filters GF/A) using a pipette. Pipetting resulted in a variable
131 number of seeds per paper square, i.e. between 8 and 155 seeds. However, positioning a similar
132 number of seeds onto the paper squares would have required using a stereoscopic microscope
133 to transfer the seeds one by one because of their small size (350 × 250 μm, Gibot-Leclerc et al.
134 2012), which was not possible under sterile conditions under the laminar flow hood. The paper
135 squares were placed on a Whatman® GF/A paper sheet (Ø 90 mm) at the bottom of a Petri dish
136 (Ø 90 mm) and moistened with 3 mL of sterile distilled water. These Petri dishes were sealed
137 with Parafilm® (American Can Company), wrapped in aluminum foil and placed in a dark
138 growth chamber, at 20°C for 14 days, to condition *P. ramosa* seeds (Gibot-Leclerc et al. 2004).

139

140 **Amino acids**

141

142 Fourteen amino acids were tested in this study, including eight amino acids which had already
143 been tested on *P. ramosa* populations harvested from infested fields of tobacco and tomato in
144 Italy (Vurro et al. 2006): These chosen amino acids: alanine, arginine, glutamine, glycine,
145 histidine, lysine, methionine and proline, reduced the germination rate by at least 60%, except
146 for glutamine. Moreover, five amino acids had only been tested on other parasitic weeds, i.e.
147 *Orobanche minor* (Fernández-Aparicio et al. 2017) and/or *Striga hermonthica* (Delile) Benth.
148 (Nzioki et al. 2016): aspartate, isoleucine, phenylalanine, tryptophan and tyrosine, and one
149 amino acid, asparagine, had never been tested on parasitic plants of the genera *Orobanche*,
150 *Phelipanche* or *Striga*. All amino acids were supplied by the Sigma-Aldrich company (Sigma-
151 Aldrich, St. Louis, MO, United States).

152

153 **Seed germination assay**

154

155 The germination stimulant strigol analogue GR24 was added to each Petri dish at 1 mg/L
156 (Mangnus et al. 1992), a suitable concentration for this population of *P. ramosa* to achieve a
157 maximum percentage of germinated seeds 10 days after application higher than 90%. The
158 GR24 was provided by Dr. Binne Zwanenburg (Radboud University, Nijmegen, The
159 Netherlands). The concentration of amino acids was set at 2 mM, based on the results of Vurro
160 et al. (2006) on the application of amino acids to seeds of *P. ramosa* harvested from tobacco
161 fields. We have grouped the 14 amino acids into four sessions, with each session including a
162 positive control (only GR24, hypothesizing a high percentage of *P. ramosa* germinated seeds)
163 and a negative control (only water, hypothesizing no germinated seeds). A seed was considered
164 germinated when the radicle had pierced the seed coat (Gibot-Leclerc et al. 2004). In the
165 following, viable seeds correspond to germinated seeds and seeds colored with TTC (see

166 section *Determination of seed viability*). In order to identify the optimal concentration of GR24
167 and the minimum duration of the exposure time before counting, two concentrations of GR24
168 were tested (1 mg/L and 10 mg/L) and the number of germinated seeds of *P. ramosa* was
169 counted at two periods after application of the GR24 (5 and 10 days). The results obtained
170 highlighted, for the concentrations 1 mg/L and 10 mg/L respectively, a germination rate of
171 67.2% and 86.5% 5 days after GR24 application, and of 99.2% and 94.7% 10 days after
172 application, thus leading to retain a concentration of GR24 of 1mg/L and a minimum exposure
173 time of 10 days after application of this concentration.

