1   Plant-plant communication and community of herbivores on tall goldenrod

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14

15   Abstract

16

17   1. The volatiles from damaged plants induce defense in neighboring plants. The phenomenon is

18   called plant-plant communication, plant talk or plant eavesdropping. Plant-plant communication has

19   been reported to be stronger between kin plants than genetically far plants in sagebrush.

20   2. Why do plants distinguish volatiles from kin or genetically far plants? We hypothesize that plants

21   respond only to important conditions; the induced defense is not free of cost for the plant. To clarify

22   the hypothesis, we conducted experiments and investigations using goldenrod of 4 different

23   genotypes.

24   3. The arthropods community on tall goldenrods were different among 4 genotypes. The response to

25   volatiles was stronger from genetically close plants to the emitter than from genetically distant plants

26   from the emitter. The volatiles from each genotype of goldenrods were different; and they were

27   categorized accordingly. Moreover, the arthropod community on each genotype of goldenrods were

28   different.

29   4. Synthesis: Our results support the hypothesis: goldenrods respond to volatiles from genetically

30   close plants because they would have similar arthropod species. These results are important clues

31   elucidating adaptive significance of plant-plant communication.

32

34   Plant communication, arthropods community, goldenrod, volatiles, genotypes

35

36 **INTRODUCTION**

37

38 Because plants are sessile, they must adjust to new growing conditions by detecting and responding

39 to changes in surrounding environment. Plants are known to respond to abiotic environment, such as

40 water stress (Hsiao, 1973; Chaves, 2002; Jaleel et al., 2009), light environment (Demmig-Adams

41 III., 1992), or temperature change (Levitt, 1980, Buntgen et al., 2015). They also sense and respond

42 to changes in neighboring biotic environment, such as presence of herbivores and competitors. Upon

43 detection of herbivory, plants may induce resistance to herbivores to minimize further damage. This

44 induced resistance, in contrast to constitutive production of defense, is thought to be a cost-saving

45 mechanism under infrequent and unpredictable herbivory (Karban, & Baldwin, 1997).

46   Plants may sense the presence of herbivores in the community prior to the actual damage

47 using volatile communication, and thereby prime themselves for future attack. For example,

48 Arabidopsis thaliana induces defense gene expression and increases resistance to insect herbivores

49 when they are exposed to plant volatile organic compounds (VOCs) from the neighbouring plants

50 (Bate, & Rothstein, 1998; Kishimoto et al., 2005). Such plant-plant communication has been

51 reported in more than 30 plants species so far (Heil, & Karban, 2010).

52   Recent studies suggest that communication among plants can be specific: Sagebrush (Artemisia

53 tridentate) distinguishes volatiles from self- and non-self-clones. The plants which received volatiles

54 from self-clones got less damage than the plants which received volatiles from non-self-clones

55 (Karban, & Shiojiri, 2009). Moreover, when they received volatiles from genetically closer

56 individuals, they became more resistant than when they received volatiles from genetically distant

57 individuals (Karban et al., 2013). It has been reported that the similarity in the blend of volatiles is

58 related to genetic similarity (Ishizaki et al., 2012). Goldenrod (Solidago altissima L) also responds to

59 self-clones stronger than non-self-clones by volatiles under the low herbivore population (Kalske et

60 al. 2019). Thus, plants may be able to perceive and respond to volatiles that are similar of their own.

61   Why should such specificity of plant signalings and communication evolve? Induced plant

62 response is thought as one of the plant's strategies to save defense cost. Agrawal et al. (1999) have

63 demonstrated that induced responses to herbivore damages and leaf tissue removal had additive

64 effects on plant fitness in wild radish plant (Raphanus raphanistrum)(Agrawal et al., 1999). Plant

65 communication, the response to volatiles of damaged neighboring plants to become resistant to

66 herbivore, is one of the induced plant responses. The merit of plant communication is to be able to

67 induce defense before plants get damage. Kalske et al., demonstrated that goldenrods that

68 experienced high pressure by herbivory induced resistance in all neighboring conspecifics by

69 volatiles, whereas those experiencing herbivore exclusion induced resistance only in neighbors of

70 the same genotype (Kalske et al., 2019). Plants would adapt to respond to necessary information.

71 Previous studies indicate that genetically related individuals are similar in leaf chemistry, and thus

72 share similar herbivore communities (Kagiya et al., 2018). VOC signals from close relatives could

73   provide accurate information about future herbivory on the receiver plant, whereas VOCs from

74   distantly related individuals may provide misleading information. Thus, for receiver plant, tuning

75   into VOC signals from close relatives is predicted to be more beneficial than that from unrelated

76   individuals.

77   To test this hypothesis, we conducted three studies using tall goldenrods (<u>Solidago</u>

78   <u>altissima</u>) as the first step. The tall goldenrod is one of the plants which are known to do plant

79   communication with volatiles (Morrell, & Kessler, 2017, Kalske et al. 2019). 1) Do tall goldenrods

80   respond more from closer genetic plant than genetically far plant? 2) Are the volatiles different

81   among genotypes? 3) Are the arthropod community different among genotype? And we analyzed the

82   relationship between plant genetic dissimilarity and the herbivore community.

