Allelopathic persistence of Helianthus tuberosus L. residues in the soil

Allelopathic persistence of *Helianthus tuberosus* L. residues in the soil

Tesio F.[*], Vidotto F., Ferrero A.

[1*]*Dipartimento di Agronomia, Selvicoltura e Gestione del Territorio, Università degli Studi di Torino, Italy. Via L. da Vinci, 44 – 10095 Grugliasco – tel: +39 0116708785, fax:+39 0116708798, e-mail: franco.tesio@unito.it*

Abstract

Helianthus tuberosus (Jerusalem artichoke) has been reported to be highly invasive in European cropping systems; simultaneously, there is a growing interest in it as a cultivated crop in middle and southern Europe. This study investigated the allelopathic effect of Jerusalem artichoke residues on the germination and early growth of crop and weed species with two experiments carried out in greenhouse. Experiment 1 was conducted by incorporating 0.5, 1.0 or 2.0 t ha^{-1} of dried *H. tuberosus* leaf tissue into pots filled with sand at 0, 7, 14, 21, 28, and 35 days prior to seeding lettuce, pea, or *Digitaria sanguinalis*. Experiment 2 was carried out by incorporating 2.5 or 5.0 t ha^{-1} of total plant residues into pots filled with naturally infested soil collected from *H. tuberosus*-free fields. Of the species considered, *D. sanguinalis* showed the highest sensitivity to the allelopathic activity of Jerusalem artichoke; germination reductions of more than 30% were observed in almost all residue incorporation times. Weed community experiment provided good evidence of the allelopathic potential of *H. tuberosus* residues. Incorporating various amounts of *H. tuberous* remains into sand planted with test seeds showed weed development and growth were progressively more inhibited with increasing residue substrate exposure time. The results of this study do not only increase the knowledge on the allelopathic potential of *H. tuberosus*, but bring the attention to the residual effect overtime.

Keywords: Jerusalem artichoke; residue degradation; plant invasion, weed infestation

1. Introduction

Weeds are one of the major constraints to plant yield worldwide, and herbicide use has risen significantly over the recent decades. Much of that growth has been driven directly by increased

labour costs and inversely by available and effective alternative weed controls (Brethour and Weersink, 2001). To invert this trend of reliance on chemical weed managements, alternative strategies are under development which tend toward finding biological solutions to minimize the unsafe impacts of herbicide and insecticide use in agriculture (Xuan et al., 2005).

One means by which to optimise pest and weed control is allelopathy, which is defined as the ability of a plant to release chemical compounds that have a depressive impact on the development and/or growth of other plants or species (Weston, 1996). The study of allelopathy and allelochemicals is considered an attractive method for weed control because of its environmental friendliness. Explanations of not only the allelopathy mechanism, but also the influence of secondary metabolites from plant synthesized phytochemicals (Reigosa et al., 1999; Petchey, 2003) have been attempted and put forth. In fact, several studies of the allelochemical mode of action and role in plant interactions have outlined how allelopathy could be developed through breeding and/or genetic manipulation to increase crop cultivar competitiveness (Weston and Duke, 2003) despite the considerable hurdles of time and monetary investment. By contrast, the simple introduction of allelopathic species into the crop rotation or utilising allelopathic plants as living/green mulches has been suggested as a cost-effective way to reduce weed presence (Tesio and Ferrero, 2010). Unfortunately, historical allelopathic research in this area has limited value due to poor methods rigor and inappropriate allelochemical concentration range choices used in several cases (Olofsdotter et al., 2002). Interesting findings show an approximate reduction of 70 – 80% of *Echinochloa crus-galli* (L.) Beauv. growth, observed after incorporating 1 or 2 t ha^{-1} of plants with strong allelopathic activity into the soil (Khanh et al., 2005a; Khanh et al., 2005b; Tesio et al., 2010). Generally, the selective effects of allelopathic materials on weed species are demonstrated regardless of growing conditions—laboratory, greenhouse, or in the field (Tesio et al., 2011). However, there is a need for more strictness in natural condition experiments and more laboratory experiment validation under field settings; in the interim, our work made use of an established knowledge base.

If allelopathy is to be a profitable weed control measure, then its research requires more accuracy. For example, the mere existence of a phytotoxic extract does not indicate allelopathic potential. That is, even though large quantities of organic solvent-rich extracts enable allelochemical compounds and their associated metabolites to be identified, their presence alone is not proof of allelopathy. From an allelopathic perspective, phytotoxic compounds are not considered suitable if they are not released into their environment, and the fate of the allelochemical into the soil should be considered. Consequently, inhibitory activity should be assessed as a function of not only the size of the phytotoxic exudates, but also its release into its surroundings. In fact, in order to have an allelopathic effect against weeds, it is important that the presence of the phytotoxic compounds released by a donor plant coincide with the germination or emergence period of the sensitive weed. If the moment of exudation/release of the allelochemicals does not coincide with the uptake period of the sensitive species, then the allelopathic compounds should at least persist sufficiently long in the soil to develop its depressive effect.

Helianthus tuberosus L. (Jerusalem artichoke), a member of the Asteraceae family, was introduced into Europe at the end of 1500s from North America, and was widely cultivated for both human and livestock consumption (Swanton et al., 1992). The economic importance of this species has varied across countries, and its success as a food crop has been hampered by the massive diffusion of alternative tuber crops such as potato. In addition to its importance as a crop, its easy propagation by tubers and stolons transformed the species into an invasive plant in numerous environments and a significant weed of field crops (Török et al., 2003). *H. tuberosus* has been found in several European countries in natural settings such as riverbanks (Schnitzler et al., 2007). Tall and dense stands of it have even been observed to depress native taxa in Austria, so much as to result in the formation of a new vegetation type (Wadsworth et al., 2000).

In cultivation, the emergence of volunteer shoots of Jerusalem artichoke, even when buried at a depth of about 30 cm, may result in crop yield and quality losses, as well as unintended spread by subsequent cultivation. For example, soybean yield reductions from 31 to 71% have been reported

77 with a medium infestation of *H. tuberosus* tubers. In corn, a density of approximately four tubers
78 m^{-2} caused a yield reduction of 16 to 25% (Wyse et al., 1980). Northern Italian field observations
79 have shown the weed arises in almost all open-field row crops, particularly when *H. tuberosus* was
80 cultivated in preceding years or in uncropped fields (Tesio et al., 2011).

81 Despite its invasive tendency, Jerusalem artichoke is of interest today as a food for direct
82 human consumption, as row material for industrial sectors using inulin, and as a sweetener, or as an
83 input for ethanol production (Swanton et al., 1992). The species can be profitably grown for
84 livestock silage feed (Swanton et al., 1992; Seiler, 1993; Seiler and Campbell, 2004). Another value
85 of Jerusalem artichoke is to sunflower cultivar (*H. annuus* L.) breeders who use it to transfer
86 *Sclerotinia sclerotiorum*-resistant genes (Cassells and Walsh, 1995). Jerusalem artichoke has
87 detrimental effects as well; several papers have reported them. These related to resource
88 competition, but ironically, also to its allelopathic potential (Khanh et al., 2005b). Previously
89 published studies demonstrated the strong potential allelopathic activity of *H. tuberosus*, which was
90 associated with its aqueous shoot extracts (Tesio et al., 2008), dried residues (Vidotto et al., 2008;
91 Tesio et al., 2010) or root exudates (Follis et al., 2010). Tesio et al. also (2011) also proved the
92 severe intensity of the allelopathic activity of this plant under open field conditions.