174

175 **Potential inhibitory effect of amino acids on the germination of *P. ramosa* seeds**

176

177 The amino acids were weighed and introduced into volumetric flasks with the GR24 solution
178 at 1 mg/L, and then 3 mL of this solution were taken in order to moisten the paper at the bottom
179 of the Petri dishes. Four paper squares with the conditioned seeds of *P. ramosa* were transferred
180 into a Petri dish. Each modality (i.e. amino acids and the positive control) included three Petri
181 dishes, for a total of 12 small paper squares, i.e. between 48 and 391 seeds per Petri dish, except
182 for the negative control modality which had only one Petri dish with 4 paper squares, i.e.
183 between 171 and 272 seeds. These Petri dishes were sealed with parafilm and wrapped in
184 aluminum foil, and then placed at 20°C in the dark for 10 days. The number of germinated
185 seeds of *P. ramosa* was counted 10 days after application of the treatment carried out in this
186 first step using a stereoscopic microscope (1.95x–250x).

187

188 **Potential reversibility effect of amino acids on the inhibition of germination of *P. ramosa*** 189 **seeds**

190 The aim of this second step was to assess whether the seeds of *P. ramosa* that did not germinate
191 during the first step of the experiment can germinate after the removal of the amino acid from
192 the medium. New Petri dishes were moistened with 3 mL of GR24 solution at 1 mg/L. Each
193 paper square with the seeds of *P. ramosa* tested during the first step was transferred into these
194 new Petri dishes. These dishes were sealed with parafilm and wrapped in aluminum foil, and
195 then placed at 20°C in the dark for 10 additional days. The number of germinated seeds of
196 *P. ramosa* was counted 10 days after application of the treatment carried out during the
197 previous step, i.e. for 20 days after the beginning of the experiment, using a stereoscopic
198 microscope (1.95x–250x). Given the small size and large number of *P. ramosa* seeds, it was
199 technically impossible to wash the seeds during this step.

200

201 **Determination of seed viability**

202

203 The viability of the *P. ramosa* seeds that did not germinate after these two steps was assessed
204 by adding 3 mL of 2,3,5-triphenyl tetrazolium chloride (TTC, Sigma-Aldrich) at 1% in each
205 Petri dish. The Petri dishes were wrapped in aluminum foil, and placed in a drying oven at
206 40°C for 48 h. The seeds were then placed onto a new Whatman® GF/A paper sheet (Ø 90
207 mm) soaked with a saline solution (1% NaOCl). Viability was assessed after 20 min.
208 Ungerminated viable and ungerminated non-viable seeds were counted under a stereoscopic
209 microscope, seeds characterized by an embryo exhibiting no carmine red staining being
210 considered as non-viable (Gibot-Leclerc et al. 2004).

211

212 **Statistical analysis**

213

214 For the subsequent statistical analyses and graphical representation, the number of seeds on the
215 four squares per Petri dish and then on the three Petri dishes of a same modality was summed
216 for each session, except for the negative control modality (only water), which had only one
217 Petri dish for each session. In order to validate the absence of any session effect on the
218 germination rates for the positive control between the four different sessions, a 4-sample test
219 for equality of proportions without continuity correction was applied, the null hypothesis being
220 that the proportions in these four sessions are the same. All analyses were made with R version
221 4.0.2 (R Core Team 2020) and the stats package. Raw data of this experiment are available in
222 Supporting Information (Table S1).

223

224

225 **Results**

226

227 **Control analysis**

228

229 In each session, no seed germination was observed for the negative control (only water), but
230 the seeds were still viable 20 days after the beginning of the experiment. The viability rate was
231 always above 85% based on TTC test. In the absence of differences between sessions for the
232 two controls, the raw results are presented for the entire experiment (refer to Supporting
233 Information for details).

234

235 **Inhibitory effect of amino acids on the germination of *P. ramosa* seeds**

236

237 Considering the germination rate of *P. ramosa* seeds after the application of amino acids, we
238 observed variable responses depending on the amino acid considered (Fig.1a). Based on the

239 confidence intervals (Table 1), four amino acids were characterized by a very strong inhibitory
240 effect with germination rates below 10% (phenylalanine, arginine, lysine and tryptophan; Fig.
241 1a; Table 1). Six amino acids (aspartate, glycine, proline, histidine, methionine and isoleucine)
242 had a low inhibitory effect with a germination rate above 70% (Fig. 1a; Table 1). Two amino
243 acids (asparagine and glutamine) had no inhibitory effect with a germination rate comparable
244 to that of the positive control (only GR24).