83

84   **MATERIAL and METHODS**

85   **Study system**

86   Tall goldenrod, <u>Solidago altissima</u> L.(Asteraceae), which was introduced to Japan from North

87   America around 1900, is a dominant and well-studied perennial herb found throughout Japan. Tall

88   goldenrod is host to diverse arthropod communities (Ando et al. 2011). It is rhizomatous and its

89   clones exhibit considerable inter-clonal genetic variation in many plant traits (Maddox, & Rootm

90   1987; Abrahamson, & Weis, 1997; Crutsinger et al., 2006; 2008).

91   In early May 2008, rhizomes were collected from 4 tall goldenrod ramets growing at 4 sites 4.5-

92   17.5 km apart in Shiga Prefecture (Table 1). Rhizomes directly attached to one another were

93   considered as the same genotype. We propagated clones of each genotype from rhizome cuttings into

94   7 cm in open-air large cage covered with small-sized mesh-net preventing from herbivore attack.

95   Watering as needed, 4 clones were kept in a large cage until our experiments in 2008, 2011, and

96   2012.

97

98   **Field experiments:**

99   *Herbivore community census*

100   In early May 2008, 10 rhizome-cuttings from each of four genotypes (total of 40 ramets) were

101   individually planted in pots (ca.18cm, hight20cm), and were grown in the large cage until late May.

102   All potted plants were then randomly transplanted into an experimental plot in a 6 m × 16 m grid in

103   the common garden.

104   The field survey was conducted in our study site at the Center for Ecological Research,

105   Kyoto University, in Otsu, Shiga Prefecture, Japan. To examine how herbivorous insects respond to

106   different clones, we conducted herbivore community censuses three times in June 2008. Abundance

107   of each herbivorous insect species was recorded. The census data for each arthropod species were

108   averaged, respectively.

109

110   *Plant communication experiment*

111   We conducted the field experiments for 2 years at our study site. In the first year (2011),

112   we compared the effectiveness of communication between plants of the self- and non-self-genotypes.

113   One potted receiver plant for each of the 4 genotypes (genotypes A, B, C and D) were placed around

114   an emitter plant (genotype A) in 2011. We removed half of each leaf from 25 % of the emitter distal

115   leaves with scissors on 29th June. Thirty replicas for each, communication between an emitter plant

116   and four receivers. We counted the number of leaves with any visible damage caused by herbivores

117   on receiver plants on 10th August. We also counted the number of all leaves.

118   In following year (2012), we measured the number of natural damages on untreated tall

119   goldenrod for each genotype, to confirm equal damage rate of each genotype. Twelve plants from

120   each genotype set up on 20th June in the same field, and counted the number of damaged leaves of

121   receiver plants on 7th November as control. Unfortunately, we did not have genotype D because of

122   artificial mistakes.

123

124   **Genetic dissimilarity of tall goldenrods**

125   To assess genetic dissimilarity among five clones, we extracted DNA from green leaf tissue of each

126   clone using the CTAB method (Milligan, 1992). Following protocols of supporting online material

127   in Crutsinger et al. (2006), we assessed genetic variation among five clones by using the AFLP

128   (amplified fragment length polymorphisms) technique (Vos et al., 1995). AFLP markers were

129   generated by using four selective primer pairs: EcoRI-AGT and MseI-CTA, EcoRI-AGT and MseI-

130   CTT, EcoRI-AGT and MseI-CTC, EcoRI-ACA and MseI-CTA, and EcoRI-ACA and MseI-CTT, and

131   EcoRI-ACA and MseI-CTC. Amplicons were separated by ABI PRISM 3130 genetic analyzer

132   (Applied Biosystems, Foster City, CA, USA). GeneScan was used to visualize AFLP bands. We

133   scored the presence and absence of 113 AFLP amplicons for 4 clones. Genetic distance among

134   clones was calculated by Nei's genetic distance (Nei 1972, 1978), using POPGENE 1.31 (Yeh et al.,

135   1999).

136

137   **Volatiles collection and analysis**

138   VOCs from artificially damaged tall goldenrods were collected. We planted 5 tall goldenrods of each

139   genotype in a laboratory room (16L8D, 24±1°C) for around 1 month. We damaged three leaves of

140   each plant with scissors. VOCs from one damaged plant were collected in a glass container (2 L)

141   using Tenax 60/80 (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany) in a laboratory room

142   (24±1°C, light intensity of 6500lux) for 30 mins. We collected volatiles from each genotype 5 times.

143   Clean air flowed through the glass bottles, and VOCs from the headspace of the bottle were collected

144   at a flow rate of 100 ml min-1. n-Tridecane (0.1 μg), infiltrated onto a piece of filter paper (1 cm^2),

145   was added as internal standard to the glass container at the onset of VOC collection.

146   The collected volatile compounds were analyzed by gas chromatograph-mass spectrometer

147   (GC-MS) (GC: Agilent Technologies, Santa Clara, CA, USA; 6890 with HP-5MS capillary column:

148   30 m long, 0.25 mm I.D., and 0.25 μm film thickness; MS: Agilent Technologies, 5973 mass

149   selective detector, 70 eV) equipped with a thermal desorption system, a cooled injection system, and

150   a cold trap system (Gerstel GmbH & Co. KG). The headspace volatiles were identified and

151   quantified by comparing their mass spectra and retention times with those of authentic compounds

152   (see above). Quantification of each compound was carried out on the basis of their GC peak areas

153   and expressed as percentages in the total ion chromatogram.