93 In summary, this research project was designed to evaluate the potential phytotoxic effects
94 of Jerusalem artichoke relative to its persistence on crop and weed species commonly associated
95 with it. That is, our investigation sought to determine whether or not the moment of release of the
96 allelopathic compounds from *H. tuberous* matched the moment of uptake by pea (*Pisum sativum* L.)
97 and lettuce (*Lactuca sativa* L.) crops or *Digitaria sanguinalis* (L.) Scop. weed, or at least, if the *H.*
98 *tuberous* allelopathic activity remained present for a sufficient period of time so as to cause a
99 suppressive effect.
100

101 # 2. Material and methods

102 *2.1. Plant material*

103 Tubers of wild *H. tuberosus* L. were collected during August 2005 in heavily infested corn
104 fields in northwestern Italy. After collection the tubers were transplanted into plastic pots (20 cm
105 diameter) filled with commercial potting media (Metromix 360). The pots were placed in a
106 greenhouse in which temperatures were maintained at 23 - 30°C daily. Supplemental metal halide
107 lighting of 12 hours per day was applied in the fall and winter months as needed. Plants were
108 watered from overhead and fertilized as needed with soluble fertilizer (NPK 21-5-20). Jerusalem
109 artichoke shoots were harvested periodically (generally each month over ten weeks) by cutting
110 stalks 10 cm above the soil surface, and selecting vigorous individuals. The leaves were separated
111 from the stalks, placed in open trays and dried in the laboratory oven (35°C) until constant weight
112 was achieved. The material was then stored in tightly closed plastic containers until use to maintain
113 dryness.

114 A total of two crops (lettuce and pea) and a weed (*D. sanguinalis*) were used as indicator
115 species. These plants were chosen as they represent crops in which Jerusalem artichoke may present
116 as a weed, or by contrast, weed species infesting *H. tuberosu*s cultivation. The weed seeds were
117 purchased from Herbiseed Company (Twyford, UK).

118

119

120 *2.2. Allelopathic persistence of* H. tuberosus *leaf tissue (experiment 1)*

121 This experiment was conducted from June 2006 to December 2006 in the experimental
122 greenhouse of the Department Agroselviter, Università degli Studi di Torino (Italy), using plastic
123 trays (28 x 18 cm, 5 cm height) filled with sand. The experiment was set up following a 3 x 6
124 factorial design, in which the residue quantity and the residue incorporation time represented the
125 first and second factor, respectively. Quantities of 49.14 g, 98.26 g, and 196.56 g *H. tuberous* dry

126 leaves were added to the trays. These amounts correspond to about 0.5, 1, and 2 t ha^{-1} of dry
127 residue, which are the same rates as those used in previously published experiments for evaluating
128 the allelopathic potential of this species (Tesio et al., 2010). The ground material was mixed well
129 with the sand before filling the trays, and 300 mL of water was then added to each tray. Trays
130 preparation were carried out at 35, 28, 21, 14, 7 and 0 days before seeding (hereafter called timing I,
131 II, III, IV, V, and VI). After each preparation, the trays were sealed in a plastic bag to avoid
132 moisture loss, then left in a dark growth chamber at a constant 25°C. The controls were represented
133 by trays filled with sand only. When the last trays were prepared (timing VI), all the trays, from all
134 times of residue incorporation and the control, were directly seeded with *D. sanguinalis* (Herbiseed,
135 30 seeds), lettuce (*Lactuca sativa* L. cv. "Trocadero la preferita", 25 seeds), and pea (*Pisum sativum*
136 L. cv "Alderman", 9 seeds). Only one indicator species was seeded per tray. For each combination
137 of residue quantity, incorporation time, and indicator species, four replications were performed such
138 that the tray represented the experimental unit. Then the entire experiment was carried out twice.

139 The greenhouse temperature during the experiment averaged 18.7°C. Natural light was
140 supplemented by metal halide lamps adjusted to produce 14 h day length by delivering about 55
141 μmol/cm^2. Pots were arranged on greenhouse benches according to a completely randomized design
142 and rotated every week to minimize spatial variation. Germination percentage was determinated
143 daily; plant height and fresh weight were assessed 15 days after sowing. Plant height was measured
144 individually while fresh weight was assessed for the entire pot, and then averaged per plant.

145

146

147 *2.3. Allelopathic effects of* Helianthus tuberosus *leaf tissue on a natural weed community*
148 *(experiment 2)*

149 This experiment was conducted from September 2008 to February 2009 in the experimental
150 greenhouse of the Department Agroselviter, Università degli Studi di Torino, using pots (11 x 13
151 cm, 5 cm height) filled with soil. Sandy loam soil samples were collected from the University

experimental farm in northwestern Italy, where *H. tuberosus* plants were absent as weed and as crop. Quantities equivalent to about 2.5 and 5.0 t ha^{-1} of plant dry residues, including leaves, stalks, and stems, were added to the pots. The ground material was thoroughly mixed with the soil before filling the pot. Pots filled solely with soil represented the control treatment. Seven replications were performed for each residue quantity in which the pot represented the experimental unit. The entire experiment was then carried out twice. The pots were arranged in a completely randomized design and rotated every week to minimize spatial variation. Greenhouse parameters were adopted and maintained as described in experiment 1.

Emergence percentage was determined at 30 and 55 days for each species; plant fresh weights were evaluated after density counting by assessing the whole pot, and then averaged per plant.

2.4. Statistical analysis

The values of daily germination obtained with the evaluation of the allelopathic persistence of *H. tuberosus* leaf tissue (experiment 1) were used for the regression analysis with the log-logistic model:

$$Y = c + \left\{(d \quad c)/[1+(x/g)^{b}]\right\}$$

where Y is percent germination, c is the response at very high extract rates, d is the response when the extract rate is near zero, b is the slope of the line in the point of inflection, g is the extract rate at the point of inflection halfway between c and d, and x is the extract rate. The regression analysis was performed using data from all the replicates using the regression utility of the *drc* package of R software (Ritz and Streibig, 2005). The days needed to obtain a germination of 25%, 50% and 90%, (GR_{25}, GR_{50} and GR_{90}, respectively) were calculated on the curves obtained for each indicator species, incorporation timing and rate of residues.

176 Main effects of the variable “experiment”, “timing”, “species”, “residue quantities”, as well as
177 the interactions were detected with the multivariate anova (software SPSS, version 16) on total
178 germination, plant height and plant fresh weight. Due to the absence of significance among the two
179 replications of experiment 1, observed if the “experiment” was considered a variable in the main
180 effect analysis, data coming from the two sets were pooled together. A t-test ($p< 0.05$) was
181 subsequently performed on total germination, plant height and plant fresh weight to evaluate the
182 effects of *H. tuberosus* dried residues (experiment 1) in comparison with control values. P values
183 were not corrected for multiple-comparison analysis. Groups of homogeneity among incorporation
184 timings and rate were detected with the Tukey post-hoc test, using the statistical software SPSS
185 (version 16).

186 Differences of weed density and biomass of experiment 2 were detected with anova analysis,
187 and groups of homogeneity were separated with the Tukey post-hoc test (SPSS software). In this
188 experiment weed composition was assessed with a t-test ($p<= 0.05$).

189

190 # 3. Results

191 *3.1. Allelopathic persistence of* Helianthus tuberosus *leaf tissue under (experiment 1)*

192 The effect of the *H. tuberosus* residues on plant germination was assessed through a
193 comparison of GRs. In the case of *D. sanguinalis* at times I and II, incorporating 0.5 t ha^{-1} of *H.*
194 *tuberosus* leaf tissue stimulated germination (Table 1). For example, at this level of residue, 90%
195 germination was observed at least 2 days before it was observed in the control. Overall, at the
196 highest levels of residue (1.0 and 2.0 t ha^{-1}), no important effects were observed while at the lowest
197 levels of residue, when the dry residue was present in the tray for the longest duration (Timing I), an
198 important delay in lettuce germination was produced (Table 2). In fact, more than 3, 4, and 7 days
199 were required to obtain 25%, 50%, and 90%, respectively, of germination compared to the control.

In general for lettuce, reducing the time that residues were buried in the growth substrate corresponded to a reduction in the inhibition of germination.

