Sustainable leaf harvesting and effects of plant density on wild leek cultivation plots and natural stands in Southern Quebec, Canada.

Pierre-Paul DION[1], Julie BUSSIÈRES and Line LAPOINTE

Centre d'étude de la forêt (CEF) and Département de biologie, Université Laval, Québec (QC) G1V 0A6, Canada.

[1]Corresponding author: pierre-paul.dion.1@ulaval.ca

10   **Abstract**

11   Overharvesting has caused major population declines in North America in non-timber forest

12   products species such as ginseng and wild leek. Sustainable exploitation could be achieved

13   through forest farming. Density reduction following bulb harvest could improve yield in natural

14   wild leek stands that reach growth-limiting densities. Limiting the harvest to leaves may also

15   provide an alternative form of exploitation, but could slow growth by reducing both carbon and

16   nutrient reserves depending on the timing and intensity of such harvest. Our objectives were to

17   assess the effects of (1) planting density and post-harvest density reduction, and (2) the timing

18   and intensity of leaf harvest on subsequent growth and reproduction of wild leek.

19   Three experiments were established. Bulbs were planted at densities from 44 to 356 bulbs m^{-2},

20   covering the range surveyed in natural populations. Plots in dense populations were subjected to

21   up to 40% bulb harvest. In cultivated plots, either half or all the leaves on each plant were

22   harvested, from 15 to 25 days after complete unfolding.

23   Plants growing in higher density plots exhibited slower growth and reproduction rates, but greater

24   productivity per cultivated area. A similar effect, albeit marginal, was obtained following bulb

25   harvests in natural populations. Harvesting leaves did not affect survival, but delaying the harvest

26   and harvesting only half of the leaves favored subsequent plant growth. We recommend

27   harvesting down to a fixed bulb density rather than harvesting a fixed percentage of bulbs, and

28   harvesting leaves only as ways to ensure sustainable exploitation of leeks.

29   **Keywords** : *Allium tricoccum*; Bulb harvest; Planting density; Forest farming; Leaf harvest

INTRODUCTION

30

31 There is a growing interest in non-timber forest products in North America, but population

32 growth of many of these species can be impaired by harvests (Ticktin 2004; Jones and Lynch

33 2007; Schmidt et al. 2011). Forest herbs are particularly sensitive to overharvesting, given that

34 low light availability often limits their growth (Rothstein and Zak 2001a), and population

35 recovery occurs slowly (Nantel et al. 1996). Over the last few decades, overharvesting has caused

36 a substantial decline in natural populations of wild leek (*Allium tricoccum* Ait.), both in southern

37 Quebec (Dagenais 1985; Couillard 1995) and in the southern Appalachians (Davis and

38 Greenfield 2002; Rock et al. 2004). Wild leek is considered to be endangered in three American

39 states (Special Concern status) and in the Canadian province of Quebec (Vulnerable status).

40 Recent studies have shown this species to be a good candidate for forest farming (Davis and

41 Greenfield 2002; Facemire 2008; Bernatchez et al. 2013), which could help alleviate harvesting

42 pressure on natural populations (Chamberlain et al. 2009). Although forest farming may improve

43 sustainability of forest herb exploitation, we need to significantly improve yield and reduce

44 maintenance costs in forest farming systems to ensure their long-term success in eastern North

45 America (Burkhart and Jacobson 2009).

46 Wild leek or ramp is a forest spring ephemeral very popular in the cuisine of eastern North

47 America for the flavor of its bulbs. Its one to three leaves unfold early in spring, right after

48 snowmelt (late April in Quebec), and senesce a few weeks later following canopy closure (late

49 May). Anthesis occurs in July and seeds are mature in late August (Jones 1979). Seven to 10

50 years can elapse from seed germination to mature plant stage (Nantel et al. 1996), and most

51 reproduction occurs asexually through division of the bulb (Nault and Gagnon 1993).

52 Average densities recorded in natural wild leek stands are around 90 bulbs m^{-2}, which includes all

53 size classes (Dagenais 1985; Nault and Gagnon 1993). These size classes were defined by Nault

54 and Gagnon (1993), based on total leaf width (TLW), as follows: class 1 (TLW $\leq$ 0.8 cm); class 2

55 (0.8 < TLW $\leq$ 2.0 cm); class 3 (2.0 < TLW $\leq$ 3.8 cm); class 4 (3.8 < TLW < 6.5 cm); class 5 (6.5

56 < TLW < 10.0 cm); class 6 (10 < TLW < 15 cm) and class 7 (TLW < 15 cm). Previous trials in

57 which wild leek were planted at very low densities of 11 and 44 bulbs m^{-2} indicated no effect of

58 density on bulb yield (Ritchey and Schumann 2005). However, wild leek populations can reach

59 much higher densities; we have counted dense patches containing 350-400 bulbs m^{-2}, without

60 even taking into account the seedlings (i.e. class 1 plants; PP Dion, *personal observations*). In a

61 forest-farming context, such densities could adversely affect growth by inducing intra-specific

62 competition. Indeed, Nault and Gagnon (1993) have reported increased annual mortality in the

63 centers of dense wild leek patches due to overcrowding. Crowding effects have been well

64 documented over a wide range of densities in the commercial onion, *Allium cepa* L. (Brewster

65 and Salter 1980; McGeary 1985; Herison et al. 1993; Leskovar et al. 2012). The usual method for

66 harvesting bulbs is also of great concern when exploiting natural populations of wild leek.

67 Harvesting individual bulbs rather than bunches of bulbs improve population recovery (Nault and

68 Gagnon 1993). However, careful excavation is virtually impossible to perform in dense stands,

69 without damaging some bulbs. Harvesting procedures that minimize bulb damage thus need to be

70 worked out.

71 This species is mainly sold as bulbs, but its leaves are also edible and their harvest is usually

72 considered a more sustainable form of exploitation of wild leek (Dagenais 1985). Nevertheless,

73 leaves are a major sink for carbon and nutrients early in the season. After complete unfolding, the

74 leaves usually account for 50 % of total biomass and contain more than 60 % of P, K, and Mg,

75   and up to 90 % of total N and Ca within the plant (Nault and Gagnon 1988). However, wild leek

76   efficiently translocates nutrients to the bulb during leaf senescence. Even calcium, which cannot

77   be translocated, appears to be reabsorbed by the roots following leaf decomposition, as the

78   former remain active during summer (Nault and Gagnon 1988; Rothstein and Zak 2001b; Hewins

79   et al. 2015). Thus, leaf harvesting could be deleterious to wild leek through the loss of nutrients

80   and carbon fixation capacity. Indeed, studies on other *Allium* species have assessed the negative

81   effects of defoliation. Following defoliation, yield of *Allium sativum* L. (garlic), *A.*

82   *ampeloprasum* L. ssp. *porrum* (leek) and *A. cepa* L. decreased (Muro et al. 1998, 2000; Irigoyen

83   et al. 2010), especially when defoliation occured as leaves had just completed unfolding and bulb

84   formation was being initiated (Irigoyen et al. 2010). Early defoliation can also substantially affect

85   subsequent growth in other spring flowering species, such as *Trillium erectum* L. (red trillium),

86   *Clintonia borealis* Aiton (Raf.) (blue-bead lily), *Claytonia virginica* L. (spring beauty) and

87   *Maianthemum canadense* Desf. (Canada mayflower) (Whigham and Chapa 1999; Lapointe et al.

88   2010). Partial harvests of the foliage could be less deleterious than a total harvest. Partial harvest

89   would allow the remaining leaves to continue photosynthesizing, even during leaf senescence

90   (Bernatchez and Lapointe 2012), and to translocate their nutrients to the bulb during leaf

91   senescence. Moreover, partial defoliation may induce compensatory photosynthesis in the

92   remaining leaves (Nowak and Caldwell 1984; Meyer 1998; Maurin and DesRochers 2013).

93   The first objective of this study was to determine if high densities, approximating those recorded

94   in some natural stands, would impede wild leek growth when cultivated according to forest

95   farming principles. Four densities were compared: 44, 88, 178 and 356 bulbs m^{-2}. The second

96   objective was to assess the effect of density reduction following partial bulb harvest of up to

97   40 % of the bulbs on the subsequent growth of wild leek in natural populations; a secondary

98   objective was to evaluate the impact of the harvesting method itself on subsequent growth. For

99   these first two objectives, we posit that competition among individuals rises with increasing

100   density, and predict a reduction in both individual plant growth and population growth. The third

101   objective was to evaluate the effects of 50 % and 100 % leaf harvesting, taking place from 15 to

102   25 days after complete unfolding, on the survival and growth of wild leek. We predict that

103   harvesting a higher percentage of leaf area early in the season will negatively affect wild leek,

104   both by reducing the total amount of carbon fixed and by preventing the recycling of nutrients

105   present in the leaf.

MATERIAL AND METHODS

107   **Study sites**

108   All experiments were conducted in the Basses-Laurentides (Lower Laurentian) region of

109   Southern Quebec, Canada, except for two sites of the Bulb Harvesting experiment, which were

110   located in Estrie (the Eastern Townships), also in Southern Quebec. Climate in the Lower

111   Laurentian region is characterized by a mean annual temperature of 5.0 °C, total precipitation of

112   1065 mm, and a total of 1866 degree-days (5 °C base temperature) per year. Estrie has a mean

113   annual temperature of 4.1 °C, 1144 mm of precipitation, and 1637 degree-days per year

114   (Environment Canada 2013). These regions are close to the northern limit of wild leek

115   distribution (Jones 1979; Dagenais 1985), but encompass several large natural populations (PP

116   Dion, *personal observations*). Exact locations of the sites have not been disclosed in order to

117   prevent their unauthorized harvesting. All sites were located in mature temperate deciduous

118   forests dominated by maple trees (*Acer saccharum* Marsh.), the natural ecosystem where wild

119   leek occurs most often in southern Quebec. Soils of the four sites for the Leaf harvesting

120   experiment were thoroughly detailed by Bernatchez et al. (2013), along with light available in the

121 understory during summer. The Planting density experiment was set up on sites B and C of

122 Bernatchez et al. (2013), nearby the other transplanted plots. None of those sites had been

123 exploited for agriculture, but there has been maple syrup production in site B, and there still is in

124 site C. Site B and C are subjected to some thinning while site D is located in a conservation area.