154

155   **Statistical analyses**:

156   *Variation in herbivore community among genotypes*

157   To examine whether herbivore community differ between the treatments, we used non-metric

158   multidimensional scaling analysis (NMDS) with the Bray-Curtis dissimilarity coefficients. Points

159   that are close together represent samples that are very similar in community composition, based on

160   the number of species and relative abundance of each species. Individual numbers of each species

161   were log(n+1)-transformed and standardized by variance before calculating the coefficients. Then,

162   difference in community compositions of herbivores among plant clones were determined using the

163   R-value in an analysis of similarity (ANOSIM; Clarke, 1993). This analysis uses non-parametric

164   permutation/randomization methods with a dissimilarity matrix. We conducted NMDS and ANOSIM

165   analysis in MASS and vegan packages of the software R Studio ver. 1.1.383 (R development core

166   Team 2017).

167   *Comparison of herbivore damaged-leaves after communication*

168   To compare the number of damaged leaves on each genotype, we used Tukey-Kramer test (JMP

169   7.0.2) after Box-Cox transformed. Because the number of the total leaves were different among

170   plants, total leaves were used as a weighting average.

171   *Relationship between plant genetic dissimilarity and the herbivore community*

172   Mantel correlations (XLSTAT version 2010.5.02; Addinsoft SARL, Paris, France) were conducted to

173   examine the hypothesis that clones that are more genetically similar support more similar herbivore

174   communities. These genetic correlations were conducted between distance matrix of the Bray-Curtis

175   dissimilarity between herbivore communities and Nei's genetic distance between clones. Also, we

176   created UPGMA tree using Nei's genetic distance.

177   *Plant volatile compounds relevant to clonal identification*

178   To identify the volatiles compounds that are related to clonal identification, we conducted

179   discriminant analysis (DA) to detect the differences in composition ratio of each compound among

180   clones.

181   However, our volatile profile data included variables whose number (40 compounds) are

182   more than the number of observations (20 individuals) and some of volatile compounds were highly

183   correlated. These situations did not fulfill the condition of DA. Therefore, before conducting DA we

184   transformed the data using principal component analysis (PCA). This procedure allowed us to

185   perform DA with the variables that are uncorrelated and that their number is less than analyzed

186   individuals (Jombart et al. 2010). PCA was performed using prcomp function in stats package of R

187 ver. 3.5.2 (R development core Team 2018). We chose 7 principal components that explained 90.2 %

188 of variance to submit DA (Appendix Table S1). DA was performed using lda function in MASS

189 package. Leave-one-out cross-validation by using CV option of lda function was used to calculate

190 error rate. Error rate was calculated by the number of misclassified samples divided by the total

191 number of samples. The contributions of each compound to linear discriminants were calculated as

192 the sum of products of coefficients of linear discriminant and principal components loadings of each

193 volatiles.

194

195 **RESULTS**

196

197 **Herbivore community**

198 We recorded 5 herbivorous insect species in 4 orders on tall goldenrods in June (Appendix 1). The

199 herbivore community consisted of one Coleoptera (Erateridae sp.), one Diptera (Agromyzidae sp.),

200 two Hemiptera (Uroleucon nigrotuberculatum, Corythucha marmorata), and one Lepidoptera

201 (Ascotis selenaria). The main leaf chewers were a geometrid moth caterpillar, *Ascotis selenaria*

202 *cretacea* NMDS analysis of the dissimilarity of herbivore community composition revealed that

203 herbivore community was clearly distinct among 4 clones (Figure 1; ANOSIM: R = 0.12, P < 0.05).

204 NMDS showed that herbivore communities between clone A and clone B were the most similar pairs

205 of the 4 clones.

206

Plant resistance after receiving volatiles in the field

208   Tall goldenrod plants that received volatiles from the same genotype experienced less damage

209   than other plants. In 2011 when the emitter was genotype A, leaf damage was the lowest on genotype

210   A receiver. The greatest damage was found in genotype D with 45 % of leaves damaged by

211   herbivores; twice as high damage as genotype A (Figure 2). In control (2012), in which the emitter

212   plants were not damaged, the natural damaged leaves were similar among the genotypes in 2012 (P =

213   0.932, df = 2, F = 0.145 One-Way ANOVA). The average of damage was 0.10 + 0.02.

214

Genetic dissimilarity of tall goldenrods

216   In 59 loci for 4 clones, the number of polymorphic loci was 39. Mean genetic distance between

217   clones was 0.40 (range: from 0.29 to 0.49). UPGMA tree showed the most similar genetic distance

218   between clone A and clone B (Figure 3, Table2)

219

Relationship between plant genetic dissimilarity and the herbivore community

221   Significant Mantel correlations (r) occurred between Nei's genetic distance and community

222   dissimilarity in 2008 (r =0.88, P = 0.001, Figure 4), indicating that genetically related genotype pairs

223   have similar herbivore community.

224

Volatiles from four genotypes.

226   The volatiles from tall goldenrods were comprised of 40 compounds including 4 unidentified ones

227   (Table 3). Because the amount of volatiles were similar, we compared the ratio of compounds among

228   four genotypes.