245

246 **Permanent vs. temporary effect of amino acids on the inhibition of germination of** 247 ***P. ramosa* seeds**

248

249 For some of the four amino acids with a strong inhibitory effect, the removal of the amino acid
250 from the medium highly increases this germination rate, thus suggesting a temporary effect of
251 the amino acid on the inhibition of germination of *P. ramosa* seeds. This is the case for arginine
252 and lysine, for which the germination rate after the removal of the amino acid was 85.0% and
253 56.9% respectively (Fig. 1b). For these two amino acids, the inhibitory effect would therefore
254 be transient, whereas for phenylalanine and tryptophan, the germination rate remains very low,
255 suggesting a permanent effect of the amino acid.

256

257 **Determination of seed viability**

258

259 Among the ungerminated seeds after the two previous steps of this experiment, the application
260 of TTC showed that only a small number of seeds were still viable, from 0.0% at 2.2% with a
261 mean of 0.9% similar to that of the positive control (only GR24). On the other hand, the
262 mortality rate of ungerminated seeds varied very strongly depending on the amino acid, from
263 about 7% to almost 100% (Fig. 2). For some amino acids, this rate was close to that observed

264 for the negative control (only water) but for most of the other amino acids, it appeared much
265 higher. This result suggests that the amino acids for which the application decreased the overall
266 germinated seed rate therefore induced the death of *P. ramosa* seeds (Fig. 2).

267

268

269 **Discussion**

270

271 **Inhibitory effect of amino acids on the germination of *P. ramosa* seeds**

272 Our study showed that *in vitro* application of several amino acids leads to an inhibitory effect
273 on the germination of *P. ramosa* seeds. Among the 14 amino acids tested, only two, asparagine
274 and glutamine, had no effect on the germination rate while only four, phenylalanine, arginine,
275 lysine and tryptophan, had a strong effect on the germination rate of *P. ramosa* seeds. For these
276 four amino acids, the germination rate is lower than 10%. When considering the results of the
277 study by Vurro et al. (2006), carried out on *P. ramosa* seeds harvested from tobacco, i.e. the
278 same pathosystem as in our study, we found that these results differed for most amino acids.
279 As an example, Vurro et al. (2006) highlighted that arginine, histidine and proline showed the
280 higher inhibitory effect of *P. ramosa* seeds, but only arginine had the same effect in our study
281 (Table S2). We hypothesize that these differences may be due to differences in the experimental
282 protocols concerning GR24 concentration and exposure time to amino acids before counting,
283 or may be due to genetic differences between *P. ramosa* populations (Gibot-Leclerc et al.
284 2021).

285 For lysine and arginine, the inhibitory effect was transient, disappearing partially for
286 lysine and completely for arginine when these amino acids are removed from the medium. This
287 was not the case for the amino acids phenylalanine and tryptophan. Recently, L-tryptophan has
288 been identified as a molecule characterized by a high inhibitory effect of the germination of

289 *Orobancha minor* seeds (Kuruma et al. 2021). Our study is the first to demonstrate the transient
290 effect of some amino acids, while the inhibitory effect alone had been tested before (Vurro et
291 al. 2006; Nzioki et al. 2016; Fernández-Aparicio et al. 2017).

292

293 **Killing effect of amino acids on *P. ramosa* seeds**

294 All the amino acids had a killing effect on the *P. ramosa* seeds, this effect varying from 7% to
295 99%. The lower the overall germinated seed rate, the higher the mortality rate for a given amino
296 acid, suggesting especially that the low overall germinated seed rate (with and after removal of
297 amino acid) of some amino acids is due to a high seed mortality rate. In any case, whatever the
298 amino acid applied, the number of ungerminated but still viable seeds was low. For two amino
299 acids, phenylalanine and tryptophan, the mortality rate was greater than 90%. Recent studies
300 (Dor et al. 2017; Shilo et al. 2017) have shown that the effective control of *P. aegyptiaca* by
301 glyphosate was due to a complex mechanism of action including inhibition of the aromatic
302 amino acid biosynthetic pathway and, as a side effect, limitation of translocation of mobile
303 solutes from the phloem to the parasite. According to Rubiales et al. (2018), some amino acids
304 may have similar effects to such an herbicide due to feedback inhibition of metabolic pathways.
305 Phenylalanine and tryptophan could then be used as alternatives to these synthetic herbicides
306 through their direct effect on broomrape pre-attached stages, mainly during seed germination
307 and development of the attachment organ (Vurro et al. 2006; Fernández-Aparicio et al. 2020;
308 Kuruma et al. 2021).