A similar effect was observed for pea at 0.5 t ha^{-1} of dry residue at Timing I (Table 3). Overall, the inhibition of the pea species was slightly smaller; its germination suffered a delay of 1.79, 2.22, and 3.78 days to reach 25%, 50% and 90% of germination, respectively. No differences were recorded when germination rates were measured for timings closest to the seeding period given the data measurements displayed high variability from which no conclusion could be drawn.

The results coming from the two set of experiments were pooled together as no differences were observed between them (table 4). If the response of all species, timings and residue quantities were considered together, significant effects were observed on total germination only depending on residues quantities.

D. sanguinalis inhibition of total germination was observed from Timing I through Timing VI (Fig. 1), particularly at the highest concentration of *H. tuberosus* dry material. No differences were observed relative to the various incorporation timings and the average germination reduction was about 40% at 2.0 t ha^{-1} of residue in Timings I – V. At timing VI, no germination effects for the species were observed. For lettuce, no total germination effects were observed compared to the control, at any rate or at any incorporation timing. Actually, the average total germination of the control and the *H. tuberosus* residue-containing trays was 43.74%, and 40.03%, respectively (Fig. 2).

For pea while a general total germination effect was not found, a minor effect was observed. Specifically, significant effects were recorded only in two instances—for Timing II at 2.0 t ha^{-1} and Timing VI at 1 t ha^{-1}. Total germination reductions relative to the control were 37% and 47.60%, respectively (Fig. 3).

On the other hand, fresh weight was affected by the presence of *H. tuberosus* residues in each of the species considered. Almost all incorporation timings showed a strong effect on *D*

225 *sanguinalis* fresh weight at 2.0 t ha^{-1} of residues (Fig. 4), which yielded a growth inhibition of more
226 than 50%. With this indicator species at the highest residue rate, only timing II was not significantly
227 different from the control, a result that is likely due to the higher variability recorded in the data for
228 this treatment. The quantity of 1.0 t ha^{-1} caused a similar inhibition level as 2.0 t ha^{-1} did in Timing
229 V and VI. For lettuce and pea, the presence of *H. tuberosus* residues in their growth media showed
230 important stimulatory effects on their fresh weights (Fig. 5 and Fig. 6); in particular, near Timing III
231 in lettuce and starting from Time II in pea.

232 Results revealed plant height to be the parameter least affected by *H. tuberosus* residues. No
233 effects were observed after residue incorporation, either with the control or among timings and rates
234 in the case of *D. sanguinalis* (data not shown). A significant inhibition was recorded only in the
235 case of incorporation Timing I at 0.5 t ha^{-1} in pea, resulting in an approximate reduction of 73%.

236

237 *3.2. Allelopathic effects of* Helianthus tuberosus *leaf tissue on natural weed community*
238 *(experiment 2)*

239 The weed composition observed in the soil samples collected in the field was characterized by the
240 typical flora recorded in the cropping systems of the area. Among the dicot weeds the most
241 represented species were *Veronica persica* Poiret, *Lamium purpureum* L., *Polygonum aviculare* L.,
242 *Galinsoga quadriradiata* Ruiz & Pav., *Chenopodium album* L. and *Portulaca oleracea* L., and the
243 most present grass weeds were *Alopecurus myosuroides* Huds and *D. sanguinalis*. The species
244 compositions was similar among treatments.

245 On average, few effects were observed among different treatments with *H. tuberosus* residues,
246 while significant differences between the control and the presence of *H. tuberosus* were always
247 present. Even if the effect on each single species was not significant, the inhibition became
248 significant when weed species were pooled together or in the groups of mono- and dicot species
249 (Table 5).

250 Total weed seedling density resulted inhibited by about 37% and 66% at 30 and 55 days after
251 the incorporation of residues, respectively (Fig. 7), while if species were considered separately, no
252 inhibition was observed. Dicot species were particularly depressed at the highest quantities of
253 residues (5.0 t/ha), with 60 and 75% emergence reduction at 30 and 55 days, respectively (Fig. 8).
254 Considering all monocot species, important density reduction was observed after 55 days, with an
255 inhibition higher than 60% at all residue quantities (Fig. 9).

256 No significant effects were detected on weed biomass, either considering the per species weight
257 or the whole biomass of all the pot species (data not shown). This could likely be due to the higher
258 competition occurred in the control pots, in which the higher number of species had to compete
259 more for the limited resources.

260

261 # 4. Discussion

262 The results from this study have yielded valuable information on the allelopathic activity of
263 *Helianthus tuberosus* plant residues incorporated into the substrate. The effect of this knowledge
264 could be helpful in two ways. In the case of the crops, that suppressive effect informs crop rotation
265 planning while in the case of the weed, this work could lead to a more positive role for allelopathy
266 as part of integrated weed control strategy.

267 These effects varied — from stimulation to noticeable inhibition — on both germination and
268 seedling growth in weed and crop species. Specifically, in pea, fresh weight was significantly
269 inhibited when residues were present in the pot for a long period (42 days) while stimulation was
270 recorded during shorter periods (from 6 to 36 days). The stimulation may likely be due to an
271 enhanced water retention due to the organic material, even if the positive effect disappeared with an
272 increasing presence of residues, showing by contrast inhibition on pea. This crop suffered a
273 germination delay caused by residue soil incorporation despite observing little inhibition from the
274 perspective of total germination. Similarly, lettuce showed no significant total germination effects
275 in spite of a significant existence of a germination delay.

These findings suggest that reliance on only the total germination value during evaluation of the phytotoxic potential of an allelopathic species against an indicator species may not provide, in some circumstances, an exhaustive picture of the germination dynamic. Even if the results of final germination coming from the addition of allelopathic residues into the substrate are similar to that of the control, different germination pattern may occur. In fact, for treatment comparing the regression analysis results of the germination values can potentially raise the likelihood of finding a negative germination effect of certain species. In environments characterized by a high competition for resources together with a high weed potential, even small germination delays and the emergence pattern of a species or community may give a competitive advantage over a less-aggressive neighbour and establish a new stability within the plant community after a period of adaptation (Callaway and Walker, 1997).

D. sanguinalis demonstrated the most sensitivity, of the studied species, to the allelopathic activity of the Jerusalem artichoke. In fact, reduction values higher than 30% were observed in almost all timings of residue incorporation. The biomass of *D. sanguinalis* was also strongly depressed at all timings if 2.0 t ha^{-1} of residues were added to the substrate. These results agree with other studies that focused on the allelopathic activity of species belonging to the *Helianthus* genus, or to *H. tuberosus* in particular (Wilson and Rice, 1968; Hall et al., 1982; Vidotto et al., 2008; Tesio et al., 2011), in which an important depression of monocot weeds was observed.

Even though these preliminary experiments focusing on the *H. tuberosus* effect on the weed community give only an indication of the allelopathic potential of its residues, a significant inhibition was found in terms of seedling emergence. It should be noted that in this case, not only the leaves, considered the most toxic part of the plant (Khanh et al, 2005b; Tesio et al. 2008), were buried into the soil, but the entire aboveground biomass.

In previous studies the authors investigated the phenomenon of allelopathy of *H. tuberosus*, describing the effects of different varieties of Jerusalem artichoke, and the effects on 6 crop and 6 weed species, both in sand and in soil conditions (Tesio et al.,2010); and in these cases the

302 differences observed in the two media resulted irrelevant. Afterwards, other than the identification
303 of some allelochemicals, the strong suppressive effect of *H. tuberosus* cultivation residues on
304 several weeds was reported at field scale, on *Digitaria sanguinalis* in particular (Tesio et al., 2011).
305 As the allelopathic potential of this plant is already well described, the authors tried to stress the
306 attention on the persistence of the allelopathic potential, an aspect that is generally not considered in
307 other studies.