229   Volatile profiles from different genotypes were profoundly different, while that of the same

230   genotype were similar. Twenty-five compounds of 40 volatile compounds were emitted from all

231   genotypes, while the others were not found in one or more genotypes (Table 3). Discriminant

232   analysis revealed that first and second discriminant functions explained 79.1 % and 15.6 % of

233   variance respectively (Table 4), and showed clear discriminations of clones (Figure 5), with the error

234   rate 0.15. First discriminant function was mainly contributed by PC2 and PC4, and second

235   discriminant function was contributed by PC3 and PC7 (Table 4). First discriminant function which

236   was positively contributed by γ-Gurjunune, unknown 2 and Isoledon discriminated genotype B that

237   emitted those 3 compounds highly (Table 3 and 5, Figure 5). Second discriminant function

238   discriminated genotype D that emitted less 2-β-Pinene and Bicyclo2.2.1heptan-2-ol, and more γ-

239   Terpinene than other clones (Table 3 and 5, Figure 5).

240

241

242   **DISCUSSION**

243   In the filed experiments, we showed that tall goldenrod which received volatiles from same clone got

244   the least damage than the other genotypes. Some plants such as sagebrush (Karban, & Shiojiri,

245   2009), Ambrosis dumosa (Mahall, & Callaway, 1996) and Cayratia japonica (Fukano, & Yamawo,

246   2015) can distinguish between self and non self by volatiles. Our result partially supports previous

247   tall goldenrod study in recognizing the same genotype (Kalske et al. 2019). Kalske et al. (2019)

248   showed that plants induced resistance in the same genotype under lower herbivore pressure and in all

249   genotypes under higher herbivore pressure. On the other hand, the goldenrod which received

250   volatiles from closer genotype got less damages in our experiments. The results suggest that the

251   goldenrod could recognize the volatiles of genetically closer plants. As for whether the induction of

252   plant resistance is limited to closer relatives, it may depend on the history of the degree of herbivore

253   pressure. Herbivore pressure in our field is likely to be lower than in the original habitats with many

254   natural enemies (e.g. the enemy-free hypothesis), so all genotypes did not need to respond to

255   volatiles of damaged-leaves in the same way. Kin-recognition through volatiles also has been

256   reported in Sagebrush (Karban et al., 2013).

257   To distinguish volatiles from kin from non-kin in goldenrod, the volatiles should be

258   different among genotype. Actually, volatiles of goldenrod were different between clones (Figure 5).

259   Our result of discriminant analysis indicated that a few volatile compounds are associated with clone

260   identification. In our study, γ-Gurjunune, unknown 2, Isoledon, 2-β-Pinene, Bicyclo2.2.1heptan-2-ol

261   and γ-Terpinene were suggested to contribute to clone identification (Table 4). More study is needed

262   to clarify whether these compounds actually cause clonal distinction. In our analysis of volatile

263   profile, clone A and clone B, which are genetically close, showed considerably different volatile

264   profiles. Therefore, we could not find the correlation between similarities of volatiles and genetics.

265   Why plants distinguish volatiles information? Because of the cost of induced defense,

266   plants want to respond only to serious information (alarm). There are significant positive correlations

267   between community dissimilarity and neutral molecular genetics in foundation tree species (Barbour

268   et al., 2009). Johnson and Agrawal have demonstrated in evening primrose, <u>Oenothera biennis</u> L.

269   (Onagraceae), that genetic variation in plant traits such as plant size, architecture and reproductive

270   phenology affect arthropod community (Johnson, & Agrawal, 2005). In tall goldenrods, the

271   herbivorous communities were significantly different among genotype and the community

272   dissimilarity was correlated with genetic distance (Figure 1, 4). This means that the herbivore

273   species for plants are different among genotypes, but genetically closer genotypes have a more

274   similar insect community structure, suggesting that future herbivory is more likely to be similar.

275   Plants should not respond to information from far genotypes. They must respond to serious dangers,

276   such as when kin plants are damaged.

277   The volatiles must be useful information to the neighbor plant. They could predict the

278   level of danger from volatiles' information. There are at least 15 different compounds in volatiles

279   between these 4 genotypes. This suggests a clone distinguishing based on difference in blend. We

280   use the volatiles of artificially damaged plants. However, the plants are known to release different

281 blend volatiles depending on damages (Aljbory, & Chen, 2018). A future work will be to discover

282 whether plants can distinguish among damage varieties.

283   These experiments and survey are the first step for understanding why plants distinguish

284 among volatiles, especially from kin and non-kin. Our results supported the hypothesis: goldenrods

285 respond to volatiles from close-genotype plants because they would have similar arthropod species.

286 These results are important clues elucidating adaptive significance of plant-plant communication.

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295 **Authors' contributions** K.S. designed and conducted the experiments, analysed the data, and wrote

296 the manuscript. S. I. and Y. A. designed and conducted the experiments, and analysed the data. All

297 authors gave final approval for publication.