125 Bulb harvesting experiments were conducted in sugar maple forests not described previously.

126 Details regarding soil characteristics of the sites for the Bulb harvesting experiment are presented

127 in the supplementary material.

128 **Planting density experiment**

129 The experiment was designed as randomized complete blocks and was established at two

130 locations in the Basses-Laurentides in spring 2008 (hereafter referred to as Yr 1 for this

131 experiment). Each block contained 12 plots, *i.e.*, three replicates of each of the four densities

132 tested (44, 88, 178, 356 bulbs m^{-2}). Each replicate consisted of 100 bulbs that were planted in

133 2008, (10 bulbs per row, 10 rows in total) in a plot of appropriate size to achieve target density;

134 the transplants were monitored each year for the five subsequent years (until 2013; referred to as

135 Yr 2 to Yr 6). The resulting distance between bulbs was 15 cm for the 44 bulbs m^{-2} density, 10.7

136 cm for the 88 bulbs m^{-2} density, 7.5 cm for the 178 bulbs m^{-2} density, and 5.2 cm for the 356

137 bulbs m^{-2} density. The 12 plots were randomly distributed within a block, each plot spaced by at

138 least 30 cm. The plant material came from a seizure by governmental authorities of illegally

139 harvested bulbs. All transplanted bulbs were in good condition; they belonged to the size class 3-

140 4, they carried at least one intact root (wild leek bulbs usually produce only a few coarse roots)

141 and were of the variety *tricoccum* (Jones 1979).

142 Plots were plowed to 15 cm depth with a Pulaski (Garant, St-François, QC); rocks and tree roots

143 were removed. Litter was raked before plowing then put back in place after transplantion was

144   completed. The plots were fertilized with 12-25-19 kg ha^{-1} of N-P$_2$O$_5$-K$_2$O (Bio-Jardin 4–3–6

145   [N–P$_2$O$_5$–K$_2$O], which also contains 3 % Mg, mixed with Fossil Bone 0–13–0, McInnes Natural

146   Fertilizers Inc., Stanstead, QC) and 1350 kg ha^{-1} of gypsum (Uncalcined Gypsum Products,

147   CaSO$_4$, Georgia-Pacific Gypsum Corporation, Atlanta, GA, USA) in spring 2008. Additional

148   application of 15-30-23 kg ha^{-1} of N-P$_2$O$_5$-K$_2$O and 1650 kg ha^{-1} gypsum occurred in 2009. From

149   2010 to 2013, plots were fertilized at higher application rates, *i.e.*, 27-55-42 kg ha^{-1} of N-P$_2$O$_5$-

150   K$_2$O and 3000 kg ha^{-1} of gypsum, following the recommendations of Bernatchez et al. (2013)

151   based on fertilization trials conducted on the same sites. At planting (in 2008), fertilizers were

152   incorporated in the soil. In subsequent years, they were broadcasted on top of the litter to prevent

153   plant disturbance.

154   From Yr 2 to 5 (2009 to 2012), Total Leaf Width was measured with a ruler on each plant

155   (TLW$_i$) of three randomly chosen rows in each plot following complete leaf unfolding. TLW$_i$ is

156   the sum of the widths of all leaves present on an individual plant measured at their broadest point.

157   Individual Bulb Width (BW$_i$) was measured with calipers on four rows per plot in July of Yr 5

158   only. BW$_i$ was measured soon after leaf senescence, *i.e.*, when translocation of leaf carbon and

159   nutrient to the bulb had been completed (Nault and Gagnon 1988). Bulb survival was estimated

160   in spring of Yr 2 and 3 by counting the number of initial transplants that had produced a least one

161   leaf. From Yr 4 to 6, the total number of shoots per plot was counted instead of transplant

162   survival, since crowding in denser plots rendered distinctions between independent bulbs and

163   bulbs issued from divisions of the same mother bulb difficult. Care was taken to make sure all

164   plants that had produced at least one leaf were counted regardless of the presence of damage or

165   herbivory. If necessary, bulb was partly dug to confirm its healthiness. The number of floral

166   scapes (elongated, leafless flowering stems) was noted in Yr 4, which was a good flowering year

167 (P.-P. Dion, pers. obs.); flowering rates of wild leek tend to vary greatly among seasons (Nault

168 and Gagnon 1993).

169 Two measures of bulb yield were calculated: (1) by estimating the annual increase in bulb fresh

170 mass in g yr^{-1} per bulb that was initially planted, and (2) by expressing it in g yr^{-1} m^{-2}. Individual

171 bulb fresh mass (FMB$_i$, g) was estimated using the equation:

$$172 \quad FMB_i = 0.250 \times TLW_i - 0.254 \tag{1}$$

173 ($r^2 = 0.728$; $P < 0.001$; $n = 214$; 2.6 cm $<$ TLW$_i$ $<$ 23.3 cm). FMB$_i$ was then summed for the

174 whole plot, and divided either by the number of bulbs that were initially planted to estimate a

175 mean FMB$_i$ per plot, or by the surface area of the plot. Annual biomass increment was then

176 calculated for each plot.

177 In addition to individual bulb growth, total growth of all daughter bulbs originating from

178 divisions of a same mother bulb should be considered. Since simple addition of the BW$_i$ would

179 inflate the effect of bulb division, the sum of daughter bulb widths originating from the same

180 mother bulb (BW$_d$) was calculated by adding up the transversal bulb areas at their broadest

181 points. Bulb areas were calculated from the diameter measured in the field, after which the

182 diameter of the larger virtual bulb was calculated using equation 2.

$$183 \quad BW_d = 2 \times \sqrt{\frac{\sum_{i=1}^{n} \pi r_i^2}{\pi}} \tag{2}$$

184 where n is the number of daughter bulbs and r_i is the radius of the ith daughter bulb. TLW for all

185 daughter bulbs originating from the same mother bulb (TLW$_d$) involved direct addition of all leaf

186 widths. TLW$_d$ and BW$_d$ provide insights into the overall production of each initial bulb, even

187 after some of them have divided.

188   In Yr 5, there was an outbreak of a pest, which was identified as the millipede *Blaniulus*

189   *guttulatus* (Spotted snake millipede). This species is common in gardens and cultivated fields in

190   North America, having been unintentionally introduced from Europe. While this millipede is a

191   known agricultural root pest (Hopkin and Read 1992; Fraval 2014), we did not find any reports of

192   it attacking *Allium* species. Because of the important damage caused by this pest, TLW and scape

193   production from Yr 5 and onward were excluded from the statistical analyses. BW was only

194   measured in Yr 5, and included only plot sections which had not yet been affected by *B.*

195   *guttulatus*. The number of bulbs per plot was noted in Yr 5 and 6 to determine if survival

196   improved at the lower density plots.

197   **Bulb harvesting**

198   Twelve plots were initially delimited in each of four natural, high-density wild leek populations

199   (two in Lower Laurentides, two in Estrie), and their area were measured (48 plots in total). Each

200   plot contained about 100 bulbs. Four different harvesting treatments were randomly assigned to

201   the plots in spring 2011 (hereafter referred to as Yr 1 for this experiment), before leaf senescence.

202   The selected harvesting method consisted of digging out all bulbs in a small plot enclosing 100

203   bulbs, handpicking the ones to harvest, and replanting the others within the same plot. We opted

204   for this method to reduce bulb damage and thus improve population recovery (Nault and Gagnon

205   1993). Harvested bulbs belonged to classes 4 and 5, *i.e.*, medium-sized plants (3.8 cm < TLW ≤

206   10 cm). We replanted those bulbs too small for commercial harvesting, together with the large

207   reproducing bulbs, to promote regeneration of the plot. Two of the harvesting treatments

208   consisted of respectively harvesting 20 and 40 % of the bulbs, which means that 80% and 60% of

209   the bulbs were replanted within the plot. After counting and sorting, the harvested bulbs were

210   replanted within the same natural populations, but outside the experimental plots. The remaining

211   treatments were two different controls, namely, a positive and a negative control. In the positive

212   control, referred to as "0 %," all bulbs were dug out and replanted within the same plot; in the

213   negative control, referred to as "Control," the bulbs were left untouched. These two controls were

214   necessary to test the effect of the harvesting method itself.

215   During the springs of Yr 2 to 4 (2012 to 2014), TLW_i of all individuals was measured after their

216   leaves had completely unfolded. The number of floral scapes and number of seeds produced per

217   plot were recorded in Yr 3, which was a good flowering year (P.-P. Dion, personal observation).

218   The juvenile ($TLW_i < 1$ cm) and non-juvenile plants were counted to assess population growth in

219   the years following bulb harvesting. We counted the number of juveniles to take into account

220   population regeneration from seeds. According to Nault and Gagnon (1993), daughter bulbs

221   resulting from a recent division have a TLW_i of at least 2.1 cm (size class 3) and it is very

222   unlikely that a seedling would reach a TLW_i of 1 cm within the time the experiment lasted.

223   **Leaf harvesting**

224   The leaf harvesting experiment was conducted in plots that had been established in 2008 (for

225   details, see Bernatchez et al. 2013). Each 90×315 cm plot consisted of 100 bulbs that were

226   planted in five rows of 20 plants each. There were six plots in each of four sites, for a total of 24

227   plots. Each plot received a combination of different N-P-K fertilizer and gypsum doses in spring

228   2008 and 2010. By autumn 2010, plots no longer differed in terms of soil extractable P, Ca^{2+} and

229   Mg^{2+} (Bernatchez et al. 2013). From 2011 to 2013, fertilizers were applied annually in each plot

230   in early spring, at the same rates as in the planting density experiment for the same years.