309

310 **Using phenylalanine and tryptophan for their biocide effect**

311 Our study highlighted the interest of phenylalanine and tryptophan for their biocide effect on
312 *P. ramosa* seeds. However, this effect has been observed only in *in vitro* conditions, and now
313 its effectiveness remains to be demonstrated on the *P. ramosa*/tobacco pathosystem under

314 controlled conditions. Indeed, previous studies have shown that various factors must be taken
315 into consideration to ensure appropriate use of this agronomic lever in field conditions (Vurro
316 et al. 2006; Nzioki et al. 2016; Fernández-Aparicio et al. 2017).

317 The potential variability of the efficacy of amino acid application, as well as the
318 associated costs, could be a major concern and would limit their use in field crops on a large
319 scale. An optimization of the way amino acids are used could thus help improving their
320 development in agriculture. Because of technical difficulties, it has so far been impossible to
321 develop on a large scale the applications put forward by Vurro et al. (2006). However, we
322 suggest proposals to deal with these difficulties for the coming years in the present study.

323 As a first option, we can use the allelochemical potential of some species and/or
324 varieties to identify those most able to produce the amino acids of interest (Cimmino et al.
325 2018; Qasem and Issa 2018). As an example, *Avena sativa* L. seems to excrete L-tryptophan
326 in these root exudates, which inhibits the growth of other plants (Kato-Noguchi et al. 1994).
327 Then it might be possible to include this species in a crop mixture (Fernández-Aparicio et al.
328 2007; Fernández-Aparicio et al. 2010) or as a cover crop in a crop sequence (Rubiales and
329 Fernández-Aparicio 2012). If this option proved effective, this lever could be used in cropping
330 systems as a part of integrated strategy to reduce the seedbank of *P. ramosa*.

331 As a second option, the production of amino acid may be due to certain fungi that
332 overproduce and excrete the relevant amino acid. For instance, Nzioki et al. (2016) developed
333 amino acid overproducing lines of *F. oxysporum* f. sp. *strigae* and demonstrated their enhanced
334 biocontrol action during management of *Striga hermonthica* on smallholder farms. Screening
335 for fungi occurring in the soil rhizosphere (Vurro et al. 2018) or as endophytic community of
336 *P. ramosa* and having deleterious effects of this weed is a promising approach. Currently,
337 certain species have been isolated from *P. ramosa* and their pathogenic power has been
338 characterized, as is the case for *Talaromyces trachyspermus* (Boari and Vurro 2004; Hemmati

339 and Gholizadeh 2019) and *Fusarium venenatum* (Gibot-Leclerc et al. 2022). At this stage,
340 relying on these fungal species to create amino acid overproducing lines could be a relevant
341 management option for *P. ramosa*.

342 As a last option, the biocide effect could be tested by encapsulating the amino acid in a
343 matrix that would permit a targeted treatment and a prolonged release of the product onto the
344 *P. ramosa* seeds. The encapsulation of traditional synthetic herbicides has already been
345 proposed with this objective (Sopeña et al. 2009). Many formulations are based on the use of
346 alginate gels (Ramos-Campos et al. 2015). This type of matrix provides a particulate form that
347 appears suitable for use in the field during sowing or can be used as a seed coating material.
348 The state of the art has also shown the interest of formulating these hydrogels by adding
349 adsorbent agents of inorganic (silica/clays) or organic (cellulose fibers) nature to control the
350 degree of release (Wang and Caruso 2005; Flores-Cespedes et al. 2007; Alila and Boufi 2009;
351 Fernández-Pérez et al. 2011). Therefore, the approach would be to determine a formulation
352 method combined with a determination of the lifetime of the amino acids in the environment.
353 The successful application of phytotoxic amino acids on *P. ramosa* would depend mainly on
354 how they are formulated. Among the new techniques that could be used, biopolymeric
355 nanomaterials could make it possible to deliver active agro-compounds in a targeted,
356 biocompatible, biodegradable and non-toxic way, while maintaining or even improving the
357 efficacy of the product (Namasivayam et al. 2023).