308 These results, together with those obtained from the authors' previous studies on *H. tuberosus*
309 dried residues, provide ecologically-relevant evidence for the phytotoxicity of these residues and for
310 the potential allelopathic activity of this species. They also partially explain the ecological and
311 agronomical advantage of this plant in natural and agricultural environments. The increasing spread
312 of *H. tuberosus* in cultivated fields across Europe and Italy in particular, may be ascribed to this
313 allelopathic effect as well as other factors such as its ability to reproduce by tubers (Swanton et al.,
314 1992).

315 In natural environments, *H. tuberosus* has acted as a weed in several crops, and when cultivated
316 in high density, its advantage is also due to the production of great quantities of residue. Dense
317 plantations of Jerusalem artichoke can also affect the crops that follow, especially by impacting the
318 establishment of a crop highly sensitive to its allelochemicals such as rice or tomato (Vidotto et al.,
319 2008).

320 In the our experiments, the amount of residue used was almost equivalent to a reasonably high
321 aboveground biomass production, similar to that achieved in the field settings. The introduction of
322 *H. tuberosus* into the crop rotation as an edible plant, as an energy crop for ethanol production, or
323 even as a living rotational crop, may be of concern due to the weediness of the species, and for the
324 possible injury suffered by a succeeding sensitive crop. Several studies have pointed out that the
325 allelopathic activity of the residue degradation of this plant upon a following crop is strongly related
326 to the seed dimension of the crop. For example, no inhibition was observed when species with large
327 seeds such as green beans, maize, zucchini, and partially winter wheat crops were seeded after the

328 incorporation of *H. tuberosus* residues (Tesio et al., 2010). The results of this study suggest that an
329 important reduction of weed density can be obtained, for up to 55 days, if the cultivation remains of
330 Jerusalem artichoke are buried into the soil after tuber harvest.

331 The incorporation of various amounts of *H. tuberous* remains into the soil in which test seeds
332 were planted resulted in inhibition of the growth and development of the plants, which varied
333 according to the period of residue substrate permanence. This behaviour was especially made
334 evident in the greenhouse pea experiment, in which, for example, the residue permanence had a
335 greater effect on growth than did the residue quantities. A similar behaviour was observed on
336 natural flora. A significant, increasing reduction of weed density was assessed after 30 and 55 days
337 of residue incorporation. These data indicate that allelopathic agents may be present in, or formed
338 during, the decay of Jerusalem artichoke leaves, which are capable of inhibiting weed growth.

339 With all these considerations in mind, the use of crop plants with allelochemical production
340 could limit the need for conventional herbicides to early season application solely. And that when
341 then combined with other agronomic, mechanical, and physical weed control strategies, late season
342 weed control can be provided with a significant reduction of chemical input. Furthermore, our
343 results hold several implications for community ecology, as the ability of the allelopathic effect to
344 persist can maximize the invasiveness of *H. tuberosus*.

345

346

347 # References

348 Brethour, C., Weersink, A., 2001. An economic evaluation of the environmental benefits from
349 pesticide reduction. Agric. Econ. 25, 219-226.

350 Callaway, R.M., Walker, L.R., 1997. Competition and facilitation: a synthetic approach to
351 interactions in plant communities. Ecology 78, 1958-1965.

352 Cassells, A.C., Walsh, M., 1995. Screening for Sclerotinia resistance in *Helianthus tuberosus* L.
353 (Jerusalem artichoke) varieties, lines and somaclones, in the field and in vitro. Plant Pathol. 44,
354 428-437.

355 Follis, F., Tesio, F., Crivellari, A., Vidotto, F., Ferrero, A., 2010. Evaluation of allelopathic
356 potential of *Helianthus tuberosus* L. root exudates, in: Ltd, A.C.B. (Ed.), EWRS 15th
357 Symposium, Kaposvár, Hungary, p. 100.

358 Hall, A.B., Blum, U., Fites, R.C., 1982. Stress modification of allelopathy of *Helianthus annuus* L.
359 debris on seed germination. Am. J. Bot. 69, 776-783.

360 Khanh, T.D., Chung, I.M., Xuan, T.D., Tawata, S., 2005a. The exploitation of crop allelopathy in
361 sustainable agricultural production. J. Agron. Crop Sci. 191, 172-184.

362 Khanh, T.D., Hong, N.H., Xuan, T.D., Chung, I.M., 2005b. Paddy weed control by medicinal and
363 leguminous plants from Southeast Asia. Crop Prot. 24, 421-431.

364 Olofsdotter, M., Jensen, L.B., Courtois, B., 2002. Improving crop competitive ability using
365 allelopathy; an example from rice. Plant Breed. 121, 1-9.

366 Petchey, O.L., 2003. Integrating methods that investigate how complementarity influences
367 ecosystem functioning. Oikos. 101, 323-330.

368 Reigosa, M.J., Sanchez-Moreiras, A., Gonzales, L., 1999. Ecophysiolocal approach in allelopathy.
369 Crit. Rev. Plant Sci. 18, 577-608.

370 Ritz, C., Streibig, J.C., 2005. Bioassay Analysis Using R. J. Stat. Softw. 12, 1-18.

371 Schnitzler, A., Hale, B.W., Alsum, E.M., 2007. Examining native and exotic species diversity in
372 European riparian forests. Biol. Conserv. 138, 146-156.

373 Seiler, G.J., 1993. Forage and tuber yields and digestibility of selected wild and cultivated
374 genotypes of Jerusalem artichoke. Agron. J. 85, 29-33.

375 Seiler, G.J., Campbell, L.G., 2004. Genetic variability for mineral element concentrations of wild
376 Jerusalem artichoke forage. Crop Sci. 44, 289-292.

377 Swanton, C.J., Cavers, P.B., Clements, D.R., Moore, M.J., 1992. The biology of Canadian weeds.
378 101. *Helianthus tuberosus* L. Can. J. Plant Sci. 72, 1367-1382.

379 Tesio, F., Ferrero, A., 2010. Allelopathy, a chance for sustainable weed management. Int. J. Sust.
380 Dev. World Ecol. 17, 377-389.

381 Tesio, F., Vidotto, F., Weston, L.A., Ferrero, A., 2008. Allelopathic effects of aqueous leaf extracts
382 of *Helianthus tuberosus* L. Allelopathy J. 22, 47-58.

383 Tesio, F., Weston, L.A., Ferrero, A., 2011. Allelochemicals identified from Jerusalem artichoke
384 (*Helianthus tuberosus* L.) residues and their potential inhibitory activity in the field and
385 laboratory. Sci. Hortic. 129, 361-368.

386 Tesio, F., Weston, L.A., Vidotto, F., Ferrero, A., 2010. Potential allopathic effects of Jerusalem
387 artichoke (*Helianthus tuberosus*) leaf tissues. Weed Technol. 24, 378-385.

388 Török, K., Botta-Dukát, Z., Dancza, I., Németh, I., Kiss, J., Mihály, B., Magyar, D., 2003. Invasion
389 gateways and corridors in the carpathian basin: biological invasions in Hungary. Biol. Invasions
390 5, 349-356.

391 Vidotto, F., Tesio, F., Ferrero, A., 2008. Allelopathic effects of *Helianthus tuberosus* L. on
392 germination and seedling growth of several crops and weeds. Biol. Agric. Hortic. 26, 55-68.

393 Wadsworth, R.A., Collingham, Y.C., Willis, S.G., Huntley, B., Hulme, P.E., 2000. Simulating the
394 spread and management of alien riparian weeds: are they out of control? J. Appl. Ecol. 37, 28-38.

395 Weston, L.A., 1996. Utilization of allelopathy for weed management in agroecosystems. Agron. J.
396 88, 860-866.

397 Weston, L.A., Duke, S.O., 2003. Weed and crop allelopathy. Crit. Rev. Plant Sci. 22, 367-389.

Wilson, R.E., Rice, E.L., 1968. Allelopathy as expressed by *Helianthus annuus* and its role in old-field succession. Bull. Torrey Bot. Club 95, 432-448.

