Rosy apple abundance is shaped by vegetative growth and water status

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1   **Rosy apple aphid abundance on apple is shaped by vegetative growth and water status**

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10   **Key message:** Rosy apple aphid abundance is positively correlated to plant vegetative growth at both the shoot and

11   tree scales. Water restriction has a negative impact on aphid abundance only at the tree scale.

12

13   **Highlights**

14   Rosy apple aphid abundance on apple is related to growth at both the shoot and the tree scale

15   This relation is modulated by genotype and water supply

16   At the shoot level the effect of water restriction on aphid abundance depends on genotype

17   At the tree level, water restriction always has a negative impact on aphid abundance

18

19   **Abstract**

20   Regulated deficit irrigation, which is a common practice to cope with water scarcity, can impact plant-aphid

21   interactions, and possibly lead to a reduction in the use of pesticides. To test the possible effect of water restriction

22   on the apple tree-rosy apple aphid (*Malus domestica-Dysaphis plantaginea*) system, we performed a factorial

23   experiment with two levels of water supply and two genotypes on artificially infested trees. Plant growth and aphid

24   abundance were characterized during the entire infestation period at two scales of analysis: the apical shoot scale

25   and the tree scale, and additional measures were performed to evaluate plant water status. Aphid abundance

26   increased with plant vegetative growth at both scales of analysis, which is consistent with the Plant Vigor

27   Hypothesis (i.e. with the fact that most of the phloem feeders prefer fast growing plants). At the tree scale, aphid

28   abundance was lower on trees that underwent water restriction, but at the shoot scale, aphid abundance responded

29   differently to water restriction depending on the tree genotype. Water restriction modified the relationship between

30   aphid abundance and growth, thus indicating that host suitability for aphids was affected by different plant

31   variables susceptible to water stress, among which growth. The different response patterns at the two scales of

32   analysis highlight the importance of scale choice in the study of plant-insect interactions.

33

34   **Keywords:** *Dysaphis plantaginea*, *Malus domestica*, pest management, drought stress, Plant Vigor Hypothesis

35

1 INTRODUCTION

37   Reducing the use of pesticides and coping with water scarcity are two of the main challenges in

38   Mediterranean horticulture. The use of less drought susceptible plant varieties and the implementation of regulated

39   deficit irrigation represent possible solutions to decrease water consumption in horticulture. Interestingly, due to

40   cross tolerance mechanisms between abiotic and biotic stresses, the implementation of deficit irrigation can also

41   reduce host plant suitability for pests and especially aphids (Foyer et al., 2016). Host plant suitability for aphids

42   encompasses multi-aspects, which can be modulated by plant water status. The four main ones are (i) nutrition (or

43   settlement) site availability, *i.e.* the number of growing apices (Forrest and Dixon, 1975), (ii) phloem nutritional

44   quality, e.g. the secondary metabolites (Czerniewicz et al., 2011) and the amino acid profiles (Ryan et al., 2014),

45   (iii) phloem accessibility (Mody et al., 2009) and (iv) phyllosphere microenvironment (Pangga et al., 2012). As the

46   effects of water scarcity on host plant characteristics vary with stress timing, intensity, and duration (Tariq et al.,

47   2012), the published results on the effect of host plant water stress on aphid performance, are often contradictory.

48   Water stress has been shown to have a positive (Archer et al., 1995; Mewis et al., 2012), a negative (Agele et al.,

49   2006; King et al., 2006; Simpson et al., 2012) or no effect (Bethke et al., 1998; King et al., 2006; Mewis et al.,

50   2012) on aphid performance. The plant genotype is also a factor that is expected to influence the plant-aphid

51   interaction under water restriction. Yet, the studies evaluating the effects of drought stress on aphid performance on

52   different plant genotypes generally considered genotypes contrasted for their resistance to the insect (Agele et al.,

53   2006; Dardeau et al., 2015; Verdugo et al., 2015) rather than genotypes contrasted for their response to drought

54   stress.

55   In the present work, the apple tree-rosy apple aphid system [*Malus domestica* Borkh. – *Dysaphis*

56   *plantaginea* (Passerini)] was chosen as the study case, because apple trees are cultivated worldwide under a wide

57   range of climatic conditions and also in semi-arid areas such as the Mediterranean basin. Moreover apple is the

58   major deciduous fruit tree production worldwide (FAO 2016). *Dysaphis plantaginea* is a major apple tree pest

59   (Forrest and Dixon, 1975). It causes leaf roll (Forrest and Dixon, 1975), shoot and fruit deformations (Marchetti et

60   al., 2009), and populations resistant to pesticides have already appeared (Delorme et al., 1999). Two apple

61   genotypes with different drought response mechanisms were identified from a "Starkimson"×"Granny Smith"

62   cross progeny (Lauri et al., 2016). The first genotype (referred to as DAG: Drought Avoidance Genotype) is

63   characterised by drought avoidance strategy, with reduced stomatal conductance and photosynthesis under water

64   deficit, growth being affected to a smaller extent. The second one (referred to as GCG: Growth Cessation

65   Genotype) is characterised by a high percentage of shoots experiencing growth cessation under drought stress. We

66   intend to use these contrasted genotypes to test how far the mechanism involved in drought resistance affects the

67   apple tree – rosy apple aphid interactions, namely under water stress conditions whose effects on shoot growth may

68   be modulated by the genotype. Other determinants of plant suitability to aphids, such as leaf water potential and

69   gas exchange rates, may also be affected to a greater of lesser extent according to genotype. The related

70   physiological traits, namely leaf temperature (Satar et al., 2008), turgor pressure (Verdugo et al., 2015), phloem sap

71   soluble sugars content (Zehnder and Hunter, 2009) and viscosity (Sevanto, 2014) to which aphids are sensitive,

72   could by modified in turn. The relationship between shoot growth and aphid performances may therefore be

73   disentangled by water stress conditions.

74   Thus, aphid abundance has been positively correlated to vegetative growth at the shoot scale (Stoeckli et al.,

75   2008; Rousselin et al., 2016). Yet, the existence of a similar relationship at the tree scale remains unclear. Indeed,

76   the susceptibility of tree organs to aphids may vary within the tree, between long and short shoots and between

77   fruiting and non-fruiting shoots (Simon et al., 2011). This could affect the patterns of aphid dispersion within the

78   crown. We will therefore consider simultaneously two study scales: the infested shoot and the whole tree, thus

79   verifying if the relation between aphid abundance and growth still holds at the tree scale, and how far it is affected

80   by water stress conditions.