298 **DATA AVAILABILITY STATEMENT**
299 Data from this manuscript were archived in the publicly accessible repository Dryad (https://doi.org/
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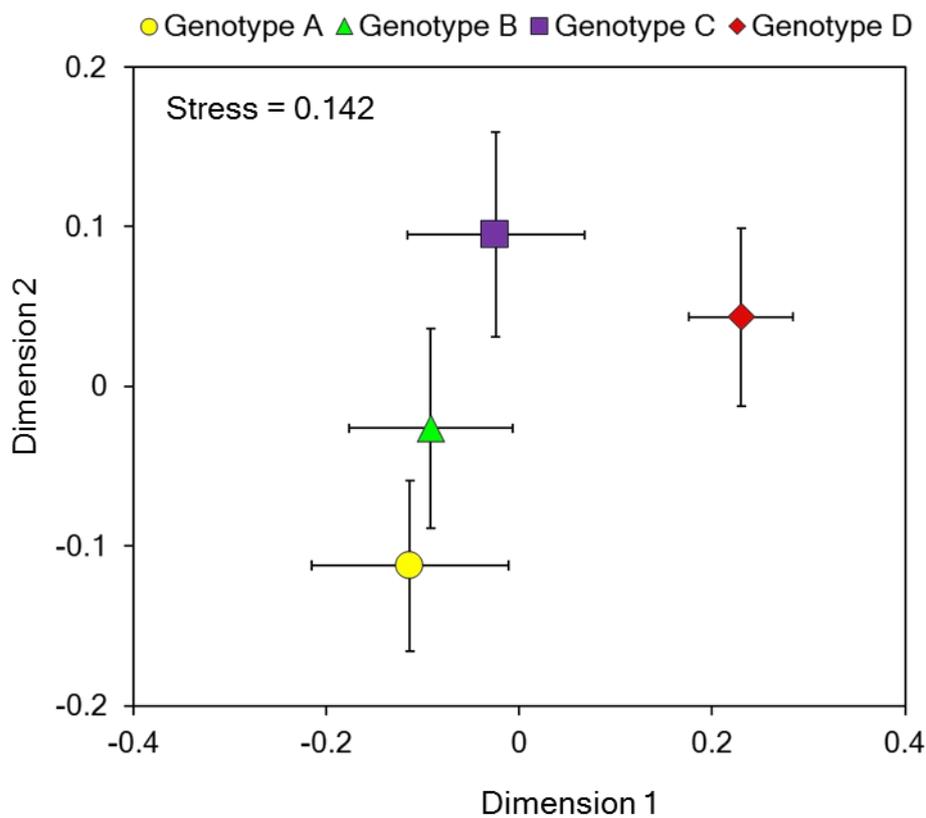
379

380   Figure 1.

381   Non-metric multidimensional scaling (NMDS) ordination of herbivore insect communities on four

382   genotypes of tall goldenrods. The herbivore communities were significantly different among

383   genotypes. Each symbol indicate the mean (± SE) of the herbivore community on each genotype.