Wyse, D.L., Young, F.L., 1980. Jerusalem artichoke interference in corn [=maize] and soybeans., Proceedings North Central Weed Control Conference, 48.

Wyse, D.L., Young, F.L., Jones, R.J., 1986. Influence of Jerusalem artichoke (*Helianthus tuberosus*) density and duration of interference on soybean (*Glycine max*) growth and yield. Weed Sci. 34, 243-247.

Xuan, T.D., Shinkichi, T., Khanh, T.D., Chung, I.M., 2005. Biological control of weeds and plant pathogens in paddy rice by exploiting plant allelopathy: an overview. Crop Prot. 24, 197-206.

Figure captions

Fig. 1. Response of *D. sanguinalis* germination to the presence of variable quantities of Jerusalem artichoke dried residues at six different incorporation times (experiment 1). Bars represent the standard errors (n = 8). * Refers to significant differences from the control (black line: 51.88%) with p <= 0.05 or ** with p <= 0.01. Letters refer to significant differences of residue rates within a single timing (Tukey post-hoc test, with p <= 0.01).

Fig. 2. Response of lettuce germination to the presence of variable quantities of Jerusalem artichoke dried residues at six different incorporation times (experiment 1). Bars represent the standard errors (n = 8). Black line refers to average germination of control (43.74%). Lower case letters refer to significant differences of residues rate within a single timing (Tukey post-hoc test, with p <= 0.01).

Fig. 3. Response of pea germination to the presence of variable quantities of Jerusalem artichoke dried residues at six different incorporation times (experiment 1). Bars represent the standard errors (n = 8). * Refers to significant differences from the control (black line: 40.63%) with p <= 0.05 or ** with p <= 0.01. Lower case letters refer to significant differences of residue rates within a single timing (Tukey post-hoc test, with p <= 0.01).

Fig. 4. Response of *D. sanguinalis* fresh weight to the presence of various quantities of Jerusalem artichoke dried residues at six different incorporation timies (experiment 1). Bars represent the standard errors (n = 8). * Refers to significant differences from the control (black line: 0.021g) with p <= 0.05 or ** with p <= 0.01. Lower case letters refer to significant differences of residue rates within a single timing (Tukey post-hoc test, with p <= 0.01).

Fig. 5. Response of lettuce fresh weight to the presence of various quantities of Jerusalem artichoke dried residues at six different incorporation times (experiment 1). Bars represent the standard errors (n = 8). * Refers to significant differences from the control (black line, 0.042g) with p <= 0.05 or ** with p <= 0.01. Lower case letters refer to significant residue rate differences within a single timing; upper case letters indicate difference among incorporation timing (Tukey post-hoc test, with p <= 0.01).

Fig. 6. Response of pea fresh weight to the presence of various quantities of Jerusalem artichoke dried residues at several incorporation times (experiment 1). Bars represent the standard errors (n = 8). * Refers to significant differences from the control (black line, 0.39g) with p <= 0.05 or ** with p <= 0.01. Lower case letters refer to significant residue rate differences within a single timing; upper case letters indicate difference among incorporation timing (Tukey post-hoc test, with p <= 0.01).

Fig. 7. Effect of *H. tuberosus* residues incorporated into the soil on total weed density (experiment 2). Bars represent the standard errors (n = 14). Letters refer to significant differences among the same assessment date (Tukey post-hoc test, with p <= 0.05).

Fig. 8. Effect of *H. tuberosus* residues incorporated into the soil on dicot weed density (experiment 2). Bars represent the standard errors (n = 14). Letters refer to significant differences among the same assessment date (Tukey post-hoc test, with p <= 0.05).

Fig. 9. Effect of *H. tuberosus* residues incorporated into the soil on monocot weed density (experiment 2). Bars represent the standard errors (n = 14). Letters refer to significant differences among the same assessment date (Tukey post-hoc test, with p <= 0.05).

Table captions

Table 1

Values of GR_{25}, GR_{50}, and GR_{90} for *D. sanguinalis* (± SE; n = 8) obtained from *H. tuberosus* dried leaf material-based substrates added at various times before seeding (experiment 1).

Table 2

Values for GR_{25}, GR_{50} and GR_{90} of lettuce (± SE; n = 8) obtained from *H. tuberosus* dried leaf material-based substrates added at various times before seeding (experiment 1).

Table 3

Values of GR_{25}, GR_{50} and GR_{90} of pea (± SE; n = 8) obtained from *H. tuberosus* dried leaf material-based substrates added at various times before seeding (experiment 1).

Table 4

Analysis of variance carried out on total germination, height and fresh weight response presenting the main effects of experiment 1.

Table 5

Analysis of variance results for weed presence at 30 and 55 days of residue incorporation into the soil (experiment 2).

Tables

Table 4

Values of GR_{25}, GR_{50}, and GR_{90} for *D. sanguinalis* (± SE; n = 8) obtained from *H. tuberosus* dried leaf material-based substrates added at various times before seeding (experiment 1).

Timing	*H. tuberosus* quantity (t ha^{-1})	GR_{25}*	GR_{50}*	GR_{90}*
0	0.0 (control)	9.74 ± 0.418	12.49 ± 0.822	20.54 ± 2.805
I	0.5	7.54 ± 0.325	9.09 ± 0.429	13.21 ± 1.554
	1.0	8.63 ± 0.806	10.93 ± 1.360	17.56 ± 4.815
	2.0	9.34 ± 0.317	11.92 ± 0.596	19.39 ± 2.082
II	0.5	7.83 ± 0.524	9.48 ± 0.681	13.88 ± 2.423
	1.0	9.37 ± 0.712	11.93 ± 1.298	19.32 ± 4.553
	2.0	10.64 1.695	13.92 ± 3.477	23.82 ± 11.505
III	0.5	8.28 ± 0.643	10.58 ± 1.248	17.28 ± 4.571
	1.0	8.92 ± 0.359	11.41 ± 0.645	18.71 ± 2.315
	2.0	11.76 0.620	14.39 ± 1.233	21.56 ± 4.032
IV	0.5	7.99 ± 0.837	9.94 ± 1.227	15.38 ± 4.410
	1.0	10.04 1.214	13.02 ± 2.514	21.90 ± 8.681
	2.0	7.29 ± 0.612	8.98 ± 1.056	13.65 ± 3.974
V	0.5	12.15 5.297	17.98 ± 10.488	39.38 ± 35.118
	1.0	8.55 ± 0.762	10.40 ± 1.041	15.41 ± 3.703
	2.0	7.29 ± 0.692	8.98 ± 1.056	13.65 ± 3.974
VI	0.5	7.67 ± 0.902	9.96 ± 1.727	16.82 ± 6.696
	1.0	9.36 ± 0.568	11.59 ± 0.892	17.80 ± 3.172
	2.0	8.25 ± 0.426	9.79 ± 0.560	13.80 ± 1.921

* GR_{25}, GR_{50} and GR_{90}: days to obtain 25, 50, 90% of total germination, respectively.

Table 5

Values for GR_{25}, GR_{50} and GR_{90} of lettuce (± SE; n = 8) obtained from *H. tuberosus* dried leaf material-based substrates added at various times before seeding (experiment 1).