81   To reach these goals, both apple genotypes were submitted to contrasted irrigation regimes, *i.e.* control vs

82   deficit irrigation, and the aphid population monitored after artificial infestation as well as tree growth, leaf gas

83   exchanges and leaf water potential. Gathered data were then analysed via hierarchical analysis of multiple

84   regression to test how far the plant genotype and the irrigation treatment affected aphid density, possibly via an

85   effect on the plant vegetative growth.

86   ## 2 MATERIAL AND METHODS

87   *2.1. Experimental design and plants*

88   The experiment was conducted in Avignon (southern France) under an 126 m^2 insect proof shelter (PEHD Cristal

89   500*600 µm mesh), insulated from the ground with a tarpaulin (PP 86gr UV stabilized), and treated with various

90   chemicals to eliminate weeds, insects, culture auxiliaries and pests. This treatment was applied first on bare soil

91 when the tunnel was built (2013) then repeated without herbicide every year (in February). The trees were

92 therefore moved for two weeks to a nearby clean shelter which also allowed control of the tarpaulin status and

93 manual weeding of the few plants that would have grown through. From 10/April/2015 until the end of the

94 experiment (1/July/2015), the temperature under the shelter was recorded every 30 min using a Hobo® Pro V2

95 logger (U23-002, Onset®, Bourne, USA).

96 For each of the two apple genotypes DAG and GCG (described in introduction), 30 scions were whip

97 grafted on M9 Pajam 2 on March 2014. They were grown in 12 L-pots filled with a medium consisting of 1:2 (v:v)

98 perlite and potting soil (Florabella® Klasmann-Deilmann®). Two drippers per pot, each with a delivery rate of 2

99 $dm^3 h^{-1}$ and connected to a different pipe, provided respectively tap water and an NPK fertilizer.

100 After one-year of growth, plants were hand-pruned in February 2015. Only the main axis (or trunk) was

101 left with 15 non latent buds (i.e. meaning that the trunk was pruned back to less than 80 cm above the grafting

102 point). Pruning wounds were protected with Phytopast®-G. The differentiation of the irrigation treatments started

103 on 24/Apr/2015. The pots were covered with a white plastic sheet to avoid penetration of rain water. Two sets of 12

104 plants of each genotype were selected for their homogeneity, and subjected to two different watering treatments,

105 denoted by W+ and W-. Plants assigned to the W+ treatment were daily irrigated until run-off and the plants

106 assigned to the W- treatment received a halved water supply. The W- treatment was adjusted by reducing the

107 duration of each of the two to four daily irrigation periods.

108

109 *2.2. Aphid rearing and infestation*

110 To ensure that individual aphids did not genetically differ in their intrinsic performance, a single clone of *D.*

111 *plantaginea* (Dp15) was used for the infestation. The aphid colony was established from a single female collected

112 on an apple tree 'Ariane' on 26/March/2015 in Avignon. Aphids were reared in the laboratory on the apple cultivar

113 M9 susceptible to aphids, under parthenogenesis-inducing conditions: 20°C +/- 1°C, 60-70% relative humidity and

114 a 16-h-day cycle (Sauge et al., 1998). Five 7-days old wingless adult females were placed on one single current

115 year axis (i.e. which had emerged on the trunk in early spring) per plant on 28/April/2015. The chosen axis for

116 infestation was positioned in the apical position of the trunk. Aphids were then free to disperse all over the plant

117 but could not move to the soil, being blocked by a glue barrier (Rampastop®, Protecta®) provided at the stem

118 base, neither to a neighbouring plant since spacing was large enough to avoid canopy contact throughout the

119 experiment.

120

121 *2.3. Data collection*

122 The infested trees were monitored weekly from the 30/April/2015 to the 01/July/2015 for vegetative growth and

123 from the 04/May/2015 to the 01/July/2015 for aphid abundance. Vegetative growth was computed by counting the

124 number of expanded leaves separately on each developing proleptic bud, or bud formed in 2014 whose

125 development was delayed by dormancy (Wheat, 1980). Two types of vegetative proleptic structures were

126 distinguished: (i) rosettes, which correspond to the expansion of the preformed leaves of the bud, and (ii) shoots,

127 which correspond to a main axis resulting from the activation of the apical meristem (*i.e.* to the transformation of

128 the rosettes into axes) and all its axillary structures. The diameter at the trunk base, considered as an accurate

129 indicator of plant vigor and classically used as a covariable to explain interplant variability (Nesme et al., 2005),

130 was measured at the start and at the end of the experiment. It varied by less than 12% within the tree populations

131 sorted by genotype (April 4) and treatment combination (July 2). This variability was too small to affect the

132 number of leaves or the aphid abundances, as shown by preliminary covariance analyses (data not shown)

133 performed at shoot and tree scale. Aphid abundance was estimated by assigning to each proleptic structure (shoot

134 or rosette) one class of infestation: C0 (no aphid), C1 (1 to 5 individuals), C2 (6 to 25), C3 (26 to 125), C4 (125 to

135 625) and C5 (more than 625) (Grechi et al., 2008, Rousselin et al., 2016). As a result, a total of 629 proleptic

136 structures (an average of 13 per tree) were monitored, among which 55% were assigned to class C4 or C5 at the

137 time of infestation peak.

138 During the same period, midday leaf water potentials were measured with a Scholander pressure bomb on

139 eight sunny dates (i.e. approximately once a week according to the weather conditions) on a subsample of 3 to 6

140 randomly chosen trees per treatment, using a non-infested sun-exposed leaf near an apex. Simultaneously, leaf

141 photosynthetic rate, leaf stomatal conductance, leaf transpiration and leaf temperature were measured on a non-

142 infested sun-exposed attached leaf with an open gasometric system LCA-4 (ADC®, Hoddesdon, UK).

143

144 *2.4. Data analysis*

145 Hereafter, dates are expressed in days after infestation (DAI). Doing so, 28/April/2015 corresponds to 0

146 DAI and 01/July/2015 corresponds to 64 DAI. Possible differences in leaf water potential, leaf photosynthetic rate,

147 leaf transpiration rate, leaf stomatal conductance, leaf temperature, the percentage of tree leaves on a shoot,

148 numbers of developing buds, shoots and rosettes, were analysed with Kruskal-Wallis tests on treatment groups

149 (water × genotype) and, when significant, they were followed by non-parametric Tukey multiple comparisons.