231   During spring 2011 (hereafter referred to as Yr 1 for this experiment), 36 bulbs per plot were

232   randomly selected from among those bulbs that had not yet divided. We selected plants with the

233   same number of leaves (2 or 3) within a plot whenever possible. We applied a combination of the

234   following treatments to each selected bulb: 0, 1 (50 % leaf area) or 2 (100 % leaf area) leaves

235   were removed, and the harvest took place 15, 20 or 25 days after complete leaf unfolding. There

236   were nine treatment combinations, which were replicated four times per plot. In the cases where a

237   bulb had three leaves and was selected for the one-leaf harvest, we removed one leaf and the

238   distal half of a second one, in order to remove 50% of the total leaf area.

239   TLW_d and BW_d were measured from Yr 1 to 3 (2011 to 2013). Floral scape emergence was noted

240   each year, and the scapes were then immediately removed to limit the energy devoted to sexual

241   reproduction and to reduce variation among individuals. Bulb division was also recorded.

242   **Statistical analysis**

243   Statistical analysis of the planting density and the leaf harvesting experiments was performed in

244   *SAS* 9.3 (SAS institute Inc., Cary, NC, USA).

245   In the planting density experiment, the effect of bulb density (44, 88, 178 or 356 bulbs m^{-2}) on

246   bulb yield, BW, TLW, survival, number of bulbs and number of floral scapes was tested with a

247   randomized complete block ANOVA with mixed models using blocks as a random variable, and

248   analyzing each sampling year (Yr 2 to 5) separately. When the bulb density effect was significant

249   ($P < 0.05$), means were compared using a Tukey HSD test. From Yr 2 to Yr 4 (2009 to 2011), the

250   analysis was also performed as two-way repeated measures ANOVA for TLW_i and TLW_d to

251   determine if there was a planting density $\times$ year interaction.

252   For the bulb harvesting experiment, mixed-effects ANCOVAs with repeated measures were

253   performed for the effect of harvesting treatment, time (years) and their interaction on the

254   following variables: TLW of the whole plot (TLW_{plot}; obtained by adding up the widths of all

255   leaves in the plot); mean TLW_i; number of bulbs; and number of juveniles per plot. Site was

256   considered as a random variable. ANCOVAs were also performed within each year from Yr 2 to

257   Yr 4 (2012 to 2014) to follow more closely population recovery from year to year. ANCOVAs

258   were performed on relative growth from Yr 2 to 4 and on scape and seed production in Yr 3. We

259   used plot size (in cm^2) as a covariate to compensate for variation in pre-treatment density (see

260   next paragraph for further details). Tukey HSD tests were used for multiple means comparisons

261   among the four treatments.

262   Despite the fact that treatments were randomly assigned among the pre-delimited plots, we noted

263   that the resulting differences in density were smaller than expected. Indeed, post-treatment

264   density did not significantly differed between the 20 % and 0 % treatments, and the 40 % plots

265   were only significantly sparser than control and 0% plots (Figure 1; $F_{3, 21} = 6.09$, $P = 0.004$).

266   Density varied greatly within a treatment, especially among the control plots, which ranged from

267   151 to 634 bulbs m^{-2}. This kind of density variation can influence the growth of wild leek (see

268   results from the planting density experiment). To more accurately assess the effect of a reduction

269   in bulb density following a partial harvest in natural populations, we decided to conduct a second

270   series of analyses where: (1) experimental treatments were set aside and the density (bulbs m^{-2})

271   that was recorded immediately after bulb harvest was used as an independent variable; and (2)

272   "control" plots were excluded from the analysis, since preliminary analyses indicated that this

273   group differed from the 0 % plots (for more detail, see Results and Discussion). We then

274   performed Pearson product-moment correlations (r) tests between the different response variables

275   and the post-treatment density recorded in Yr 1, using *Stats* package of R 2.15.1 (R Development

276   Core Team 2012).

277   In the leaf harvesting experiment, two plots were plundered in spring of Yr 1 on one site. They

278   were excluded from subsequent analyses. Two other plots were also plundered in spring of Yr 2

279   on the same site as the first two, after leaf width and before bulb width measurements had been

280   completed, and were excluded from the analysis in subsequent years.

281   The experimental design contained 3 sub-groups with no leaf harvesting, one for each date of leaf

282   harvesting. As explained by Gates (1991), we cannot analyze these data as a 3×3 factorial

283   experiment, because this would artificially inflate the interaction between the two factors. An

284   appropriate way of analyzing such an experimental design has been suggested by Lynch et al.

285   (2008). As a first step, the interaction between the two factors was analyzed in a 3×2 factorial

286   analysis ('number of days before harvest' × 'percentage leaf area removed'), excluding the plants

287   in which no leaf was harvested. Mixed-effects ANOVAs were performed on BW_d and TLW_d,

288   with repeated measures: Yr 2 and Yr 3 for TLW_d; and Yr 1 to Yr 3 for BW_d. Leaf measurements

289   of Yr 1 were not included, because they were already unfolded at the time the treatment was

290   applied. Site, plot and year were considered as random variables in the linear mixed-model. In a

291   second step, all plants that had experienced no leaf harvest were pooled and considered as a

292   'control' treatment. ANOVAs and multiple comparisons among all seven treatment

293   combinations, including the controls, were performed within each year, using Tukey HSD tests,

294   for TLW_d (Yr 2 and Yr 3), BW_d (Yr 1 to Yr 3), floral scapes (Yr 3, which was a good flowering

295   season), bulb division (Yr 2 and Yr 3), and survival after two years (Yr 3). For bulb division,

296   flowering and survival, a logit transformation was used to model the probability that the binary

297   events "division", "flowering" and "survival" occur.

RESULTS

Planting density experiment

Planting density significantly influenced most of the growth parameters that were recorded

(Table 1). Only data from the final year are presented (Yr 4 or 5), except for survival, where Yr 2

and Yr 3 results confirmed the absence of transplant shock, with survival ranging from 87.8 % to

95.5 %. Three years after transplantation (Yr 4), wild leek had higher TLW_i and TLW_d in the

lower density plots (Table 1 and Figure 2). Vegetative reproduction occurred also more

frequently in sparsely planted plots; the number of bulbs per plot in Yr 4 had increased by 47.3 %

in the lowest density plots (44 bulbs m^{-2}), by 32.7 % in the 88 bulbs m^{-2} plots, and remained at

levels similar to original planting numbers in the two highest density plots (Figure 3). This led to

a significant interaction between the effects of Density and Yr on TLW_d (Figure 2b); plant

growth and bulb division were both higher in plots with low density, whereas the Density×Yr

interaction was not significant for TLW_i (Figure 2a).

Consistent with the TLW_i and TLW_d data, bulb width (measured in Yr 5) was also higher in low-

density plots than in high-density plots, when reported both on an individual basis (BW_i; 22 %

higher) and as a sum of all bulb widths originating from the same mother bulb (BW_d; 37 %

higher; Table 1). As larger bulbs tend to flower more frequently, the 44 bulbs m^{-2} plots produced

nearly twice as many scapes in Yr 4 than the 356 bulbs m^{-2} plots. The annual yield from Yr 2 to

Yr 4 in grams (fresh mass) of bulb per year per bulb planted was 168 % higher in sparser plots

than in the 356 bulbs m^{-2} plots. The annual yield in terms of grams (fresh mass) of bulb per m^2

per year, however, followed the opposite trend, and was 196 and 91 % higher in plots with 356

bulbs m^{-2} than in plots with respectively 44 or 88 bulbs m^{-2}. The final number of bulbs m^{-2} in

Yr 4 also followed the same trend, remaining six times higher in denser than in sparser plots.

321   No significant differences were apparent between the two lowest densities (44 and 88 bulbs m^{-2}),

322   for any of the variables (Table 1). At a density of 178 bulbs m^{-2} and higher, BW_d, TLW_d, the

323   number of bulbs per plot and the annual bulb yield (g FM yr^{-1} per bulb initially planted)

324   significantly decreased compared to the lowest density (44 bulbs m^{-2}). Only the highest density

325   (356 bulbs m^{-2}) significantly decreased BW_i, TLW_i and flowering the last year prior to the pest

326   outbreak, relative to the lowest density.

327   Figure 3 presents the reduction in bulb number in Yr 5 and 6, which was probably caused by the

328   pest outbreak, compared with the numbers recorded in Yr 4. Effects of density and variation from

329   one season to the next were significant. The less dense plots seemed to be initially less affected

330   by the pest from Yr 4 to 5, but once their numbers began to drop, they did so at rates similar to

331   those of the denser plots; the interaction between density and year was not significant.

332   **Bulb harvesting**

333   One site exhibited surprisingly low survival of the replanted bulbs, probably because of high air

334   temperatures on the day transplantation took place. In Yr 3, an average of 40, 20 and 13 bulbs

335   remained alive in the 0 %, 20 % and 40 % harvest plots, respectively, while 90 bulbs survived in

336   the controls. This site was therefore excluded from further statistical analysis. Three plots from

337   another site (two "0 %" and one "20 %") were also dropped from the analysis for the same

338   reason.