Timing	*H. tuberosus* quantity (t ha^{-1})	GR_{25}*	GR_{50}*	GR_{90}*
0	0.0 (control)	4.26 0.229	4.70 ± 0.745	5.71 ± 0.519
I	0.5	7.54 0.325	9.09 ± 0.429	13.21 ± 1.553
	1.0	3.28 0.233	3.98 ± 0.205	5.87 ± 0.617
	2.0	3.33 0.296	4.17 ± 0.257	6.53 ± 0.913
II	0.5	2.55 0.662	3.79 ± 0.771	8.43 ± 4.429
	1.0	3.15 0.202	3.87 ± 0.166	5.84 ± 0.492
	2.0	3.71 0.259	4.46 ± 0.198	6.47 ± 0.642
III	0.5	3.78 0.464	4.53 ± 0.387	6.50 ± 1.479
	1.0	3.47 0.182	3.98 ± 0.126	5.24 ± 0.335
	2.0	4.48 0.546	4.70 ± 0.342	5.17 ± 0.328
IV	0.5	3.97 0.083	4.34 ± 0.075	5.20 ± 0.196
	1.0	4.17 0.173	4.63 ± 0.134	5.70 ± 0.339
	2.0	4.34 0.332	4.97 ± 0.295	6.52 ± 0.872
V	0.5	3.87 0.539	4.32 ± 0.442	5.39 ± 1.393
	1.0	4.22 0.513	4.93 ± 0.434	6.73 ± 1.456
	2.0	4.37 0.443	4.82 ± 0.351	5.87 ± 1.105
VI	0.5	4.86 0.479	5.52 ± 0.442	7.11 ± 1.428
	1.0	3.96 0.515	4.42 ± 0.395	5.48 ± 1.247
	2.0	4.53 0.321	5.13 ± 0.304	6.59 ± 0.965

* GR_{25}, GR_{50} and GR_{90}: days to obtain 25, 50, 90% of total germination, respectively.

494

495 **Table 6**

496 Values of GR_{25}, GR_{50} and GR_{90} of pea (± SE; n = 8) obtained from *H. tuberosus* dried leaf
497 material-based substrates added at various times before seeding (experiment 1).

Timing	*H. tuberosus* quantity (t ha^{-1})	GR_{25}*	GR_{50}*	GR_{90}*
0	0.0 (control)	5.75 ± 0.245	6.78 ± 0.266	9.43 ± 0.911
I	0.5	7.54 ± 0.325	9.09 ± 0.429	13.21 ± 1.554
	1.0	5.04 ± 0.263	6.56 ± 0.354	11.12 ± 1.571
	2.0	3.66 ± 1.213	5.17 ± 1.811	10.33 ± 8.875
II	0.5	4.43 ± 0.934	5.93 ± 1.183	10.61 ± 5.179
	1.0	7.47 ± 0.461	8.63 ± 0.419	11.50 ± 1.233
	2.0	3.70 ± 1.021	5.22 ± 1.853	10.38 ± 8.927
III	0.5	4.13 ± 0.259	5.64 ± 0.402	10.54 ± 1.875
	1.0	4.45 ± 0.443	5.36 ± 0.417	7.78 ± 1.364
	2.0	4.19 ± 0.452	5.64 ± 0.655	10.20 ± 2.953
IV	0.5	4.55 ± 0.422	5.64 ± 0.499	8.67 ± 2.009
	1.0	4.43 ± 0.428	6.27 ± 0.810	12.55 ± 3.945
	2.0	5.35 ± 2.263	8.17 ± 5.694	19.06 ± 19.00
V	0.5	4.81 ± 0.331	5.71 ± 0.312	8.03 ± 1.058
	1.0	4.05 ± 0.169	4.87 ± 0.143	7.05 ± 0.476
	2.0	5.38 ± 0.502	6.51 ± 0.481	9.55 ± 1.576
VI	0.5	4.22 ± 0.657	5.94 ± 1.199	11.73 ± 6.013
	1.0	7.00 ± 0.480	7.21 ± 0.811	7.67 ± 2.657
	2.0	5.21 ± 0.245	6.18 ± 0.405	8.70 ± 1.263

498 * GR_{25}, GR_{50} and GR_{90}: days to obtain 25, 50, 90% of total germination, respectively.

499

500
501

Table 4

Analysis of variance carried out on total germination, height and fresh weight response presenting the main effects of experiment 1.

Parameter	Variable	*F*	Sig. (*F*)
Experiment	total germination	0.89	0.438
	height	0.19	0.899
	fresh weight	0.30	0.922
Timing	total germination	0.31	0.933
	height	0.84	0.537
	fresh weight	14.64	0.001
Species	total germination	2.11	0.122
	height	65.70	0.001
	fresh weight	688.97	0.001
Residue quantity	total germination	3.52	0.015
	height	0.196	0.899
	fresh weight	85.92	0.001

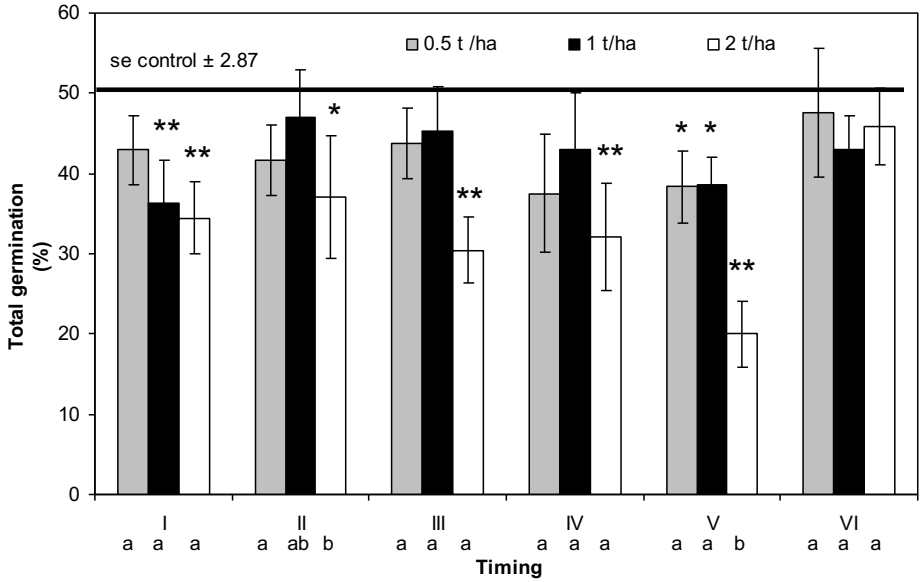
Table 5

Analysis of variance results for weed presence at 30 and 55 days of residue incorporation into the soil (experiment 2).

Days	Weed denstity	*F*	Sig. (*F*)
30	Total	5.73	0.012
	Dicot	3.73	0.044
	Monocot	3.61	0.048
55	Total	14.82	0.001
	Dicot	9.33	0.002
	Monocot	15.38	0.001

513 **Figures**

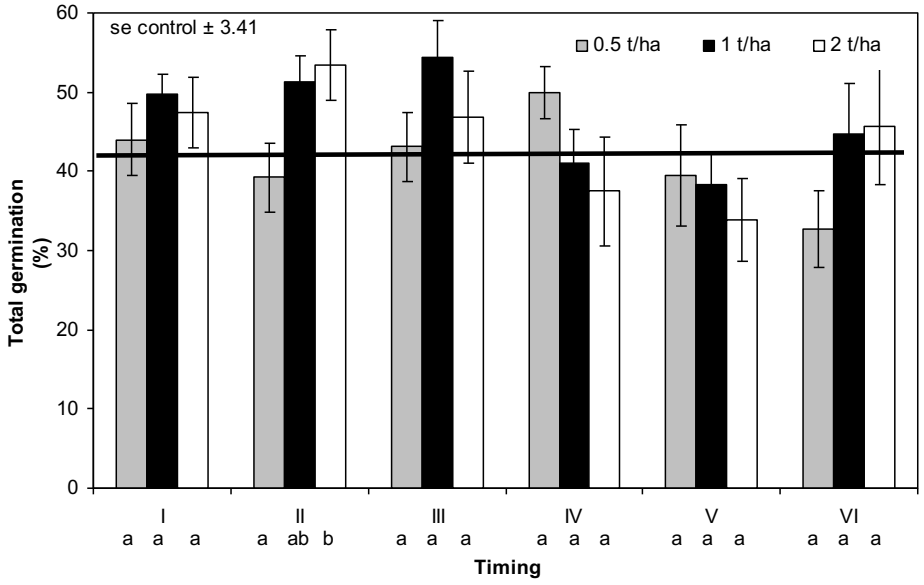
514



515

516 **Fig. 10.** Response of *D. sanguinalis* germination to the presence of variable quantities of
517 Jerusalem artichoke dried residues at six different incorporation times (experiment 1). Bars
518 represent the standard errors (n = 8). * Refers to significant differences from the control
519 (black line: 51.88%) with p <= 0.05 or ** with p <= 0.01. Letters refer to significant
520 differences of residue rates within a single timing (Tukey post-hoc test, with p <= 0.01).