150   Data were analysed at two different scales: the shoot scale, considering the primarily infested shoot (i.e.

151   the apical shoot) and the tree scale. The vegetative growth was computed as the increase in the number of fully

152   expanded leaves between two and 64 DAI, at the shoot scale considering the apical infested proleptic axis plus all

153   its axillary structures, leading to the final shoot vegetative growth (FSVG) and at the tree scale, considering all

154   types of proleptic structures, *i.e.* rosettes and shoots, leading to final tree vegetative growth (FTVG). Both

155   vegetative growth variables (*i.e.* FSVG and FTGV) were log transformed to fulfil the conditions required for

156   statistical analysis.

157   The actual aphid abundance, was estimated from the reported infestation classes by drawing a value from

158   a uniform distribution with boundaries relevant to the different abundance classes (e.g. 125 – 624 for the infestation

159   level C4), for each of the 629 proleptic structures (shoots and rosettes) and sampling dates. It is therefore evident

160   that any reconstruction of the actual aphid abundance differed from another due to the randomness of the drawing

161   process. We constructed 10,000 virtual aphid abundance curves per shoot and then per tree (by summing the values

162   obtained for the proleptic structures belonging to each individual tree). At tree scale, the rosettes contributed to less

163   than 20% to aphid abundances, despite the fact that they represented 36% of the proleptic structures whatever the

164   genotype or the irrigation treatment (see Results section). Indeed, the aphid abundances were significantly lower on

165   the rosettes than on the shoots for all measurements dates (Kurskall-Wallis tests, $p < 0.001$ for all tests, undetailed

166   data). The aphid indexes, representative of the infestation severity throughout the infestation period, were

167   calculated as the sums of the actual aphid abundances estimated for each notation date at infested shoot and tree

168   scales. This synthetic variable was representative of the diversity of the infestation dynamics among the proleptic

169   structures (and among the trees) taking into account the infestation duration and the evolution of the aphid

170   population while present on the shoots. The median values (over the 10,000 random resamples) obtained at the tree

171   level or at the infested shoot level were then used for statistical analysis. Such a resampling procedure is intended

172   to obtain continuous variables (e.g. aphid abundance and aphid index), rather than a categorical one (e.g. reported

173   classes of infestation) to describe a continuous value: the aphid abundance. Using a continuous variable simplified

174   moreover the scale change, allowing the calculation of the tree aphid abundance from the data collected on its

175   constitutive proleptic structures.

176   To test the effects of water treatment W, final vegetative growth (FSVG or FTVG) and genotype V on

177   aphid abundance, a hierarchical analysis of multiple regression models was performed, with aphid abundance as

178   the dependent variable, plant or shoot growth (i.e. FSVG or FTVG) as continuous predictor variables and W and V

179   as categorical predictor variables. All possible models were ranked according to AICc (second-order Akaike

180 Information Criterion). One model was assumed to be better than another if ΔAICc > 2 (Bolker, 2008),

181 consequently the models with a value of ΔAICc of more than 2 from the best models were ignored. After being run

182 on the median values of aphid indexes, the model selection procedure was also run on the 10,000 datasets issued

183 from the transformation of aphid abundance classes into aphid index in order to estimate possible model selection

184 sensitivity to drawings.

185 To better understand why the effects of irrigation on aphids varied with the study scale (see Results

186 section), we also analysed possible correlations between apical shoot and total tree growth. Therefore, a

187 hierarchical analysis of multiple regression models was performed with final tree vegetative growth (FTVG) as the

188 dependent variable and final shoot vegetative growth (FSVG) as continuous predictor variable and water treatment

189 W and genotype V as categorical predictor variables. We then performed the same kind of analyses with FSVG or

190 FTVG as the response variable, and the number of proleptic shoots as the continuous predictor variable and W and

191 V as categorical predictor variables. To test if the percentage of tree leaves inserted on shoots was dependant on

192 FTVG, we performed a hierarchical analysis of multiple regression models, with FTVG as continuous variable and

193 W and V as categorical variables.

194 All data analyses were carried out using R software version 3.3.1 (R Core Team, 2016) and additional

195 packages 'nparcomp' and 'glmulti'.

3 RESULTS

197 *3.1. Plant water status and leaf functioning*

198 Trees subject to water restriction, *i.e.* the W- trees, had a higher leaf water potential in absolute values than W+

199 trees (Fig. 1), the differences being significant for DAG (Drought Avoidance Genotype), at 14 (Fig. 1c) and 30

200 DAI (Days After Infestation, Fig. 1d) and for GCG (Growth Cessation Genotype) at 30 and 58 DAI (Fig. 1h).

201 Among the same watering treatment there was no significant difference between genotype for leaf water potential,

202 except for 14 and 37 DAI (Fig. 1e) between W+ trees, where GCG W+ had a higher leaf water potential in absolute

203 values than DAG W+.

204 Over the six monitoring dates of leaf stomatal conductance, leaf photosynthetic rate, leaf transpiration rate

205 and leaf temperature, the differences were significant between the treatments for only one date. On 30 DAI (Fig.

206 2), leaf stomatal conductance (Fig. 2a) and leaf transpiration rate (Fig. 2b) were reduced for the W- trees compared

207 to the W+ trees for both genotypes. For DAG, leaf photosynthetic rate (Fig. 2c) was significantly higher for W+

208 trees than W- trees. For GCG, leaf temperature (Fig. 2d) was significantly lower for W+ than W-.

209

3.2. Vegetative growth

The temporal dynamics of vegetative growth followed similar patterns at the tree and the infested shoot scales with a slowdown of growth after 30 DAI (Fig. 3). The ranking of the different treatments were identical between the two scales with, in decreasing order: the higher vegetative growth for GCG W+, followed by GCG W-, then DAG W+ and the smaller vegetative growth for DAG W-. Water restriction decreased vegetative growth for both genotypes.