339   There was no significant interaction between the effects of time (years after harvesting) and

340   harvesting treatments on TLW_{plot}, TLW_i, the number of mature bulbs, and the number of

341   juveniles per plot (Table 2). However, multiple comparisons among treatments performed within

342   each year (Table 3) highlighted post-harvest recovery over time. In Yr 2, TLW_i was 28 % higher

343 in control plots than in the three harvesting treatments, indicating a negative effect of the

344 harvesting method (Table 3). This difference was less pronounced in Yr 3, as the 20 % plots no

345 longer significantly differed from the controls, but TLW_i of the 0 % and 40 % plots remained

346 lower. There was no further difference in Yr 4 for TLW_i. As expected, the TLW_{plot} was higher in

347 the control plots and lower in the 20 % and 40 % plots, but the observed difference became

348 smaller from Yr 2 to Yr 4 ($F_{3, 20}$ decreased from 22.5 to 6.4). In Yr 4, the 0 % plots had caught up

349 with the control, but the 20 % and 40 % treatments still had 27 % and 34 % lower TLW_{plot},

350 respectively. Relative increases of TLW_{plot} and number of bulbs per plot did not differ among

351 treatments from Yr 2 to Yr 4, according to ANCOVA (Table 3). Yet the relative increase in

352 TLW_i tended to be higher in treated plots ($P = 0.094$), suggesting a positive effect of the

353 digging/replanting treatment on relative growth of individual plants (TLW_i). It is worth

354 mentioning that TLW_i increased by 14 to 40 % across treatment groups between Yr 2 (2012) and

355 Yr 3 (2013), then decreased by 16 to 26 % between Yr 3 (2013) and Yr 4 (2014). The average

356 number of scapes and seeds produced per bulb, and the number of juveniles per plot did not differ

357 among treatments. As only class 4 and 5 plants were harvested, the treatment itself could have

358 induced changes in size classes in subsequent years, and decreased mean TLW_i in the 20 and 40

359 % harvested treatments. In addition to the results presented in Table 3, we performed an

360 additional ANCOVA on the TLW_i data after simulating a harvest of 20 % (for the 20 %

361 treatment) and 40 % (for the 0 % treatment) of the total number of bulbs, removing only class 4

362 and 5 plants (not shown). This was done to properly compare mean TLW_i in response to

363 differential harvesting, without the statistical artifact incurred by the selective harvesting of mid-

364 size plants. This adjustment yielded results similar to those presented in Table 3, except that the

365 difference among treatments for TLW_i became no longer significant in Yr 3 instead of in Yr 4.

366   Correlations between post-harvest bulb density and response variables are presented in Table 4.

367   Two negative relationships significantly differed from zero: one with the relative increase of

368   TLW_i (Yr 4/Yr 2) and the other with TLW_i in Yr 4. These correlations indeed suggest that higher

369   post-harvest densities negatively affect individual plant growth.

370   **Leaf harvesting**

371   Complete defoliation (100% leaf harvesting) significantly reduced TLW_d by 21-41%, BW_d by 8-

372   24% and diminished floral scape production by 65 to 90%, relative to the control (Table 5). In all

373   years, the negative effect of complete defoliation on these growth parameters was more

374   pronounced when leaves were removed at 15 d rather than 25 d following leaf unfolding (Table

375   5). Plants that were subjected to complete defoliation still exhibited a 39-48% lower frequency of

376   bulb division two years later (Yr 3), except for the 20-day group. Surprisingly, the bulb division

377   rate was not significantly affected in Yr 2 ($P = 0.216$). Partial defoliation (50% leaf harvesting)

378   was less damaging to wild leek growth. Although 50% leaf harvesting caused a significant

379   reduction in TLW_d for the 15-d group in Yr 2, and a significant 4-11% reduction of BW_d in Yr 1

380   and 2 compared to the unharvested control, no other significant negative effects on TLW_d, BW_d

381   and scape production were observed. There was thus a significant interaction between the

382   percentage of leaves that were harvested and the number of days before harvesting on bulb and

383   leaf size (BW_d and TLW_d) during subsequent growing seasons, as illustrated in Figure 4. This

384   suggests that delaying the number of days has a greater effect when all leaves were harvested

385   than when only half the leaves were removed. Plant survival was not significantly affected by

386   leaf harvesting, regardless of treatment.

DISCUSSION

387

388 **Density effects**

389 Planting wild leek at high densities reduced individual growth of both bulb and leaf. This effect

390 most likely results from competition and crowding among bulbs. The only other density

391 manipulation experiment that has been performed on wild leek reported no density effect

392 (Ritchey and Schumann 2005), but the densities tested were 11 and 44 bulbs m^{-2}. These results do

393 not contradict ours; we only observed a density effect on bulb and leaf size at densities of 178

394 bulbs m^{-2} or greater. Nevertheless, we can conclude that planting at a density lower than 44 bulbs

395 m^{-2} does not improve wild leek growth further.

396 Annual yield per m^2 increased with planting density, whereas individual growth and yield per

397 planted bulb decreased with an increase in planting density. This response has been reported for

398 commercial onion, for which individual bulb size also decreased with increasing density, but

399 optimal yield per unit area reached its maximum value at an intermediate density (Brewster and

400 Salter 1980; McGeary 1985; Herison et al. 1993). The highest density tested in the present study

401 may have been too low to negatively affect yields per area, but the decrease in TLW, BW and

402 annual yield per transplanted bulb may nevertheless reduce the value of the crop on a per bulb

403 basis. It is important to mention that fertilizer applications were calculated on an area basis

404 (nutrients per area), at rates established for a planting density of 44 bulbs m^{-2} (Bernatchez et al.

405 2013). Increasing the amount of nutrient applied may improve growth at higher densities. Low-

406 density planting promoted bulb division and flower production. The interaction between Density

407 and Yr on TLW$_d$ corroborates the overall faster annual growth of wild leek at lower density.

408 Overcrowding and competition for resources is of concern for this species, considering the very

409 high densities that can be reached in natural populations. Indeed, mortality in the denser patches

410   was reported (Nault and Gagnon 1993). Competition also reduced sexual and asexual

411   reproduction in *Allium oleraceum* L. (Fialová and Duchoslav 2014).

412   The density effect was also noticeable in natural populations following partial bulb harvest. The

413   effect only became apparent three years after treatment, as revealed by the negative correlation

414   between post-harvest density and TLW_i ($r = -0.537$; $P = 0.007$). In Yr 2 and 3, plants were

415   smaller in the harvested plots than in the controls, regardless of the percentage of bulbs that had

416   been harvested. Plants were thus likely recovering from the transplant stress that was caused by

417   the harvesting method that had been applied. Similarly, a reduction in individual growth rates has

418   been previously reported in wild leek following transplantation (Vasseur and Gagnon 1994).

419   TLW_i no longer differed between the harvested and control plots in Yr 4, suggesting a higher

420   growth rate in harvested compared to control plots between Yr 2 and 4.

421   The maximum harvest rate that would cause no long-term decline in numbers was modeled for

422   natural populations of wild leek and estimated to be 8 to 10 % annually (Nault and Gagnon 1993;

423   Nantel et al.1996). Rock et al. (2004) suggested harvest rates as low as 10 % over a 10-year

424   period. These studies did not consider the effects of bulb density on subsequent growth in their

425   models. The present results show that overcrowding is indeed present in dense populations. A

426   substantial reduction in density by partial bulb harvest can favor increased bulb size and,

427   eventually, bulb division and seed production, as larger bulbs are more likely to flower and divide

428   (Nault and Gagnon 1993). Furthermore, the survival of seedlings could be improved since they

429   would have access to more resources.

430   To our knowledge, it is the first time that *B. guttulatus* has been reported as a pest on wild leek.

431   This pest usually affects other root vegetables such as sugar beets, carrots and potatoes (Allen

432   and Filotas 2009). Nault and Gagnon (1993) reported what they called "clump death," similarly

433   to the decline that we reported in the density plots. Further research is required to determine if the

434   cause of death could be attributed to infestation by *B. guttulatus* or if another pest or pathogen

435   preceded *B. guttalatus* and weakened the plants. Lower planting densities are known to slow pest

436   infestations of *Thrips tabaci* on onion (*A. cepa*) (Jima et al. 2013). This could explain the

437   apparent delay in the reduction of population size observed at lower density in the present

438   experiment, and constitutes another argument in favor of planting less than 88 bulbs m^{-2}.

439   Applying a specific percentage of harvesting, as previously recommended (Nault and Gagnon

440   1993; Nantel et al. 1996; Rock et al. 2004), can lead to variable yields, considering the very high

441   variability in plant density present in natural populations as in the Bulb harvesting experiment.

442   Harvesting down to a fixed post-harvest density between 44 and 88 bulbs m^{-2} should provide

443   optimal conditions for population recovery, considering the reduction in competition among

444   individuals. However, we do recommend waiting until high densities are reached, before

445   harvesting bulbs again. This conservative approach will allow populations to remain healthy

446   despite stochastic events.

447   High mortality that was recorded on one site reveals the potential deleterious consequences of the

448   harvest and transplant methods. Harvest on this particular site was performed on a particularly

449   hot and dry day. Stress on the roots may have been enough to affect survival. Nevertheless, it is

450   surprising, considering the high survival rates reported for transplanted wild leek and its tolerance

451   to transplantation shock (Vasseur and Gagnon 1994; Bernatchez et al. 2013). The reduction in

452   growth observed in the other sites in the plots where all bulbs had been harvested then replanted

453   (0 %) compared to the non-harvested plots (controls) illustrates the possible effect of transplant

454   shock on subsequent growth of wild leek. Such transplant stress occurs more frequently in arid or

455 semi-arid environments (Shinohara and Leskovar 2014), but it has been reported for transplants

456 of black cohosh *Actaea racemosa* L. in temperate forest sites (Small et al. 2014). Nevertheless,

457 we could reduce post-harvest mortality by (1) harvesting on cool and cloudy days; (2) keeping

458 plants in a cooler during the process; and (3) watering the soil after the bulbs have been

459 replanted. Selective harvests could also take place immediately after leaf senescence at a time

460 when the plants are much less sensitive to transplant shock.

461 **Leaf harvest**

462 As expected, increasing the proportion of leaves that were harvested and performing an early

463 harvest reduced growth of wild leek during subsequent years. Allowing the leaves to perform

464 photosynthesis over a longer period of time before being harvested likely lead to more carbon

465 accumulation in the bulb, whereas removing only half of the leaves likely reduced the loss in

466 carbon and nutrients. Bigger bulbs resulted at the end of the season in which the harvest was

467 performed and in subsequent years, and larger leaves in the following seasons. The significant

468 interaction between the number of leaves that were harvested and the number of days before

469 harvest suggests that the difference between harvesting half and harvesting all of the leaves was

470 less pronounced if the harvest was delayed as late as possible before senescence. Indeed,

471 harvesting all leaves at 25 days yields similar growth responses as does harvesting half of the

472 leaves.