521



522

523 **Fig. 11.** Response of lettuce germination to the presence of variable quantities of
524 Jerusalem artichoke dried residues at six different incorporation times (experiment 1). Bars

represent the standard errors (n = 8). Black line refers to average germination of control (43.74%). Lower case letters refer to significant differences of residues rate within a single timing (Tukey post-hoc test, with p <= 0.01).

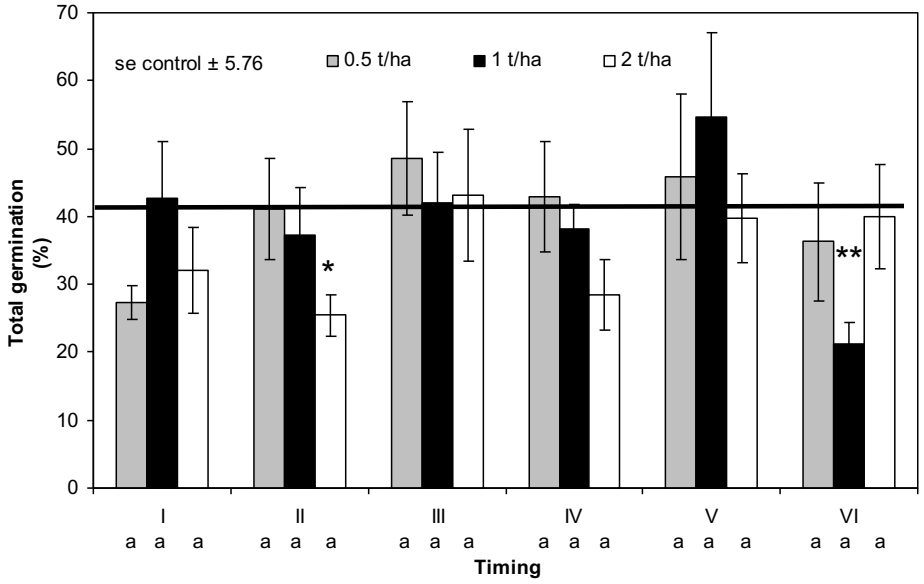
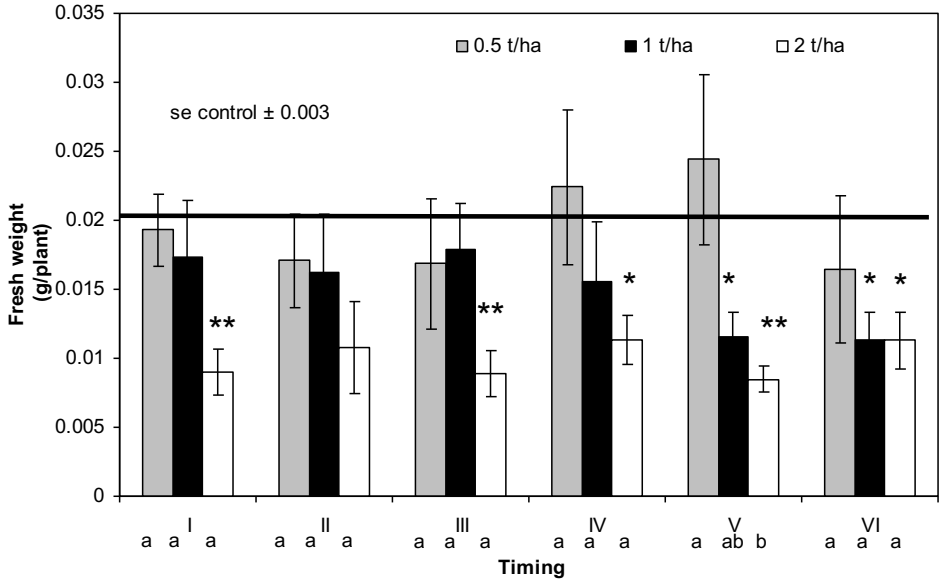
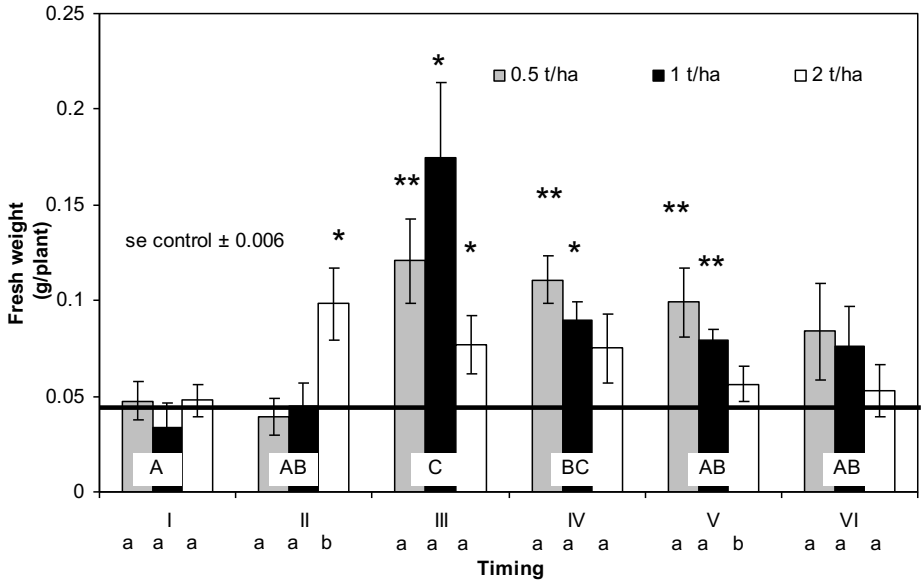


Fig. 3. Response of pea germination to the presence of variable quantities of Jerusalem artichoke dried residues at six different incorporation times (experiment 1). Bars represent the standard errors (n = 8). * Refers to significant differences from the control (black line: 40.63%) with p <= 0.05 or ** with p <= 0.01. Lower case letters refer to significant differences of residue rates within a single timing (Tukey post-hoc test, with p <= 0.01).



537

538 **Fig. 4.** Response of *D. sanguinalis* fresh weight to the presence of various quantities of Jerusalem
539 artichoke dried residues at six different incorporation timies (experiment 1). Bars represent the
540 standard errors (n = 8). * Refers to significant differences from the control (black line: 0.021g) with
541 p <= 0.05 or ** with p <= 0.01. Lower case letters refer to significant differences of residue rates
542 within a single timing (Tukey post-hoc test, with p <= 0.01).



543

544 **Fig. 5.** Response of lettuce fresh weight to the presence of various quantities of Jerusalem artichoke
545 dried residues at six different incorporation times (experiment 1). Bars represent the standard errors
546 (n = 8). * Refers to significant differences from the control (black line, 0.042g) with p <= 0.05 or **

with p <= 0.01. Lower case letters refer to significant residue rate differences within a single timing; upper case letters indicate difference among incorporation timing (Tukey post-hoc test, with p <= 0.01).