Final tree vegetative growth FTVG, estimated by the increase of the number of fully expanded leaves during the infestation period was positively correlated to final shoot vegetative growth FSVG, and the relationship was influenced by both water treatment and tree genotype (Fig 4a). Additionally, FTVG was positively correlated to the number of proleptic shoots per tree (Fig 4b), whereas FSVG was independent of this number (Fig 4c), which was probably a consequence of tree acrotony, as the infested shoot was in the apical position. At shoot and tree scales the ranking of the treatments were consistent. GCG was more vigorous than DAG.

Although the number of proleptic structures per tree was significantly higher for GCG W- than DAG W-, the number of rosettes per tree and the number of shoots per tree did not differ significantly between treatments (data not shown). The percentage of tree leaves inserted on shoots was positively correlated to FTVG since the proportion of tree leaves on shoots increased during the season. Indeed, axes expanded leaves throughout the infestation period while rosettes stopped growing after expansion of their preformed leaves. The percentage of tree leaves inserted on shoots vs rosettes was lower for the trees which underwent a water restriction after 24 DAI, suggesting that W+ shoots grew more vigorously than W- shoots.

3.3. Aphid population dynamics

There was at first a period of slow increase of aphid abundance between 0 and 13 DAI, then the aphid population peaked between 29 and 36 DAI and after that there was a quick decrease in aphid abundance at both study scales (Fig. 5). The aphid population started to decrease when the maximal daily temperatures exceeded 42.5°C. The ranking of the mean aphid abundances of the different treatments at the peak was different between the two scales. However, given the size of the standard deviation these rankings were only indicative of trends. At the infested shoot scale (Fig. 5a), GCG W+ experienced the highest infestation, followed by both genotypes under W- and the lowest infestation was on DAG W+ whereas at the tree scale (Fig. 5b), GCG W+ still experienced the higher infestation but it was followed by DAG W+ and then both genotypes under W- treatment.

240

3.4. Aphid abundance as related to water supply, genotype and vegetative growth

     3.4.1. At shoot scale

The best model to explain shoot aphid index included the final shoot vegetative growth FSVG, water treatment W, tree genotype V and the interaction term (W × V) (Fig. 6). When considering the 10,000 different estimated aphid index curves, the same model gave the best results in 82.8% of the cases. At equivalent shoot growth, DAG sustained a more abundant aphid population than GCG. The two genotypes responded oppositely to the water treatment. Thus a water restriction enhanced the positive effect of shoot growth on the aphid index for DAG, but depleted it for GCG.

     3.4.2. At tree scale

The best model to explain the tree aphid index included final tree vegetative growth FTVG, water treatment W and the interaction term FTVG×W (Fig. 7). This model was selected as the best for 68.5% of the 10,000 datasets resulting from the transformation of aphid infestation classes into aphid numbers. The second best model took into account FTVG, V, W and the interaction term FTVG×W had a ΔAICc of 0.28 and it was selected as the best in 31.4% of the 10,000 runs. Then, an effect of the tree genotype (V) on the tree aphid index cannot be ruled out. At the tree scale, aphid abundance was positively correlated to vegetative growth. The slope of the regression line was smaller for W- trees compared to W+ trees.

4 DISCUSSION

Our results showed a positive relationship between vegetative growth and aphid abundance whatever the study scale, the genotype or the water supply. At the shoot scale, tree genotype played a role on aphid abundance in interaction with water supply. Thus, the effects of water restriction on the two genotypes were opposite: it increased aphid abundance on DAG and reduced it on GCG. At the tree scale, aphid abundance was penalized by water restriction and the effect of genotype on aphid abundance was less clear.

     The positive relationship between *D. plantaginea* abundance and both shoot and tree vegetative growths is consistent with the Plant Vigour Hypothesis that predicts a better performance of phytophagous insects on vigorous plants or organs (Price 1991). Our result, at the shoot scale, is consistent with another study conducted on apple tree, evidencing that *Aphis pomi* abundance on a current-year shoot was well correlated to the shoot growth but was independent of trunk diameter and median shoot length that are indicators of tree vigour (Stoeckli et al. 2008). Final tree vegetative growth (FTVG) was positively correlated to the percentage of tree leaves inserted on shoot and also to the number of shoots. However, there was no relation between final apical shoot vegetative growth (or

270   FSVG) and the number of shoots per tree, suggesting that, due to their apical position and apple tree acrotony, the

271   infested shoots had priority for vegetative growth with respect to the other shoots. As aphids have been shown to be

272   more performant on long shoots (Grechi et al., 2008; Simon et al., 2011), the most suitable one for aphid

273   development within the tree crown, was likely the apical shoot which grew at the most rapid rate. Apical shoot

274   growth, like tree growth, varied moreover with genotype. Thus, since grafting, GCG was more vigorous than DAG,

275   which could be related either to difference in genotypic vigour or in grafting compatibility. After grafting and

276   throughout 2014, vegetative growth was more important for GCG than for DAG (data not shown). The dry mass

277   suppressed by winter pruning prior to the experiment was consequently significantly higher for GCG (Kruskal-

278   Wallis test, p < 0.001, *i.e.* 41.3 ± 16.5 g (mean ± SD) for GCG vs 25.2 ± 9.5 g for DAG). It means that in 2014,

279   GCG had not only more functional leaves but, according to the functional equilibrium theory (Brouwer, 1983), also

280   more roots than DAG, and therefore probably higher amounts of C and N stored therein (higher reservoir size and

281   higher C and N intake in 2014). Higher vegetative growth of GCG during the first growth flush of 2015, which

282   correspond to the period of aphid population development, could thus result from higher spring remobilisation

283   (Jordan et al., 2009) or higher pruning intensity (Bevacqua et al., 2012). The infestation dynamic at tree level

284   depended not only on the population increase on a single shoot, but also on aphid dispersion within the crown,

285   which was affected by other aspects of plant development and architecture than solely individual shoot growth.

286   Indeed, dispersion could first depend on tree architecture complexity. On apple-*D. plantaginea* system strong and

287   complex branching decreased infestation, as pedestrian aphids attempting to colonize other plant shoots are less

288   likely to find suitable feeding sites (Simon et al., 2011). However as the architecture of our small trees was quite

289   homogeneous in term of branching complexity, this was unlikely to have played a major role in our study.