473 Removing half of the foliage did not induce complete compensatory photosynthesis in the

474 remaining foliage, since these plants did not accumulate as much C in their bulbs as did the

475 control plants, based on the leaf and bulb widths. Tip-defoliation has been shown to induce

476 compensatory photosynthesis in the remaining leaf sections of *Oenothera biennis* L. (evening-

477 primrose; Morrison and Reekie 1995) or to maintain constant photosynthetic rates in damaged

478 leaves of bluebunch wheatgrass *Pseudoroegneria spicata* (Pursh) Á. Löve and crested wheatgrass

479 *Agropyron desertorum* (Fish.) J.A. Schutes (Nowak and Caldwell 1984). Direct measurements of

480 photosynthetic rates would be necessary to confirm whether compensatory photosynthesis occurs

481 following partial leaf harvesting.

482 Flowering was affected by the proportion of leaf area that was harvested, but not by the timing of

483 defoliation. Flowering might be more sensitive to nutrient than to carbon availability within the

484 bulb. Nault and Gagnon (1988) reported that the presence of chlorophyll allows the floral scape

485 to be partially self-sufficient. Moreover, they observed that reproductive structures contain 30

486 and 45 % of total plant Mg and Ca, respectively, while these two elements account for 15 % of

487 total biomass. Harvesting all leaves also had a negative effect on bulb division. In perennial

488 grassland herbs, defoliation sometimes reduces asexual reproduction by rhizome propagation, but

489 this effect varies with species, given that some also exhibit improved reproductive effort

490 following defoliation (Benot et al. 2009, 2010; Bostrom et al. 2013). The proportion of leaf area

491 harvested thus seems to affect to a greater extent the overall growth of wild leek than the time at

492 which harvesting took place.

493 **Stochastic environment**

494 The 2013 (Yr 3 of Bulb and Leaf harvesting experiments) growing season was apparently a bad

495 season for wild leek. It is clearly demonstrated in the Leaf harvest experiment as a decrease in

496 BW_d between Yr 2 and 3, and in the Bulb harvest experiment as a decrease in TLW_i from Yr 3 to

497 4. Leaf elongation begins in autumn (Nault and Gagnon 1993) and, thus, TLW is influenced by

498 the previous season. Spring 2013 was particularly dry during the epigeous period of wild leek

499 (Environment Canada 2013). A similar reduction in leaf longevity during a dry summer has been

500 observed in the forest herb *Trillium erectum* (Tessier 2008). Indeed, Nantel et al. (1996) reduced

501   the recommended bulb harvest rates to compensate for stochastic environments. Nevertheless,

502   plots in which 40% of the bulbs had been harvested maintain a stable bulb number in the three

503   subsequent years, which indicates that wild leek populations subjected to a high harvest rate can

504   tolerate a harsh season a few years later. Producers should be advised to wait until the population

505   has reached the pre-harvest state, in terms of plant size and number, before harvesting again.

506   The same precautions can apply to leaf harvesting. For instance, in Yr 2, plants completely

507   defoliated at 25 days already had an average BW_d comparable to control plants in Yr 1 (see Table

508   5). This response would suggest that the treatment could be repeated every two years without a

509   long-term negative effect. The growth decline that was observed in 2013 after a dry spring would

510   suggest waiting somewhat longer. Only long-term follow-ups of both harvested and control plots

511   could determine optimal leaf and bulb harvest frequency.

512   **Concluding remarks**

513   The results of the present study confirm that overcrowding can be present in natural populations

514   and that reduced bulb density can improve yield. We also confirmed that modulating both the

515   date at which leaves are harvested and the proportion of leaf harvested influence the effects of

516   leaf harvesting on subsequent plant growth. Based on the present results, the following

517   recommendations can be proposed to improve yield of wild leek plantations. When establishing a

518   new plantation, the bulbs should be planted at densities not exceeding 88 bulbs m^{-2}. In the present

519   study, this was the best compromise between individual growth, reproduction and yield per area.

520   Harvesting bulbs in natural populations should be made on cool or overcast days, with the soil

521   being watered to prevent mortality. The high survival in most plots indicates that a single high

522   harvest event (up to 40 % of bulbs being harvested) is not a threat to population survival, if done

523   under proper conditions. However, we strongly recommend that subsequent harvests should take

524 place only when the population has reached its pre-harvest density. We recommend harvesting

525 down to a fixed post-harvest density of 44 to 88 bulbs m^{-2} instead of harvesting a percentage of

526 plants to favor post-harvest growth and population recovery. Leaves should be harvested at the

527 latest possible date ($\geq$ 25 days) after complete unfolding. Recreational harvesters should limit

528 themselves to one leaf per plant. Commercial producers, for practical reasons, will harvest all

529 leaves on either patches or rows of plants. This more severe treatment does not affect survival,

530 but the producer should wait until plants have reached pre-harvest size before harvesting again,

531 which should take at least two years.

REFERENCE LIST

Allen J, Filotas M. (2009). Millipedes in carrots and sweet potatoes. Factsheet ISSN 1198-712X. Agdex 258/611, February 2009. Ontario Ministry of Agriculture, Food and Rural Affairs, Queen's Printer for Ontario. 4 p.

Benot ML, Bonis A, Mony C. (2010) Do spatial patterns of clonal fragments and architectural responses to defoliation depend on the structural blue-print? An experimental test with two rhizomatous Cyperaceae. Evol Ecol 24: 1475-1487.

Benot ML, Mony C, Puijalon S, Mohammad-Esmaeili M, van Alphen JJM, Bouzille JB, Bonis A. (2009) Responses of clonal architecture to experimental defoliation: a comparative study between ten grassland species. Plant Ecol 201: 621-630.

Bernatchez A, Bussières J, Lapointe L. (2013) Testing fertilizer, gypsum, planting season and varieties of wild leek (*Allium tricoccum*) in forest farming system. Agrofor Syst 87: 977-991.

Bernatchez A, Lapointe L. (2012) Cooler temperatures favour growth of wild leek (*Allium tricoccum*), a deciduous forest spring ephemeral. Botany 90: 1125-1132.

Bostrom U, Andersson L, Forkman J, Hakman I, Liew J, Magnuski E. (2013) Seasonal variation in sprouting capacity from intact rhizome systems of three perennial weeds. Weed Res 53: 387-398.

Brewster JL, Salter PJ. (1980) The effect of plant spacing on the yield and bolting of two cultivars of overwintered bulb onions. J Hortic Sci 55: 97-102.

Burkhart EP, Jacobson MG. (2009) Transitioning from wild collection to forest cultivation of indigenous medicinal forest plants in eastern North America is constrained by lack of profitability. Agrofor Syst 76: 437-453.

Chamberlain JL, Mitchell D, Brigham T, Hobby T, Zabek L, Davis J. (2009) Forest farming practices. In: Garrett HEG (ed) North American agroforestry, an integrated science and practice. Am Soc Agron, Madison, WI, pp. 219-256.

Couillard L. (1995) La situation de l'ail des bois (*Allium tricoccum*) au Québec. In: Direction de la conservation et du patrimoine écologique, Ministère de l'Environnement et de la Faune du Québec (eds), 31 p.

Dagenais J. (1985) Rapport sur la situation de l'ail des bois (*Allium tricoccum*) au Québec. Association des biologistes du Québec, 50 p.

Davis JM, Greenfield J. (2002) Cultivating ramps: Wild leeks of Appalachia. In: Janick J, Whipkey A (eds) Trends in new crops and new uses. Proceedings of the fifth National Symposium, New Crops and New Uses: Strength in Diversity, 8-11 November 2001, Atlanta, GA. ASHS Press, Alexandria, VA. pp. 449-452.

576   Environment Canada. 2013. Climate archives, Sherbrooke and Oka meteorological stations in
577   2013. http://www.climat.meteo.gc.ca/ Accessed [2013-11-05]

578   Facemire G. (2008) Having your ramps and eating them too. McClain Printing Company,
579   Parsons, West Virginia, 144 p.

580   Fialová M, Duchoslav M. (2014) Response to competition of bulbous geophyte *Allium oleraceum*
581   differing in ploidy level. Plant Biol 16: 186-196.

582   Fraval A. (2014) Encyclopédie des ravageurs européens. Institut national de la recherche
583   agronomique. http://www7.inra.fr/hyppz/ Accessed [2014-06-16]

584   Gates CE. (1991) A user's guide to misanalyzing planned experiments. HortScience 26: 1262-
585   1265.

586   Herison C, Masabni JG, Zandstra BH. (1993) Increasing seedling density, age, and nitrogen-
587   fertilization increases onion yield. HortScience 28: 23-25.

588   Hewins CR, Carrino-Kyker SR, Burke DJ. (2015) Seasonal variation in mycorrhizal fungi
589   colonizing roots of *Allium tricoccum* (wild leek) in a mature mixed hardwood forest.
590   Mycorrhiza 1-15.

591   Hopkin SP, Read HJ. (1992) The biology of millipedes. Oxford University Press, Oxford, 233 p.

592   Irigoyen I, Domeno I, Muro J. (2010) The effect of defoliation on the yield of leek (*Allium
593   porrum* L.). Span J Agric Res. 8: 434-439.

594   Jima TA, Tekie H, Tikubet G. (2013) Effects of manure application and host plant spacing on the
595   infestation level and damage of *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) in onion
596   (*Allium cepa*). Afr Entomol 21: 316-323.

597   Jones AG. (1979) A study of wild leek, and the recognition of *Allium burdickii* (Liliaceae). Syst
598   Bot 4: 29-43.

599   Jones, ET, K.A. Lynch. (2007) Nontimber forest products and biodiversity management in the
600   Pacific Northwest. For Ecol Manag 246: 29-37.

601   Lapointe L, Bussières J, Crete M, Ouellet JP. (2010) Impact of growth form and carbohydrate
602   reserves on tolerance to simulated deer herbivory and subsequent recovery in Liliaceae. Am
603   J Bot 97: 913-924.

604   Leskovar DI, Agehara S, Yoo K, Pascual-Seva N. (2012) Deficit irrigation and plant density
605   impact on growth, yield, quality and quercetin of short-day onion. XXVIII International
606   horticultural congress on science and horticulture for people (IHC2010); International
607   symposium on quality-chain management of fresh vegetables: from fork to farm, 22-27
608   August 2010, Lisbon, Portugal. pp. 219-226.