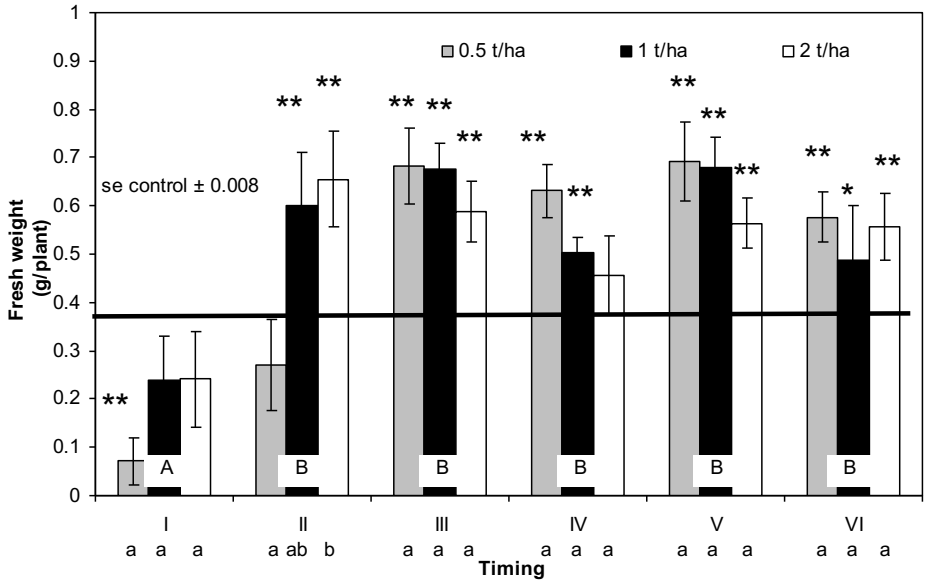
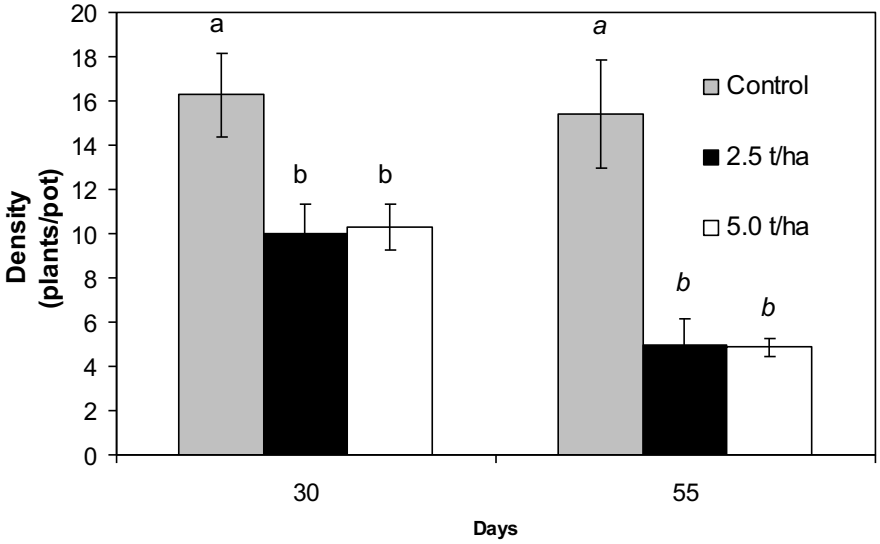


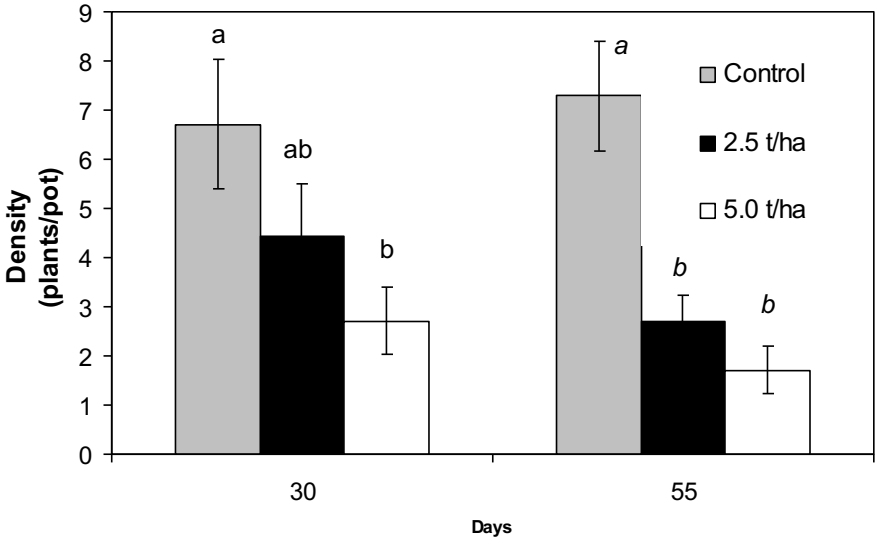
Fig. 6. Response of pea fresh weight to the presence of various quantities of Jerusalem artichoke dried residues at several incorporation times (experiment 1). Bars represent the standard errors (n = 8). * Refers to significant differences from the control (black line, 0.39g) with p <= 0.05 or ** with p <= 0.01. Lower case letters refer to significant residue rate differences within a single timing; upper case letters indicate difference among incorporation timing (Tukey post-hoc test, with p <= 0.01).



559

560 **Fig. 7.** Effect of *H. tuberosus* residues incorporated into the soil on total weed density (experiment
561 2). Bars represent the standard errors (n = 14). Letters refer to significant differences among the
562 same assessment date (Tukey post-hoc test, with p <= 0.05).

563

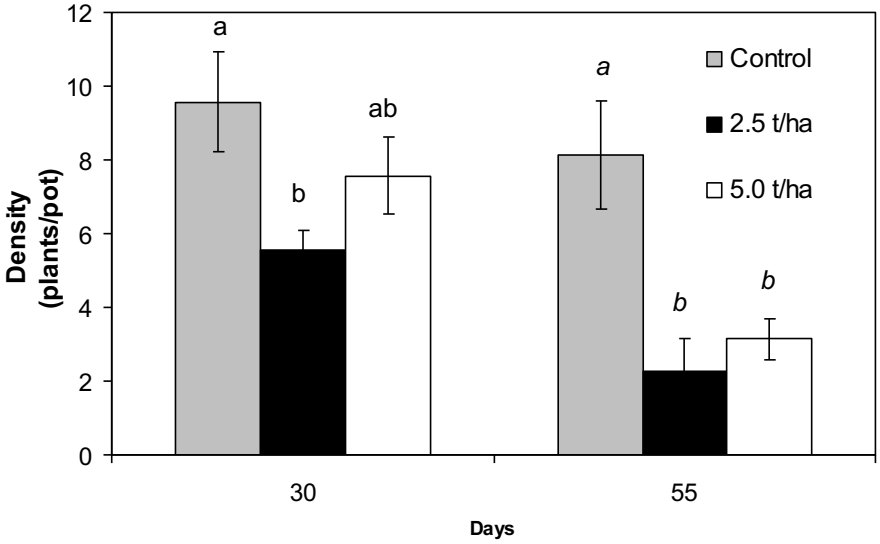


564

565 **Fig. 8.** Effect of *H. tuberosus* residues incorporated into the soil on dicot weed density (experiment
566 2). Bars represent the standard errors (n = 14). Letters refer to significant differences among the
567 same assessment date (Tukey post-hoc test, with p <= 0.05).

568

569



570

571 **Fig. 9.** Effect of *H. tuberosus* residues incorporated into the soil on monocot weed density
572 (experiment 2). Bars represent the standard errors (n = 14). Letters refer to significant differences
573 among the same assessment date (Tukey post-hoc test, with p <= 0.05).

574

575

576