290   Dispersion within the tree might also depend on the number of individuals able to feed on the apical shoot, i.e. on

291   the aphid density in relation with phloem sap quality and accessibility, but also on the attractiveness of the other

292   tree shoots compared to apical favoured one. Indeed, aphids preferentially settled on plant stratum with the highest

293   nitrogen availability (Chau et al., 2005) but nitrogen allocation and leaf transpiration (phloem sap flow) vary

294   greatly within a tree as a function of tree height (Livingston et al., 1998), leaf age (Constable and Rawson, 1980),

295   genotype in interaction with leaf area (Tausend et al., 2000), shoot orientation (Le Roux et al., 2012) or cultural

296   practices (Jordan et al., 2011). Therefore, if the phloem quality is poor on a given shoot, aphids are more likely to

297   start roaming about to test other available feeding sites (Nowak and Komor, 2010), or might even return to the

298   apical shoot. The within tree variability varies moreover with stress intensity (Jordan et al., 2012; Ballester et al.,

299   2013), which might partly explain why deficit irrigation could be oppositely related to aphid abundance at shoot

300   and tree scale.

301   The effect of water restriction on aphid abundance was partially mediated by vegetative growth, which was

302   reduced by deficit irrigation at shoot and tree scales for both genotypes. However a water restriction also modified

303   the relationship between aphid and vegetative growth, which indicated additional physiological consequences of

304   water restriction on tree suitability for aphid. However, we did not find clear difference among treatments in

305   midday leaf water potential which is correlated to predawn leaf water potential considered as the most accurate

306   indicator of plant water stress (Paço et al., 2013). We therefore hypothesize that water restriction modified several

307   variables related to plant water status, which possibly impact aphid performance. Water restriction could increase

308   sap viscosity (Sevanto, 2014), due to higher solute concentration, which may impair efficient sap uptake by the

309   aphid. Aphids are also affected by phloem carbohydrate concentrations that determine excretion costs (Zehnder and

310   Hunter, 2009), by turgor pressure (Mody et al., 2009; Verdugo et al., 2015) and cell wall thickness (Goggin, 2007;

311   Foyer et al., 2016) that influence phloem accessibility. If the host plant condition is worsened by water restriction

312   (or any other treatment), the aphids modify their feeding behaviour, spending less time in passive ingestion of

313   phloem sap (Lu et al., 2016). They are therefore smaller in size and exhibit longer pre-adult life stage and reduced

314   fecundity (Lu et al., 2016). Intermittent stress seems to impose harsher conditions than a continuous and lasting

315   drought, thus reducing the aphid performances, among which the fecundity, to a greater extent as a continuous

316   drought (Banfield-Zanin and Leather, 2015, on the Stika spruce – green spruce aphid system). Under pulsed stress

317   conditions, tree mortality was also higher when previously infested by aphids (Banfield-Zanin and Leather, 2014),

318   which is consistent with the fact that aphids have been shown to affect their host plant water potential, in ways

319   similar to drought (Cabrera et al., 1995). Notice that in our study the W+ trees were also the most infested, so the

320   differences in the severity of aphid infestation between W+ and W- might have reduced the difference in leaf water

321   potential observed between the two water treatments.

322   Water restriction seemed to impair aphid abundance first at tree scale. This negative effect can also result from

323   an increased leaf temperature consecutive to a decrease in leaf transpiration after stomata closure as evidenced by

324   Ballester et al. (2013). On 30 DAI, for instance, leaf temperature was significantly higher for GCG W- compared to

325   GCG W+, and the difference was 3.7°C. These differences could partially explain the more rapid decrease of aphid

326   abundance on the water restricted trees, all the more because the temperatures peaked above 42.5°C and up to

327   43.3°C, which is below the lethal temperature for most aphid species (Satar et al. 2008, Hullé et al. 2010).

At the shoot scale, the effect of water restriction on the relation between vegetative growth and aphid abundance was negative for GCG but positive for DAG. A modulation of genotypic resistance to aphids by water restriction has also been observed on other tree-aphid systems. On both poplar-*Phloeomyzus passerinii* (Dardeau et al., 2015) and peach tree-*Myzus persicae* (Verdugo et al., 2015) systems, water stress had no effect on the aphid population on aphid susceptible cultivar, but it increased aphid performance on aphid resistant cultivar. In the present study, deficit irrigation favoured aphid development on the DAG W- apical shoots, despite it also reduced their vegetative growth. We hypothesized therefore that the irrigation treatment modified also other determinants of plant suitability for aphids, among which shoot N concentration, thereby counterbalancing the expected negative effect of low shoot growth. Indeed, DAG W- had the lowest number of proleptic structures, the difference being significant between DAG W- and GCG W-, so the nitrogen resource (mainly amino acids) might have been less diluted in those trees. Decreased C:N ratio or increased amino acids contents have been shown to increase aphid performance (Nowak and Komor, 2010; Ryalls et al., 2014). This hypothesis relies furthermore on the assumption that the tree aphids gathered on the apical shoot because of its higher nutritional quality due to its position, as shown on the Chrysanthemum-Melon aphid system [*Dendranthema grandiflora - Aphis gossypii*] in which within plant aphid distribution is driven by nutrient availability (Chau et al., 2005).

The absence of the expected marked differences in physiological measures of the trees of the two genotypes impairs the identification of mechanisms acting differently on aphid performance between the two genotypes. According to Lauri et al. (2016), we expected DAG to close its stomata quicker than GCG. Our measured values of stomatal conductance of well-watered trees are halved compared to the values obtained by Lauri et al. (2016), which may result from the use of a different rootstock, which influenced the response of the plant to water restriction (Liu et al., 2012). Photosynthetic rate was significantly affected by watering treatment for only one date and furthermore only for DAG. In addition, we expected GCG vegetative growth to be more affected by water restriction than DAG, but we obtained the reverse.

The different effects of water restriction on the aphid performance on DAG at tree and shoot levels underlined the importance of the scale choice. Most studies on tree-aphid interaction focused on a few number of sub-sample shoots (Stoeckli et al., 2008; Rousselin et al., 2016), or even at a smaller scale, such as the individual leaf (Mace and Mills, 2015). In the present study we demonstrated that the responses to abiotic constraints might be different at apical shoot and tree scales. As far as orchard aphid management is concerned, the patterns at the tree scale seem more representative of the overall aphid induced damages. Thus the reduction of tree vigour can be a lever to control *D. plantaginea* populations. In addition, trees submitted to water restriction were less favourable to aphids

358 than well-watered trees, so it may be possible to address at the same time the water scarcity and the chemical use

359 reduction issues, by reducing water supply in apple orchards. Water restriction has to be applied preferably in

360 spring, i.e. when aphid populations were susceptible to develop on the trees. But, host plant suitability to aphids

361 could probably also be affected by regulated deficit irrigation techniques, currently applied for longer periods but

362 mainly in summer and autumn, techniques which were, to our knowledge, evaluated only for fruit production

363 (Girona et al., 2010). Indeed, a water restriction modifies determinants of plant suitability others than growth, and

364 those effects could last several months, especially when shoot composition is concerned.