609 Lynch DH, Zheng ZM, Zebarth BJ, Martin RC. (2008) Organic amendment effects on tuber
610   yield, plant N uptake and soil mineral N under organic potato production. Renew Agric
611   Food Syst 23: 250-259.

612 Maurin V, DesRochers A. (2013) Physiological and growth responses to pruning season and
613   intensity of hybrid poplar. For Ecol Manag 304: 399-406.

614 McGeary DJ. (1985) The effects of plant-density on the shape, size, uniformity, soluble solids
615   content and yield of onions suitable for pickling. J Hortic Sci 60: 83-87.

616 Meyer GA. (1998) Pattern of defoliation and its effect on photosynthesis and growth of
617   goldenrod. Funct Ecol 12: 270-279.

618 Morrison KD, Reekie EG. (1995) Pattern of defoliation and its effect on photosynthetic capacity
619   in *Oenothera biennis*. J Ecol 83: 759-767.

620 Muro J, Irigoyen I, Lamsfus C. (1998) Effect of defoliation on onion crop yield. Sci Hortic
621   (Amsterdam) 77: 1-10.

622 Muro J, Irigoyen I, Lamsfus C, Fernandez Militino A. (2000) Effect of defoliation on garlic yield.
623   Sci Hortic (Amsterdam) 86: 161-167.

624 Nantel P, Gagnon D, Nault A. (1996) Population viability analysis of American ginseng and wild
625   leek harvested in stochastic environments. Conserv Biol 10: 608-621.

626 Nault A, Gagnon D. (1988) Seasonal biomass and nutrient allocation patterns in wild leek
627   (*Allium tricoccum* Ait.), a spring geophyte. Bull Torrey Bot Club 115: 45-54.

628 Nault A, Gagnon D. (1993) Ramet demography of *Allium tricoccum*, a spring ephemeral,
629   perennial forest herb. J Ecol 81: 101-119.

630 Nowak RS, Caldwell MM. (1984) A test of compensatory photosynthesis in the field:
631   Implications for herbivory tolerance. Oecologia 61: 311-318.

632 R Development Core Team (2012) R: A language and environment for statistical computing. R
633   Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL
634   *http://www.R-project.org/*

635 Ritchey KD, Schumann CM. (2005) Response of woodland-planted ramps to surface-applied
636   calcium, planting density, and bulb preparation. HortScience 40: 1516-1520.

637 Rock JH, Beckage B, Gross LJ. (2004) Population recovery following differential harvesting of
638   *Allium tricoccum* Ait. in the southern Appalachians. Biol Conserv 116: 227-234.

639 Rothstein DE, Zak DR. (2001a) Photosynthetic adaptation and acclimation to exploit seasonal
640   periods of direct irradiance in three temperate, deciduous-forest herbs. Funct Ecol 15: 722-
641   731.

642   Rothstein DE, Zak DR. (2001b) Relationships between plant nitrogen economy and life history in
643   three deciduous-forest herbs. J Ecol 89: 385-394.

644   Schmidt IB, Mandle L, Ticktin T, Gaoue OG. (2011) What do matrix population models reveal
645   about the sustainability of non-timber forest product harvest? J Appl Ecol 48: 815-826.

646   Shinohara T, Leskovar DI. (2014) Effects of ABA, antitranspirants, heat and drought stress on
647   plant growth, physiology and water status of artichoke transplants. Sci Hortic 165: 225-234.

648   Small CJ, Chamberlain JL, Nuckols CM. (2014) Failure of black cohosh (*Actaea racemosa* L.)
649   rhizome transplants: potential causes and forest farming implications. Agrofor Syst 88:
650   815-822

651   Tessier JT. (2008) Leaf habit, phenology, and longevity of 11 forest understory plant species in
652   Algonquin State Forest, northwest Connecticut, USA. Botany 86: 457-465.

653   Ticktin T. (2004) The ecological implications of harvesting non-timber forest products. J Appl
654   Ecol 41: 11-21.

655   Vasseur L, Gagnon D. (1994) Survival and growth of *Allium tricoccum* Ait. transplants in
656   different habitats. Biol Conserv 68: 107-114.

657   Whigham DF, Chapa AS. (1999) Timing and intensity of herbivory: Its influence on the
658   performance of clonal woodland herbs. Plant Species Biol 14: 29-37.

659

660

661

662

663 **TABLES**

664 Table 1. ANOVA and multiple comparisons of wild leek growth responses among different
665 planting densities.

Variable	Density (bulb m^{-2})†				SE	$F_{3,\,15}$**	*P*-value
	44	88	178	356			
Annual bulb yield‡ (g FM yr^{-1} m^{-2})	56.1 a	86.5 a	108.5 ab	165.5 b	20.8	6.36	**0.005**
Annual bulb yield‡ (g FM yr^{-1} per bulb planted)	1.26 a	0.97 ab	0.61 bc	0.47c	0.14	9.77	**< 0.001**
BW$_i$ Yr 5 (cm)	1.62 a	1.64 a	1.50 ab	1.33 b	0.05	8.05	**0.003**
BW$_d$ Yr 5 (cm)	2.47 a	2.21 ab	2.02 bc	1.79 c	0.13	11.67	**< 0.001**
TLW$_i$ Yr 4 (cm)	10.02 a	9.22 a	9.03 a	7.36 b	0.72	10.22	**< 0.001**
TLW$_d$ Yr 4 (cm)	15.50 a	13.71 ab	11.46 bc	9.81 c	1.34	19.93	**< 0.001**
Survival Yr 2 (%)	95.5	95.5	93.8	96.0	1.18	1.28	0.318
Survival Yr 3 (%)	93.0	94.2	89.2	87.8	2.58	1.86	0.180
Bulb number per plot Yr 4*	147.3 a	132.7 ab	99.7 b	99.0 b	13.3	3.88	**0.031**
Bulb number m^{-2} Yr 4	65.5 a	116.4 ab	178.0 b	366.7 c	26.5	28.87	**< 0.001**
Scape per bulb Yr 4	0.325 a	0.193 ab	0.247 ab	0.168 b	0.05	4.53	**0.019**

666 † Within a row, values followed by different letters significantly differ according to Tukey HSD tests (α = 0.05). *P*
667 values in boldface type are statistically significant (*P* ≤ 0.05).
668 ‡ Annual yields represent the mean annual increment from Yr 2 to Yr 4. Planting took place in Yr 1.
669 *100 bulbs were initially planted in each plot.
670 **ANOVAs on bulb width performed with 12 degrees of freedom for the error term, because the mortality caused by
671 the parasite reduced the number of plots. Six, 6, 5 and 4 plots unaffected by the pest were included in the ANOVA
672 for the densities of 44, 88, 178 and 356 bulbs m^{-2} respectively.
673 Abbreviations: BW$_i$, individual bulb width; BW$_d$, sum of daughter bulbs width originating from the same mother
674 bulb; TLW$_i$, individual total leaf width; TLW$_d$, total leaf width of all daughter bulbs originating from a same mother
675 bulb; FM, fresh mass; SE, standard error.
676

677 Table 2. Effect of bulb harvesting treatment, year and their interaction on TLW_{plot}, TLW_i, number
678 of juveniles per plot and number of bulbs per plot in natural wild leek stands in which 0, 20 or 40%
679 of the bulbs were harvested. Results of repeated measures ANCOVA are reported. The actual plot
680 area was included in the model as a covariate, since it influences plant density..

		d.f.			
Variable	Effect	Num.	Den.	F-value	P-Value
TLW_{plot}					
	Harvesting treatment	3	78	13.37	**< 0.001**
	Yr	2	78	20.55	**< 0.001**
	H × Yr	6	78	2.00	*0.075*
	Plot Area (covariate)	1	78	6.84	**0.011**
TLW_i					
	Harvesting treatment	3	78	7.25	**< 0.001**
	Yr	2	78	50.40	**< 0.001**
	H × Yr	6	78	2.09	*0.064*
	Plot Area (covariate)	1	78	6.55	**0.012**
Juveniles per plot					
	Harvesting treatment	3	78	0.25	0.863
	Yr	2	78	0.80	0.453
	H × Yr	6	78	1.28	0.279
	Plot Area (covariate)	1	78	4.45	**0.038**
Bulbs per plot					
	Harvesting treatment	3	78	11.38	**< 0.001**
	Yr	2	78	0.42	0.661
	H × Yr	6	78	0.49	0.814
	Plot Area (covariate)	1	78	3.43	*0.068*

681 Note:
682 ANCOVA and multiple comparisons among treatments within each year are presented in Table 3. *P* values in
683 boldface type are statistically significant ($P \leq 0.05$); those in italic font are almost significant ($0.05 < P \leq 0.1$).
684 Abbreviations: TLW_i, mean individual total leaf width; TLW_{plot}, total leaf width of all plants with $TLW_i > 1$ cm
685 within a plot.