384
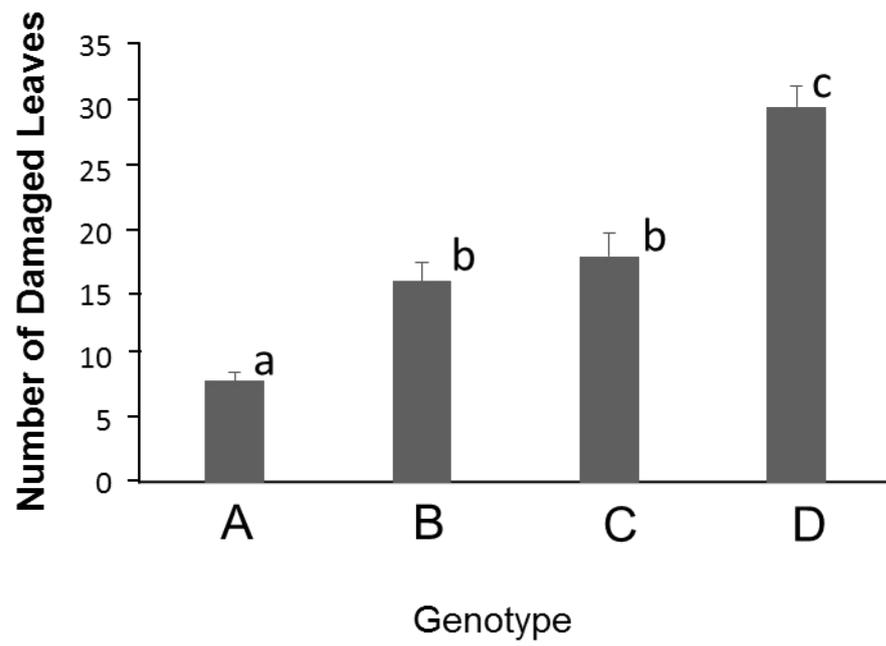
385

386 Figure 2.

387 Number of damaged leaves of goldenrods in each genotypes. Genotype A was as an emitter.

388
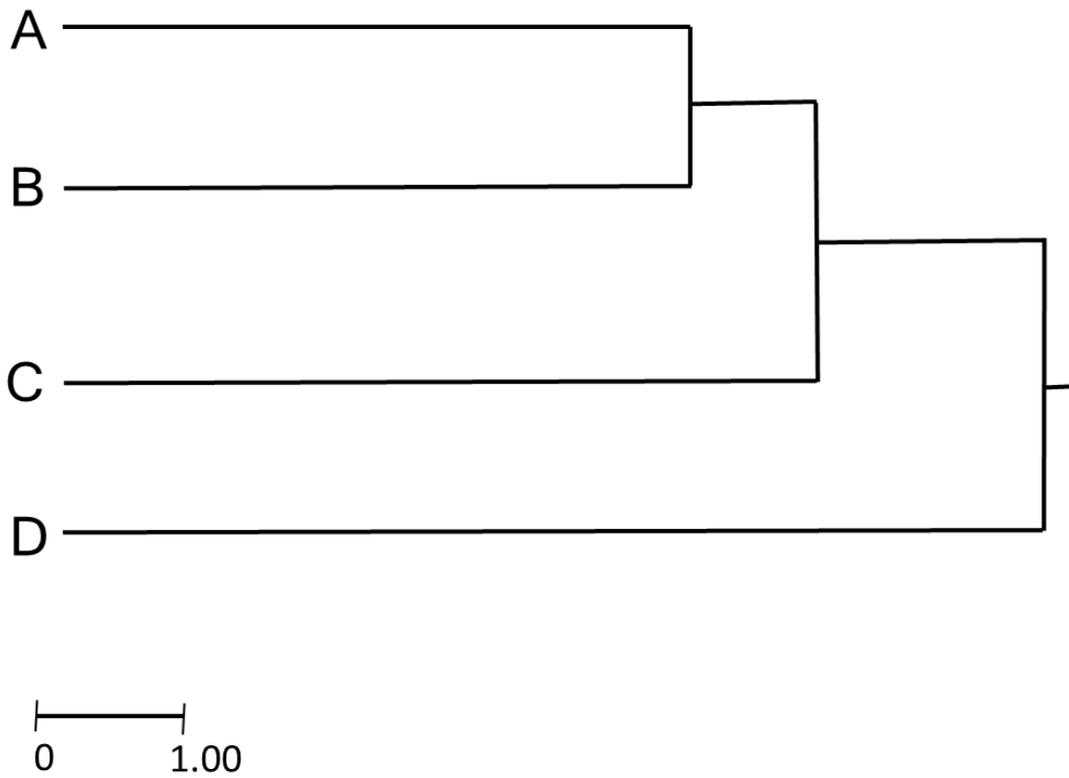
389

390

391 Figure3.

392 UPGMA dendrogram based on Nei's genetic distance between the four tall goldenrod genotypes

393 calculated from the AFLP data. The scale bars represent the genetic distance.
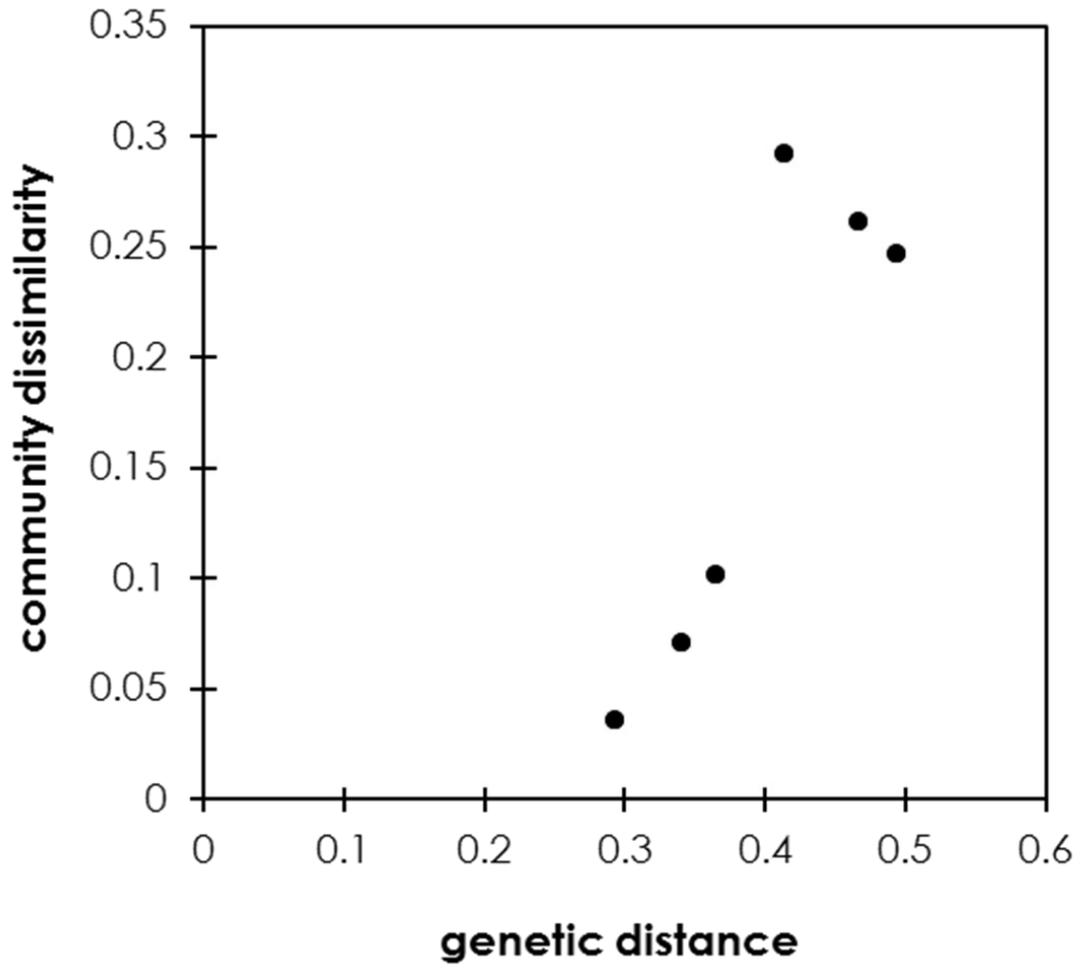
395

396   Figure 4.

397   Relationship between genetic distance and community dissimilarity. Plots describe pairwise Mantel

398   correlation comparing distance matrices summarizing herbivore community variation (Bray-Curtis

399   dissimilarity) with those for Nei's genetic distance. (Table 2)