358

359 This work in *in vitro* conditions constitutes the first step of use of amino acid to control seed
360 germination and to deplete soil seedbank of *P. ramosa*. Future work will focus on the
361 optimization of amino acid application in field conditions, including concentration, timing and
362 formulation of this application.

363

364 **Acknowledgements**

365 We thank Lucie Guinhard and Carole Reibel for their technical assistance. We thank Lucien
366 Gatard (CT2F) for the supply of broomrape seeds. This work was supported by CASDAR FAM
367 201939 ELIOT.

368

369 **Statements and Declarations**

370 **Conflict of interest** The authors have no competing interests to declare that are relevant to the
371 content of this article.

372

373 **Author contributions** Conceived and designed the experiments: SGL, FD. Performed the
374 experiments: SGL, MC. Analyzed the data: FD, RP. Wrote the paper: SGL, FD, MC, RP.
375 Supervised the work: SGL, FD. Acquired funding: SGL.

376

377 **Data availability** The datasets generated and analysed during the current study are available
378 from the corresponding author on reasonable request.

379

380

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382

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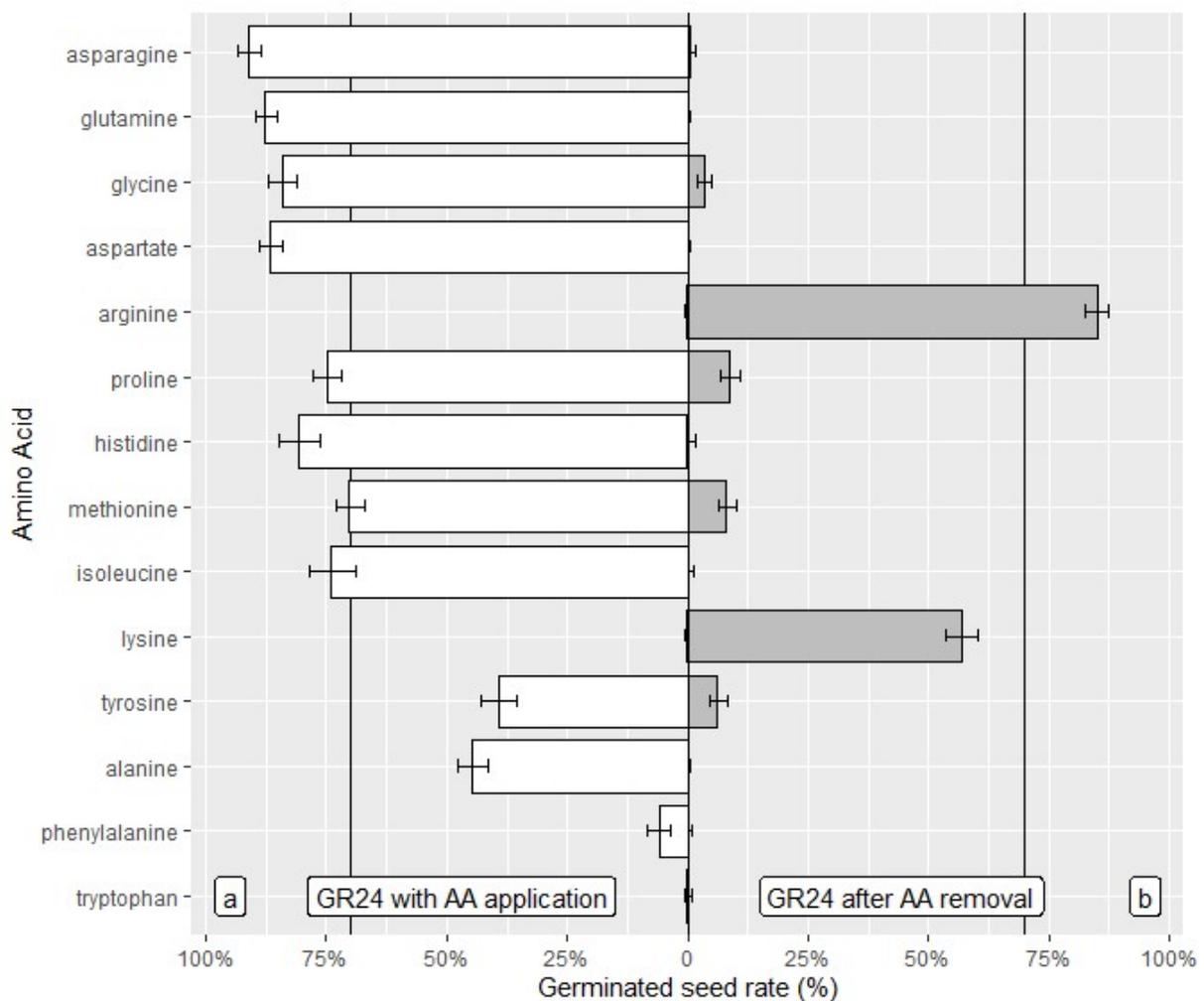
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545 **Figures**