Table 3. Effects of different percentages of bulb harvesting on growth responses in natural stands of wild leek, during the three subsequent years. Data are presented as the expected values at the mean plot area of 0.47 m^2 (covariate), as modeled by ANCOVA.

| Variable | Year(s) | Treatment | | | | $F_{3,20}$ | P-value |
		Control	0 %	20 %	40 %		
TLW$_{plot}$	Yr 2	804.5 (39.4) a	635.5 (43.9) b	506.1 (41.6) c	446.6 (39.2) c	22.50	**< 0.001**
(cm)	Yr 3	893.0 (45.3) a	795.4 (51.8) ab	674.9 (48.1) b	532.6 (45.2) c	11.62	**< 0.001**
	Yr 4	701.7 (59.0) a	715.2 (65.7) a	510.3 (62.4) b	461.37 (58.8) b	6.44	**0.003**
TLW$_i$	Yr 2	7.68 (0.33) a	5.80 (0.36) b	6.10 (0.35) b	6.11 (0.33) b	12.22	**< 0.001**
(cm)	Yr 3	8.81 (0.45) a	7.44 (0.49) c	8.57 (0.47) ab	7.71 (0.45) bc	3.37	**0.039**
	Yr 4	6.87 (0.27)	6.26 (0.30)	6.30 (0.28)	6.33 (0.27)	1.54	0.235
Juveniles per plot (TLW$_i$ < 1 cm)	Yr 2	29.94 (9.87)	19.97 (11.26)	39.57 (10.48)	26.20 (9.84)	0.59	0.628
	Yr 3	22.17 (7.27)	33.90 (8.11)	28.12 (7.69)	37.68 (7.25)	1.17	0.345
	Yr 4	23.83 (8.27)	30.58 (8.85)	23.63 (8.60)	25.72 (8.25)	0.30	0.823
Bulbs per plot (TLW$_i$ > 1 cm)**	Yr 2	104.8 (4.2) a	109.2 (4.8) a	84.2 (4.4) b	73.0 (4.2) b	15.59	**< 0.001**
	Yr 3	102.2 (5.2) a	109.7 (6.0) a	80.1 (5.5) b	69.3 (5.2) b	11.83	**< 0.001**
	Yr 4	101.5 (7.8) a	114.2 (8.9) a	78.2 (8.3) b	73.2 (7.8) b	5.66	**0.006**
Rel. increase of TLW$_{plot}$[†]	Yr 4 / Yr 2	-0.125 (0.104)	0.134 (0.117)	0.034 (0.111)	0.044 (0.104)	0.72	0.550
Rel. increase of TLW$_i$[*]	Yr 4 / Yr 2	-0.095 (0.064)	0.093 (0.071)	0.083 (0.068)	0.046 (0.064)	2.44	*0.094*
Rel. increase of bulb number[‡]	Yr 4 / Yr 2	-0.031 (0.074)	0.038 (0.085)	-0.105 (0.079)	0.021 (0.074)	0.62	0.613
Scape per bulb	Yr 3	0.214 (0.031)	0.097 (0.036)	0.158 (0.033)	0.141 (0.031)	2.12	0.130
Seeds per bulb	Yr 3	1.859 (0.344)	0.768 (0.393)	1.462 (0.365)	1.382 (0.343)	1.77	0.185

Notes:
ANCOVA performed with plot area as the covariate. Value in parentheses is the standard error. Within a line, values followed by the same letter do not significantly differ according to Tukey HSD tests ($\alpha = 0.05$). P values in boldface type are statistically significant ($P \leq 0.05$); those in italic font are almost significant ($0.05 < P \leq 0.1$)
[†] Relative increase in the sum of TLW$_i$ for the whole plot (TLW$_{plot}$): (TLW$_{plot\_Yr 4}$ − TLW$_{plot\_Yr 2}$) / TLW$_{plot\_Yr 2}$
[*] Relative increase in the TLW$_i$: (TLW$_{i\_Yr 4}$ − TLW$_{i\_Yr 2}$) / TLW$_{i\_Yr 2}$
[‡] Relative increase in the number of bulbs: (Nb Bulbs$_{Yr 4}$ − Nb Bulbs$_{Yr 2}$) / Nb Bulbs$_{Yr 2}$
[**]100 bulbs were initially planted in each plot.
Abbreviations: TLW$_i$, mean individual total leaf width; TLW$_{plot}$, total leaf width of all plants with TLW$_i$ > 1 cm within a plot.

697 Table 4. Product-moment correlations (r) between post-harvest density in Yr 1 (bulb m^{-2}) and
698 wild leek growth responses in subsequent years. Values in boldface indicate correlations that
699 significantly differ from 0 at $P = 0.05$.

Response variable	Year	Correlations with bulb density in Yr 1	
		r	P-value
Rel. increase – TLW_{plot}[†]	Yr 4/Yr 2	- 0.307	0.145
Rel. increase – TLW_i[*]	Yr 4/Yr 2	**- 0.486**	**0.016**
Rel. increase in bulb[‡] number	Yr 4/Yr 2	0.020	0.925
Seeds per bulb	Yr 3	0.145	0.499
Scape per bulb	Yr 3	0.125	0.562
TLW_i (cm)	Yr 2	0.156	0.466
	Yr 3	0.028	0.898
	Yr 4	**-0.537**	**0.007**
TLW_{plot}	Yr 2	0.017	0.936
	Yr 3	-0.173	0.420
	Yr 4	-0.181	0.397
Juveniles per plot	Yr 2	-0.034	0.873
	Yr 3	0.012	0.957
	Yr 4	0.055	0.800
Number of bulbs	Yr 2	-0.047	0.826
	Yr 3	-0.130	0.546
	Yr 4	-0.022	0.920

700
701 Notes:
702 Bulb density in Yr 1 was assessed immediately after the harvest.
703 † Relative increase of the sum of TLW_i for the whole plot (TLW_{plot}): ($TLW_{plot\_Yr\,4}$ – $TLW_{plot\_Yr\,2}$) / TLW_{plot_Yr2}
704 * Relative increase of the TLW_i: ($TLW_{i\_Yr\,4}$ – $TLW_{i\_Yr\,2}$) / $TLW_{i\_Yr\,2}$
705 ‡ Relative increase of the number of bulbs: (Nb $Bulbs_{Yr\,4}$ – Nb $Bulbs_{Yr\,2}$) / Nb $Bulbs_{Yr\,2}$
706 Abbreviations: TLW_i, mean individual total leaf width; TLW_{plot}, total leaf width of all plants with TLW_i > 1 cm
707 within a plot.

708 Table 5. Growth response of wild leek to different leaf harvesting treatments applied once in Yr 1.

Variable	Year	Half of leaves harvested			All leaves harvested			Control	d.f.		*F*-value	*P*-value
		15 d†	20 d	25 d	15 d	20 d	25 d		Num.	Den.		
TLW_d (cm)	Yr 2	10.73 bc (1.58)	11.77 ab (1.58)	11.35 abc (1.57)	7.67 e (1.58)	8.68 de (1.58)	9.99 cd (1.58)	12.91 a (1.54)	6	672	30.82	**< 0.001**
	Yr 3	11.65 ab (1.77)	12.04 ab (1.78)	12.53 ab (1.76)	8.31 d (1.77)	9.70 cd (1.77)	10.78 bc (1.77)	13.58 a (1.70)	6	536	17.96	**< 0.001**
BW_d (cm)	Yr 1	1.52 bc (0.08)	1.57 b (0.08)	1.63 b (0.08)	1.30 d (0.08)	1.43 c (0.08)	1.56 b (0.08)	1.70 a (0.08)	6	756	40.41	**< 0.001**
	Yr 2	1.79 b (0.11)	1.90 ab (0.11)	1.88 ab (0.11)	1.50 d (0.11)	1.63 cd (0.11)	1.74 bc (0.11)	1.97 a (0.11)	6	613	21.86	**< 0.001**
	Yr 3	1.56 ab (0.11)	1.55 ab (0.11)	1.62 ab (0.11)	1.28 d (0.11)	1.37 cd (0.11)	1.49 bc (0.11)	1.67 a (0.10)	6	529	17.37	**< 0.001**
Scape*	Yr 3	0.189 ab (0.063)	0.188 ab (0.066)	0.197 ab (0.063)	0.034 b (0.022)	0.114 b (0.046)	0.122b (0.048)	0.351 a (0.071)	6	521	6.26	**< 0.001**
Division*	Yr 2	0.258 (0.080)	0.303 (0.085)	0.261 (0.077)	0.158 (0.057)	0.217 (0.072)	0.187 (0.064)	0.306 (0.069)	6	613	1.39	0.216
	Yr 3	0.198 ab (0.076)	0.270 ab (0.092)	0.303 ab (0.093)	0.104 b (0.050)	0.204 ab (0.077)	0.141 b (0.063)	0.375 a (0.086)	6	521	3.57	**0.002**
Survival*	Yr 3	0.830 (0.07)	0.737 (0.09)	0.919 (0.04)	0.831 (0.07)	0.800 (0.08)	0.846 (0.06)	0.863 (0.05)	6	687	1.92	*0.076*

709 Notes:
710 Values in parentheses are standard errors.
711 ANOVA performed for each response variable among all treatment combinations. Within a row, means followed by a same letter do not significantly differ at α =
712 0.05. *P* values in boldface type are statistically significant ($P \leq 0.05$); those in italic font are almost significant ($0.05 < P \leq 0.1$).
713 † Time treatment: number of days (15d, 20d or 25d) that elapsed between complete leaf unfolding and leaf harvesting
714 * Values represent the proportion of bulbs that produced a scape, that were divided or that survived.
715 Abbreviations: TLW_d, total leaf width of all daughter bulbs issued from a same mother bulb; BW_d, total bulb width of all daughter bulbs issued from a same
716 mother bulb; d.f., degrees of freedom

FIGURE CAPTIONS

718 Figure 1. Box plots of post-treatment density immediately after the application of the different

719 bulb harvest treatments (Yr 1). Dotted line indicates the mean; the continuous line indicates the

720 median (50th percentile); box edges indicate 25th and 75th percentiles; whiskers indicate 10th and

721 90th percentiles. ANOVA was performed on log-transformed data to respect normality and

722 homoscedasticity assumptions. Treatments with a same letter do not significantly differ according

723 to Tukey HSD tests ($F_{3, 21}$ = 6.09; P = 0.004).

724 Figure 2. Interaction between planting density effect and year for individual total leaf width

725 (TLW$_i$) and TLW for all daughter bulbs arising from a same mother bulb (TLW$_d$). Mean ± SE.