400
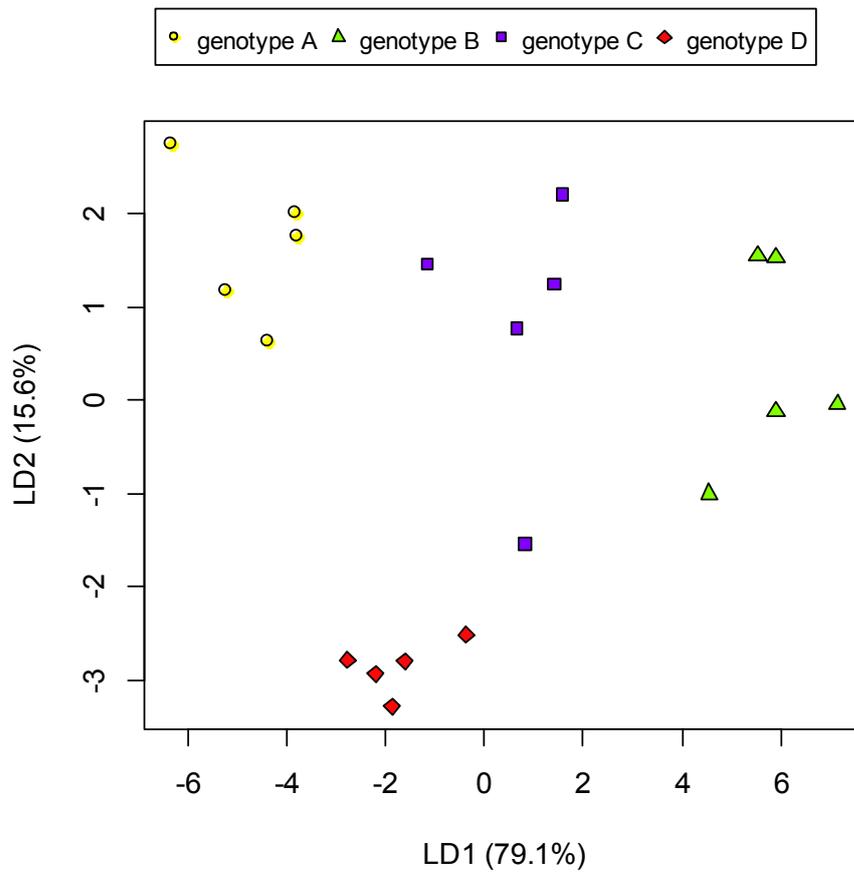
401

402 Figure 5.

403 Scatterplot for scores of volatile compounds from four genotypes of *Solidago altissima* based on the

404 first two discriminant functions. Proportion of variance explained by each function are shown in

405 parentheses. Before discriminant analysis, volatile data were transformed to 7 principal components.

406 .

407

408

409   Table 1. Original site of each genotype

410

Genotype	latitude	longitude
A	35.04	136.04
B	35.05	136.02
C	35.19	136.08
D	35.06	136.04

416

417   Table 2. Genetic dissimilarity of tall goldenrods

418

	A	B	C	D
A	*			
B	0.2933	*		
C	0.3640	0.3399	*	
D	0.4666	0.4940	0.4140	*

419
420

Table 3 Means ± SDs of composition ratio (% to total GC peak areas) of each volatile compound detected from each genotype of *Solidago altissima*