546 **Fig. 1** Effect of amino acids on the germination rate of *P. ramosa* seeds: a) with GR24 and
547 amino acid application; b) with GR24 after removal of amino acid. Amino acids are ordered
548 according to their overall germinated seed rate (i.e. the sum of the germinated seeds with and
549 after removal of amino acid). Error bars correspond to 95% confidence interval. See Material
550 and methods for experimental details



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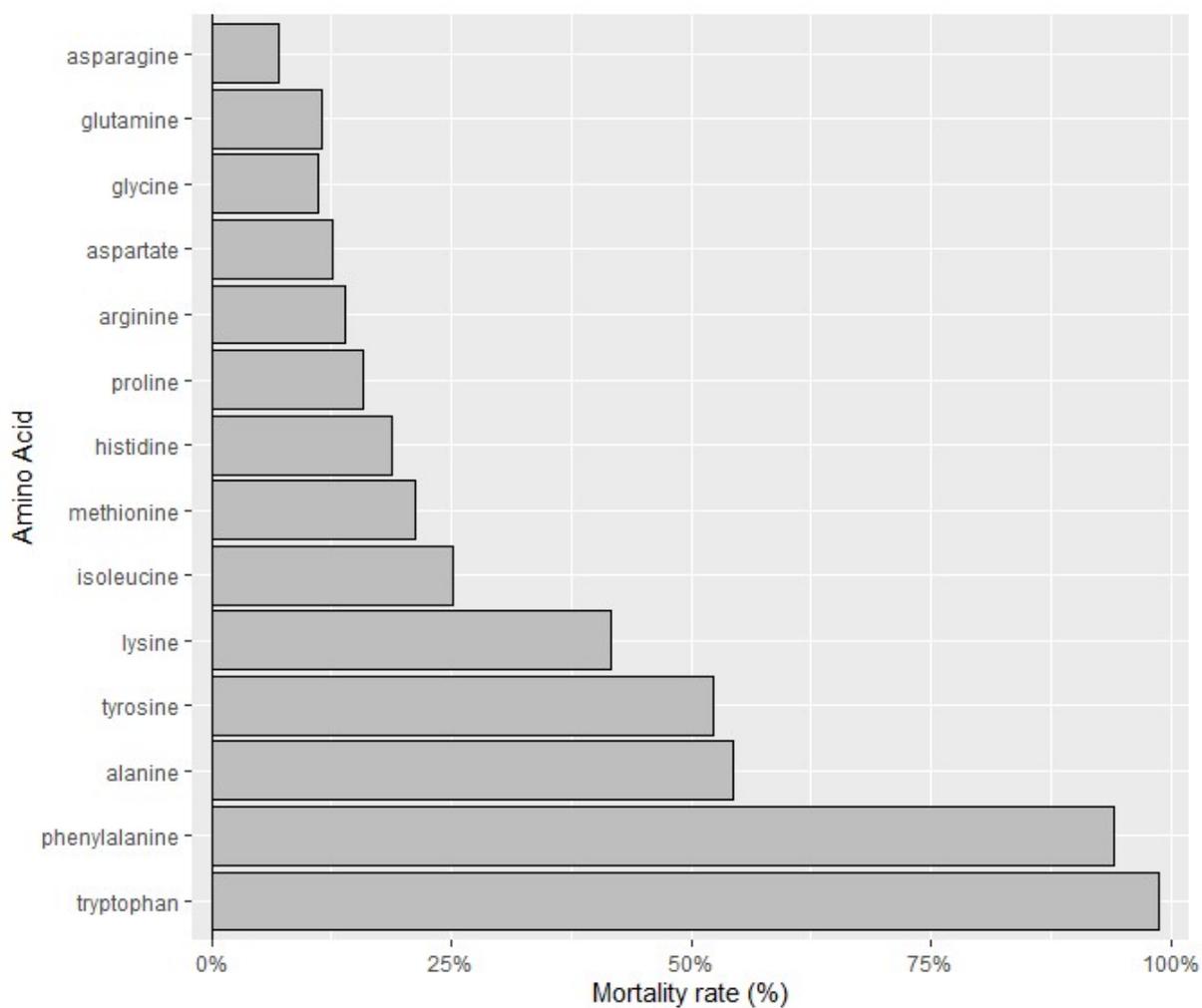
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558 **Fig. 2** Mortality rate of ungerminated seed of *P. ramosa*. Amino acids are ordered according