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Caption of figures

Fig. 1 Absolute values of midday leaf water potential of on apple trees (sample size indicated within the figure for each date), subject to four treatments, combining two levels of water supply (W+ and W-) and two tree genotypes (DAG, drought avoidance genotype and GCG, growth cessation genotype). Each panel corresponds to a date expressed in days after infestation (DAI), and to a total number of n plants. Different letters indicate significant differences between treatment combinations (Kruskal-Wallis test and Tukey multiple comparisons, performed at each date). No significant differences were observed on DAI = 1, 9 and 49.

Fig. 2 Characterisation of leaf functioning of 17 apple trees on 30 DAI (days after infestation) according to the different treatments, combining two tree genotypes: GCG (Growth cessation genotype) and DAG (Drought Avoidance Genotype) and two levels of water supply: W+ and W-. (a) Leaf stomatal conductance, (b) transpiration rate, (c) photosynthetic rate, (d) temperature. Different letters indicate significant differences between treatment combinations (Kruskal-Wallis test and Tukey multiple comparisons).

Fig. 3 Vegetative growth dynamics at both shoot (a) and tree (b) scales as an increase of leaves number from 2 to 64 days after infestation (DAI). Each point represents the mean value for a treatment combination: water supply (W+ and W-) and tree genotype (DAG: Drought Avoidance Genotype and GCG: Growth Cessation Genotype). Bars stand for standard deviation.

Fig. 4 Relationship between the number of proleptic shoots per tree and final vegetative growth variables in increase in number of leaves at tree (FTVG) and shoot (FSVG) scales. (a) FTVG as a function of FSVG, the regression lines result from the selected best model (based on AICc, R^2_{adj} = 0.74) including FSVG, W (water treatment), V (tree genotype) and the interaction FSVG × V. (b) FTVG as a function of the number of proleptic shoots per tree: the regression lines result from the selected best model (R^2_{adj} = 0.68) including the number of proleptic shoots, W, V and the interaction the number of proleptic shoots and genotype. (c) FSVG as a function of the number of proleptic shoots: the regression lines result from the selected best model (R^2_{adj} = 0.34) including W and V. Notice that FTVG and FSVG have been log transformed to fulfil the conditions required for statistical analysis

Fig. 5 Aphid abundance temporal dynamics (mean ± standard deviation) for each treatment combination at (a) the infested shoot scale and (b) the tree scale for the different dates (in days after infestation: DAI).

Fig. 6 Relationship between final shoot vegetative growth (FSVG) and shoot aphid index. Each point represents the value of the apical shoot of a tree. The regression lines result from the selected best model (based on AICc, R^2_{adj}= 0.52) including FSVG, water treatment W, tree genotype V and the interaction term (W × V) as explanatory variables and factors. Notice that FSVG has been log transformed to fulfil the conditions required for statistical analysis.

Fig. 7 Relationship between tree aphid index and final tree vegetative growth (FTVG). Each point represents the value of a single tree. The regression lines result from the selected best model (based on AICc, R^2_{adj}= 0.42) including FTVG, water treatment W and the interaction term FTVG × H, as explanatory variable and factor. Notice that FTVG has been log transformed to fulfil the conditions required for statistical analysis.