Volatile compound	Composition ratio (% to total GC peak areas)											
	genotype A			genotype B			genotype C			genotype D		
Cyclohexane	1.24	±	1.14	1.59	±	0.45	1.19	±	0.7	1.95	±	1.5
1-methoxy-2-propoxy.ethane	9.16	±	3.39	3.72	±	3.72	4.36	±	4.47	9.47	±	4.41
2-Hexenal(E)	0.35	±	0.79	0.25	±	0.56	4.08	±	3.94	2.03	±	1.98
3-Hexenol-1-ol	5.37	±	1.87	3.97	±	2.31	3.62	±	3.77	3.83	±	1.39
alpha-Thujene	0.21	±	0.47	0.57	±	1.01	1.13	±	0.85	1.05	±	0.88
Alpha-Pinene	6.19	±	1	3.63	±	1.78	8.23	±	2.68	3.42	±	0.67
Camphene	1.36	±	1.2	1.82	±	3.02	1.2	±	1.34	0	±	0
Sabinene	1.8	±	1.97	3.08	±	2.05	4.58	±	4.09	5.16	±	3.58
2-Beta-Pinene	4.32	±	1.08	1.16	±	0.91	3.91	±	2.02	0.75	±	0.43
Beta-Myrcene	5.94	±	0.89	6.04	±	1.71	6.6	±	2.39	7.66	±	2.13
1-Phellandrene	0.98	±	0.67	0.82	±	0.91	0.65	±	0.91	1.06	±	0.67
3-Hexen-1-ol,acetate	19.24	±	5.03	10.67	±	4.48	17.58	±	7.91	17.84	±	11.81
alpha-Terpinene	1.08	±	1.24	3.59	±	2.34	2.75	±	2.95	3.21	±	1.81
dl-Limonene	11.75	±	4.06	9.56	±	2.47	13.26	±	3.78	10.54	±	2.72
Cyclohexane.1-methylene-4	2.87	±	3.96	0.72	±	1.61	0.93	±	2.08	0	±	0
1.3.6-Octatriene	0.18	±	0.41	1.89	±	0.59	1.28	±	1.02	1.53	±	0.92
gamma-Terpinene	1.6	±	0.3	3.08	±	1.83	3.77	±	2.02	4.34	±	1.17
alpha-Terpinolene	0.18	±	0.4	2.3	±	1.64	1.7	±	1.23	1.8	±	1.12
Nonanal	0.53	±	0.74	0.41	±	0.4	0.22	±	0.49	0.56	±	0.55
(E)-4.8-Dimethyl-1.3.7-nonatriene	0.45	±	0.62	0	±	0	0	±	0	0	±	0
Decanal	1.33	±	0.88	0.47	±	0.54	0.32	±	0.72	1	±	0.71
Bicyclo2.2.1heptan-2-ol	3.05	±	0.85	1.35	±	0.92	1.07	±	1	0.45	±	0.46
gamma-Gurjunune	0	±	0	0.54	±	0.31	0	±	0	0	±	0
unknown1	0	±	0	0.92	±	0.57	0	±	0	0.18	±	0.4
alpha-Cubebene	1.99	±	1.82	0	±	0	0	±	0	0	±	0
alpha-Ylangene	0	±	0	0.73	±	0.46	0	±	0	0.66	±	0.64
alpha-Copaene	0	±	0	1.31	±	0.8	0.28	±	0.63	0.66	±	0.63
Alpha-Bourbonene	0	±	0	0.74	±	0.71	0	±	0	0.41	±	0.6
Beta-Bourbonene	0	±	0	2.02	±	0.79	0.7	±	0.67	1.31	±	0.84
Cedrene-V6	0.21	±	0.48	1	±	0.3	0.13	±	0.29	0.62	±	0.61
unknown2	0	±	0	7.05	±	1.07	3.39	±	0.97	0	±	0
trans-Caryophyllene	5.2	±	3.79	0	±	0	0	±	0	3.38	±	3.88
Beta-Guaiene	0	±	0	0	±	0	0	±	0	0.96	±	2.14
beta-Cubebene	0.63	±	0.9	2.09	±	1.02	1.07	±	0.68	1.18	±	1.09
alpha-Amorphene	2.87	±	1.04	2.8	±	1.25	1.49	±	1.09	2.83	±	2.07
Germacrene-D	4.26	±	3.27	4.38	±	3.36	3.42	±	3.26	1.94	±	1.35
Isoledene	0	±	0	6.5	±	1.29	3.19	±	1.61	0	±	0
alpha-Muurolene	0	±	0	3.91	±	1.62	1.86	±	1.09	3.1	±	3.45
delta-Cadinene	4.64	±	2.05	3.09	±	1.92	1.25	±	1.18	3.28	±	2.45
alpha-Cadinene	1.01	±	1.09	2.24	±	0.62	0.78	±	0.72	1.87	±	1.47

422

Table 4 Results of discriminant analysis for 7 principal components (PCs). PCs with strong coefficient (first three strongest) on a giving linear discriminant function (LD) are shown in bold

		LD1	LD2
Proportion of trace:		0.791	0.156
Coefficients of linear discriminants:			
	PC1	23.11	0.465
	PC2	**-51.746**	-4.47
	PC3	-1.823	**26.356**
	PC4	**-35.979**	18.513
	PC5	**29.444**	**26.282**
	PC6	4.448	-12.774
	PC7	15.729	**-28.696**

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Table 5 Contributions of each volatile compound to linear discriminant functions. Contributions were calculated as the sum of products of coefficients of linear discriminant and principal components loadings of each volatiles. Volatile compounds with strong contribution (first three strongest) on a giving linear discriminant function (LD) are shown in bold

	LD1	LD2
Cyclohexane	16.640	-10.557
1-methoxy-2-propoxy.ethane	-23.522	-10.034
2-Hexenal(E)	-13.291	-7.914
3-Hexenol-1-ol	-8.599	10.321
alpha-Thujene	-8.987	-18.584
Alpha-Pinene	-21.457	25.078
Camphene	4.976	26.761
Sabinene	4.497	-19.820
2-Beta-Pinene	-35.621	**38.629**
Beta-Myrcene	-9.986	-19.063
1-Phellandrene	-18.901	-1.388
3-Hexen-1-ol,acetate	-12.731	-0.167
alpha-Terpinene	16.329	-14.152
dl-Limonene	-2.561	7.321
Cyclohexane.1-methylene-4	-25.604	24.764
1.3.6-Octatriene	31.947	-20.522
gamma-Terpinene	4.730	**-29.605**
alpha-Terpinolene	21.079	-12.401
Nonanal	-8.270	-11.140
(E)-4.8-Dimethyl-1.3.7-nonatriene	-27.578	11.671
Decanal	-32.025	1.590
Bicyclo2.2.1heptan-2-ol	-26.348	**33.497**
gamma-Gurjunune	**50.783**	1.021
unknown1	38.825	-8.134
alpha-Cubebene	-40.062	20.606
alpha-Ylangene	32.304	-18.811
alpha-Copaene	44.439	-2.863
Alpha-Bourbonene	28.669	-4.286
Beta-Bourbonene	45.125	-18.010
Cedrene-V6	27.838	-2.852
unknown2	**66.484**	12.125
trans-Caryophyllene	-48.608	-4.608
Beta-Guaiene	-9.091	-22.092
beta-Cubebene	34.167	-12.427
alpha-Amorphene	-10.335	-6.831
Germacrene-D	7.787	6.783
Isoledene	**64.970**	14.491
alpha-Muurolene	36.445	-19.767
delta-Cadinene	-18.922	3.047
alpha-Cadinene	14.166	-11.449