559 to their overall germinated seed rate, i.e. the same order as in Fig. 1. See Material and methods

560 for experimental details



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562

563 **Table 1** Germination rate (%) and confidence interval of *P. ramosa* with GR24 and amino acid
564 (AA) application (step 1) and then with GR24 after removal of amino acid (step 2). Amino
565 acids are ordered according to their overall germinated seed rate, i.e. the same order as in Fig.
566 1. * Amino acid with germination rate (step 1) not significantly different from GR24 only
567

Session	Amino acid	GR24 with AA application		GR24 after AA removal	
		Germination rate	CI 95%	Germination rate	CI 95%
S4	asparagine*	91.1%	[88.5; 93.3%]	0.7%	[0.2; 1.8%]
S3	glutamine*	87.7%	[85.3; 89.8%]	0.0%	[0.0; 0.4%]
S4	glycine	84.2%	[81.0; 87.0%]	3.4%	[2.1; 5.1%]
S3	aspartate	86.8%	[84.2; 89.1%]	0.0%	[0.0; 0.5%]
S2	arginine	0.1%	[0.0; 0.7%]	85.0%	[82.4; 87.3%]
S2	proline	74.9%	[71.7; 77.9%]	8.7%	[6.8; 10.9%]
S1	histidine	80.8%	[76.2; 84.9%]	0.3%	[0.0; 1.6%]
S2	methionine	70.2%	[67.1; 73.1%]	8.1%	[6.4; 10.1%]
S3	isoleucine	73.9%	[68.7; 78.6%]	0.0%	[0.0; 1.2%]
S2	lysine	0.1%	[0.0; 0.7%]	56.9%	[53.5; 60.3%]
S4	tyrosine	39.3%	[35.6; 43.1%]	6.2%	[4.5; 8.3%]
S1	alanine	44.6%	[41.4; 47.9%]	0.0%	[0.0; 0.4%]
S3	phenylalanine	5.7%	[3.5; 8.5%]	0.0%	[0.0; 1.0%]
S2	tryptophan	0.0%	[0.0; 0.5%]	0.3%	[0.0; 0.9%]

568