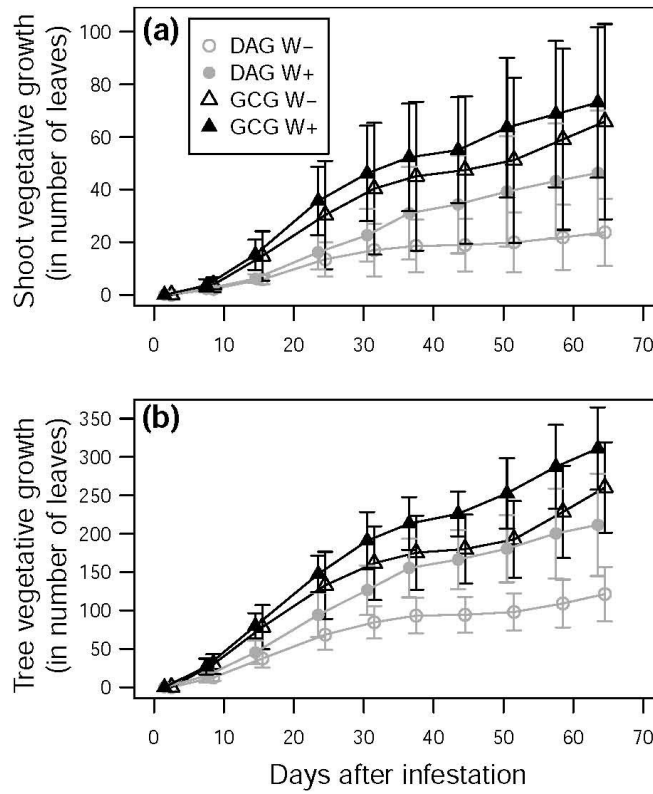**Figure 1:** 2 columns fitting image, original size (w/l) 16*21 cm in a separate eps file
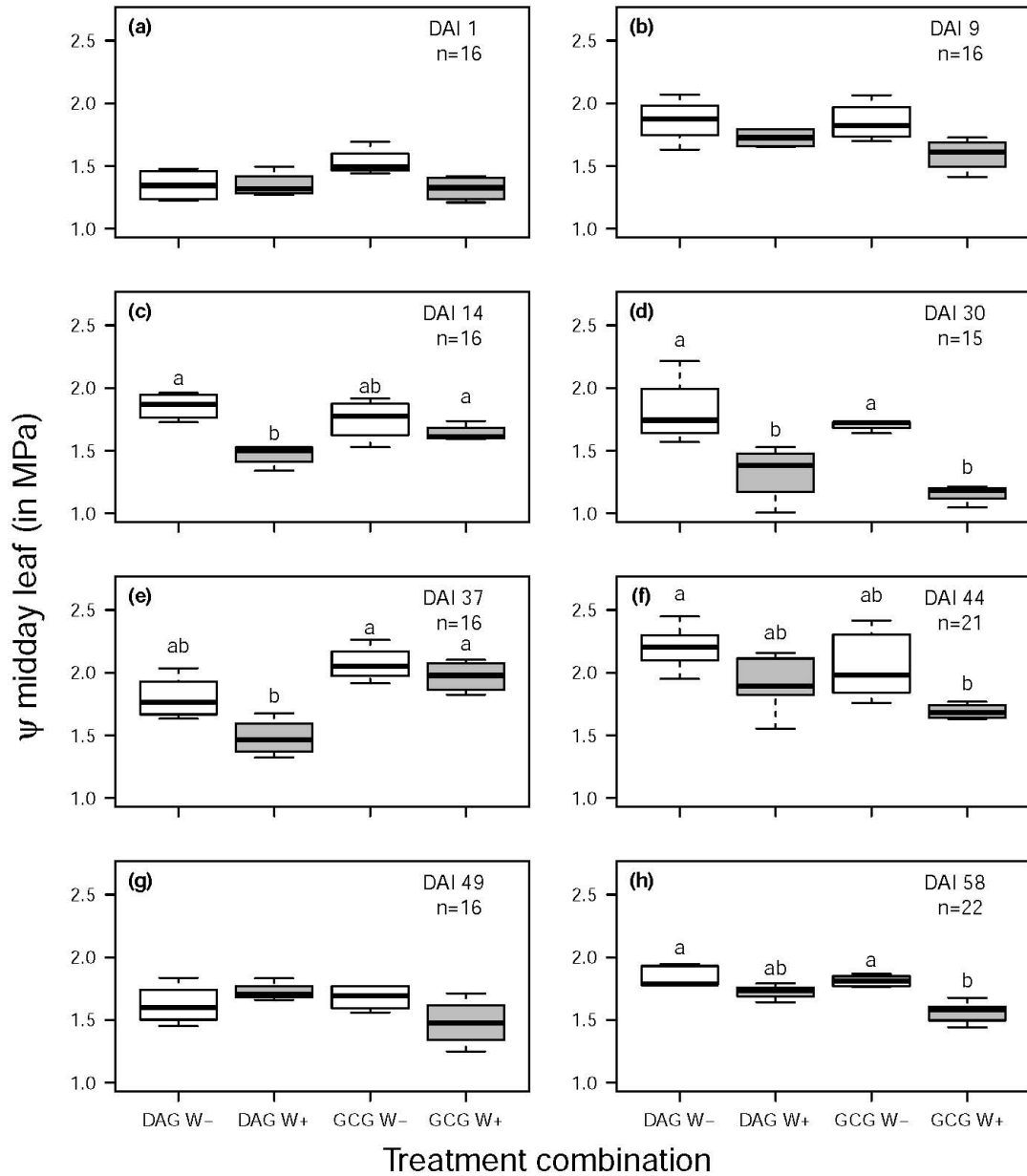
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599 **Figure 3:** 2 columns fitting image, original size (w/l) 14* 14.5 cm in a separate eps file
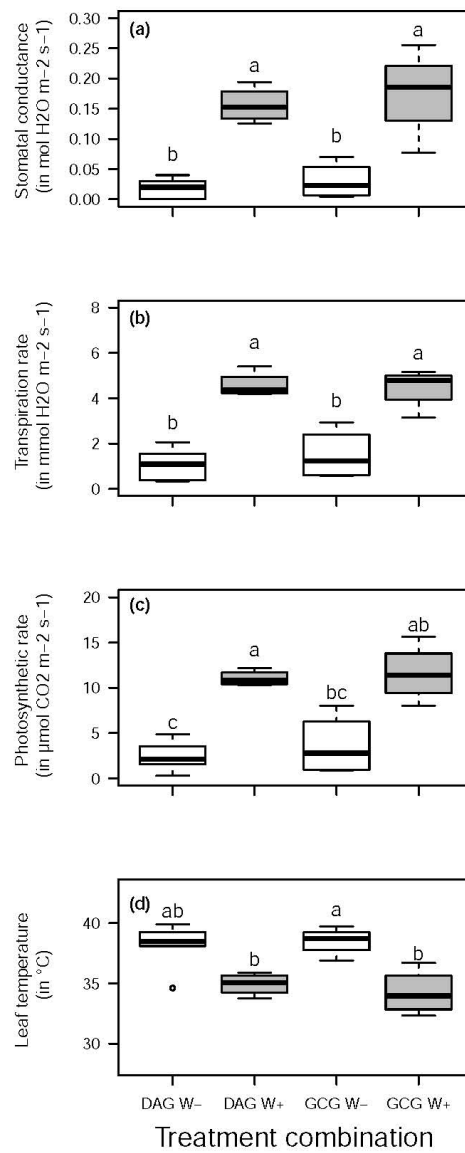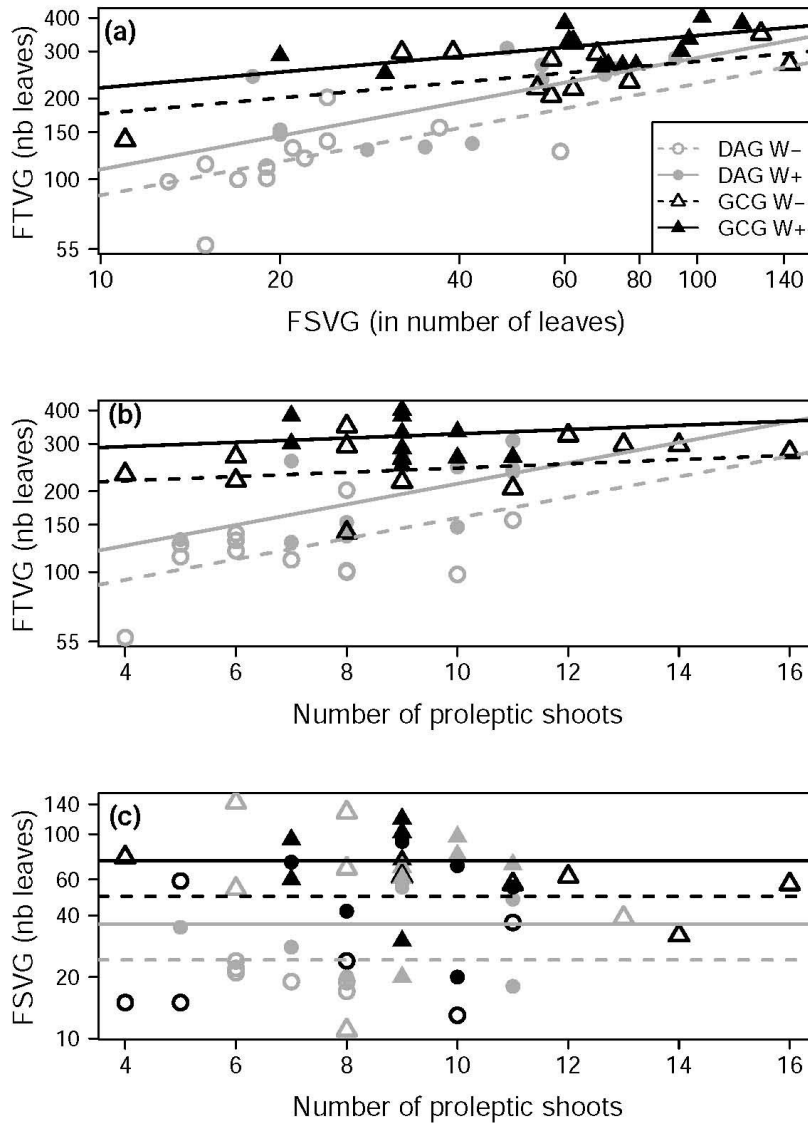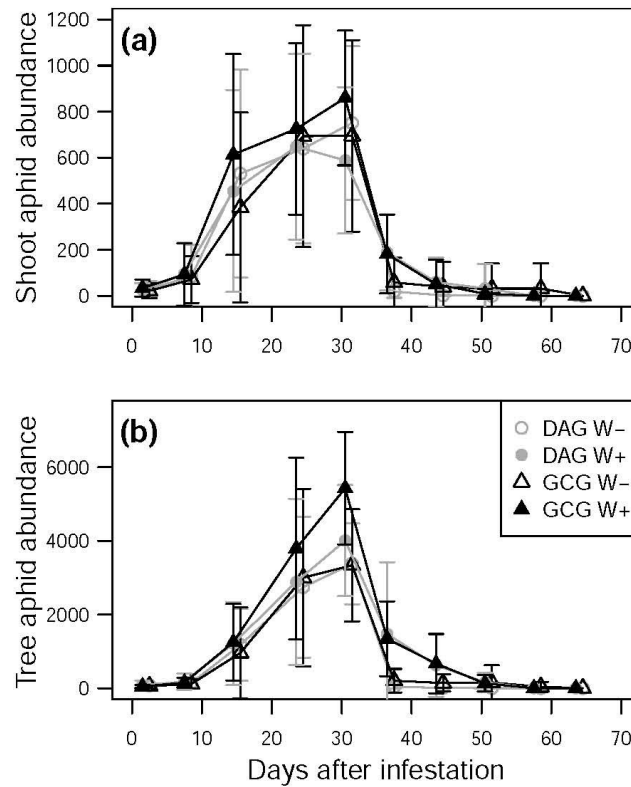