726 Results of ANOVA for TLW$_i$: Density effect (D) ($F_{3,55}$ = 12.2, $\boldsymbol{P < 0.001}$), Year effect (Yr) ($F_{2, 55}$

727 = 203.9, $\boldsymbol{P < 0.001}$), D × Yr ($F_{6, 55}$ = 1.46, P = 0.21). ANOVA for TLW$_d$: D ($F_{3, 55}$ = 12.2, $\boldsymbol{P <}$

728 $\boldsymbol{0.001}$), Yr ($F_{2, 55}$ = 136.8, $\boldsymbol{P < 0.001}$), D × Yr ($F_{6, 55}$ = 3.77, $\boldsymbol{P = 0.003}$). Planting took place in Yr
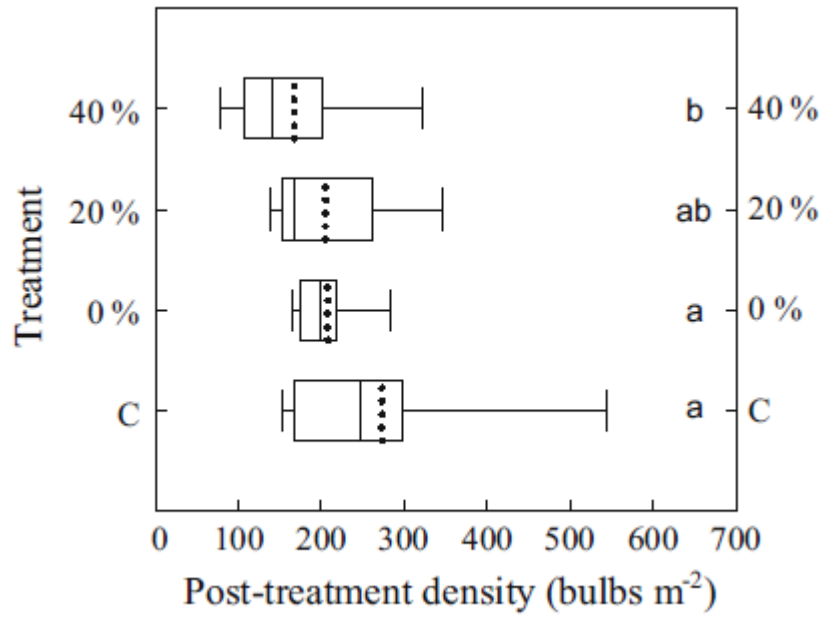
729 1.

730 Figure 3. Number of bulbs (mean ± SE) per plot from Yr 4 to Yr 6 as a function of initial planting

731 density. The horizontal line indicates the number of bulbs planted per plot (100). Results of

732 ANOVA: density effect (D) ($F_{3, 54}$ =14.1, $\boldsymbol{P < 0.001}$); year effect (Yr) ($F_{2, 54}$ = 11.7, $\boldsymbol{P < 0.001}$); D

733 × Yr effect ($F_{6, 54}$ =0.54, P = 0.774). Planting took place in Yr 1.

734 Figure 4. Interaction between the effects of the proportion of leaves harvested, the number of

735 days before harvesting and the year elapsed since the harvest took place on total leaf width

736 (TLW$_d$) and total bulb width (BW$_d$) for all daughter bulbs arising from a same mother bulb. Δ = 0

737 % leaf harvested (control); ○ = 50 % leaf harvested; □ = 100 % leaf harvested; gray = Yr 1; white

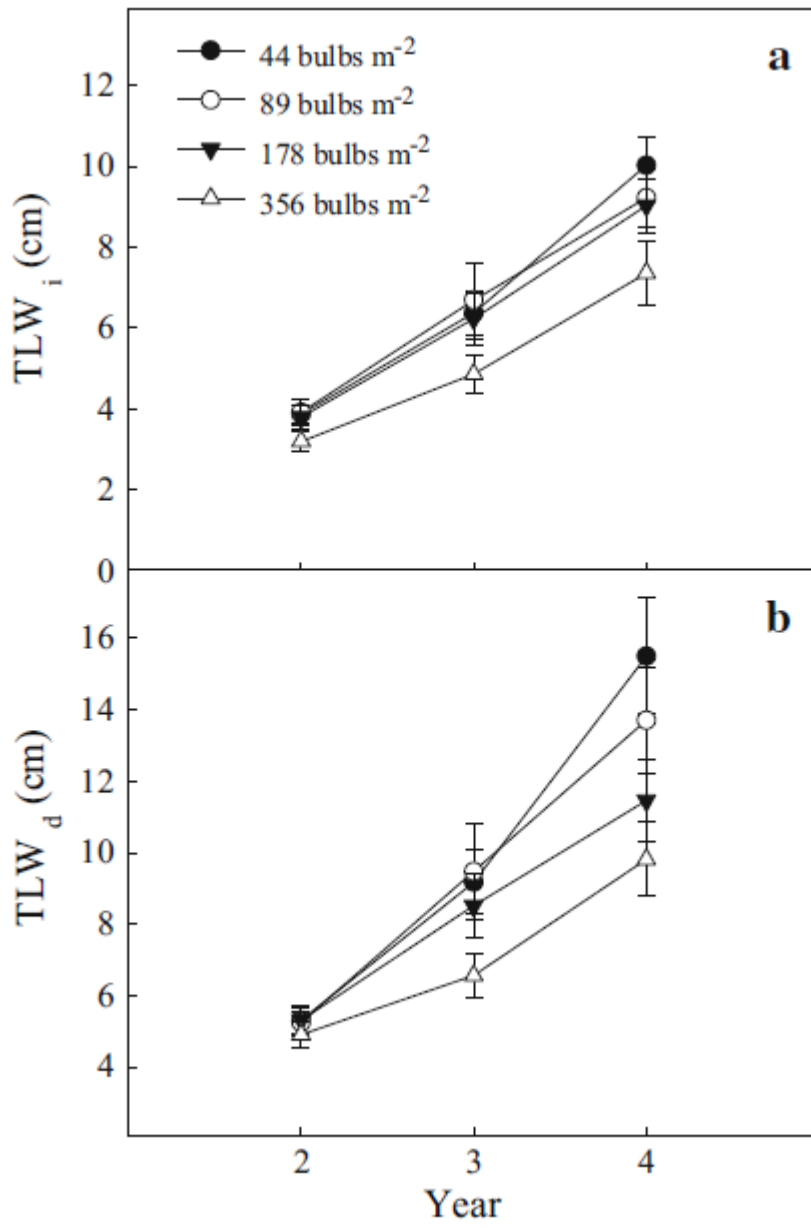738 = Yr 2; black = Yr 3. Leaf width in Yr 1 was measured before the treatment and was thus

739 excluded from the analysis. The controls (0 % leaf harvested) are presented for illustrative

740 purposes only, and were not included in the ANOVA. Results of ANOVA for BW_d: Nb leaves

741 effect (L) ($F_{1, 1208} = 138.4$; $P < \mathbf{0.001}$), Time (No. days) effect (T) ($F_{2, 1208} = 30.3$; $P < \mathbf{0.001}$),

742 Year effect (Yr) ($F_{2, 1208} = 93.6$; $P < \mathbf{0.001}$), L $\times$ T ($F_{2, 1208} = 7.21$; $P = \mathbf{0.001}$), L $\times$ Yr ($F_{2, 1208} =$

743 3.27; $P = \mathbf{0.038}$), T $\times$ Yr ($F_{4, 1208} = 0.83$; $P = 0.504$), L $\times$ T $\times$ Yr ($F_{4, 1208} = 0.28$; $P = 0.891$).

744 ANOVA for TLW_d: L ($F_{1, 738} = 110.0$; $P < \mathbf{0.001}$), T ($F_{2, 738} = 11.29$; $P < \mathbf{0.001}$), Yr ($F_{1, 738} =$

745 15.7; $P < \mathbf{0.001}$), L $\times$ T ($F_{2, 738} = 5.53$; $P = \mathbf{0.004}$), L $\times$ Yr ($F_{2, 738} = 0.02$; $P = 0.891$), T $\times$ Yr ($F_{4,}$

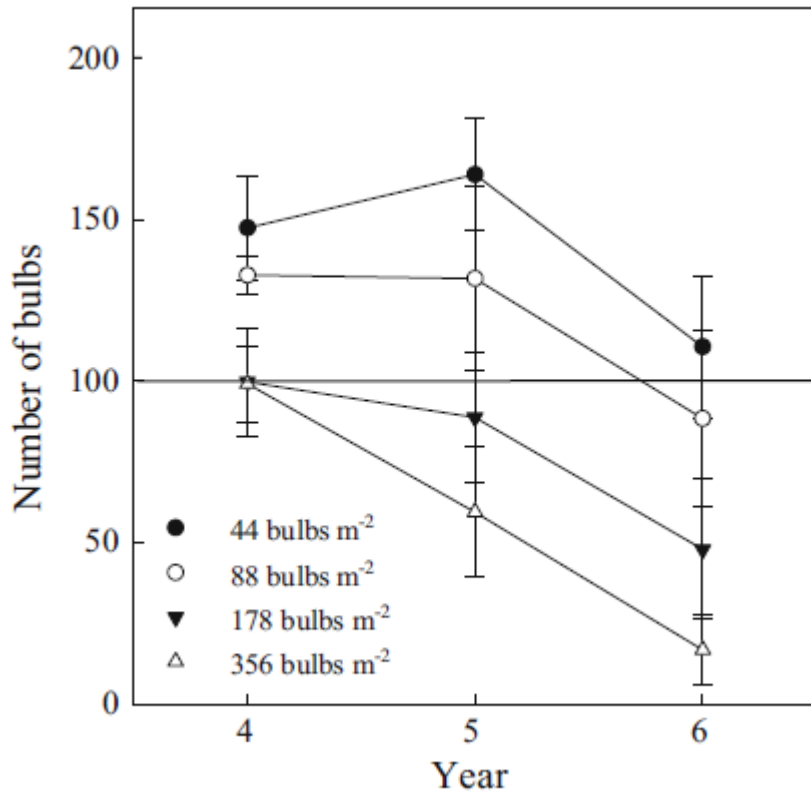746 $_{738} = 0.17$; $P = 0.841$), L $\times$ T $\times$ Yr ($F_{4, 738} = 0.31$; $P = 0.734$).

747

748   Figure 1

749

750   Figure 2

751

752   Figure 3

753

Figure legend:
- ● 44 bulbs m^{-2}
- ○ 88 bulbs m^{-2}
- ▼ 178 bulbs m^{-2}
- △ 356 bulbs m^{-2}

Y-axis: Number of bulbs
X-axis: Year

Days before harvesting