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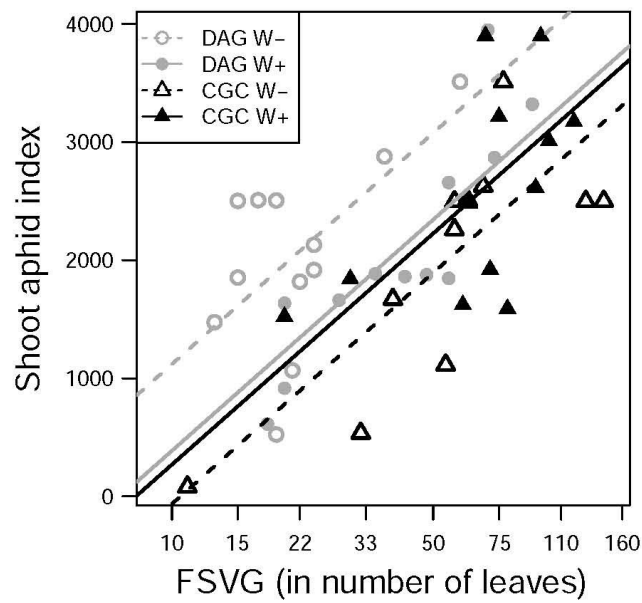602 **Figure 4:** 2 columns fitting image, original size (w/l) 17.5*21 cm in a separate eps file
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607   **Figure 5:** 2 columns fitting image, original size (w/l) 14* 14.5 cm in a separate eps file
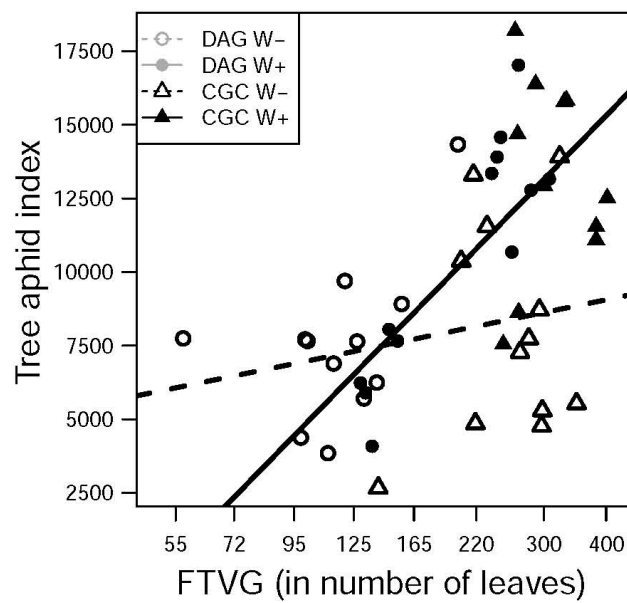608

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610 **Figure 6:** 1 column fitting image, original size (w/l) 12* 12 cm in a separate eps file

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613   **Figure 7:** 1 column fitting image, original size (w/l) 12* 12 cm in a separate eps file
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