Morphological and olfactory tree traits influence the susceptibility and suitability of the apple species *Malus domestica* and *M. sylvestris* to the florivorous weevil *Anthonomus pomorum* (Coleoptera: Curculionidae)

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The florivorous apple blossom weevil, *Anthonomus pomorum* (Coleoptera: Curculionidae), is the most economically relevant insect pest of European apple orchards in early spring. Neither efficient monitoring nor ecologically sustainable management of this insect pest has yet been implemented. To identify heritable traits of apple trees that might influence host selection of *A. pomorum*, we compared susceptibility of apple tree species using infestation rates of the domesticated apple, *Malus domestica* (Rosaceae: Pyreae), and the European crab apple, *M. sylvestris*. We evaluated the suitability of the two apple species for *A. pomorum* by quantifying the mass of weevil offspring. Because volatile organic compounds (VOCs) emitted from flower buds of domesticated apple have previously been suggested to mediate female weevil preference via olfactory cues, we conducted bioassay experiments with blossom buds of both apple species to explore the olfactory preference of adult weevils and, furthermore, identified the headspace VOCs of blossom buds of both apple species through GC-MS analysis. The infestation analysis showed that *A. pomorum* infested the native European crab apple stronger than the domesticated apple, which originated from Central Asia. The European crab apple also appeared to be better suited for weevil larval development than the domesticated apple, as weevils emerging from *M. sylvestris* had a higher body mass than those emerging from *M. domestica*. These field observations were supported by olfactory bioassays, which showed that *A. pomorum* significantly preferred the odor of *M. sylvestris* buds compared to the odor of *M. domestica* buds. The analysis of headspace VOCs indicated differences in the blossom bud volatiles separating several *M. domestica* individuals from *M. sylvestris* individuals. This knowledge might be employed in further studies to repel *A. pomorum* from *M. domestica* blossom buds.

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Abstract

The florivorous apple blossom weevil, *Anthonomus pomorum* (Coleoptera: Curculionidae), is the most economically relevant insect pest of European apple orchards in early spring. Neither efficient monitoring nor ecologically sustainable management of this insect pest has yet been implemented. To identify heritable traits of apple trees that might influence host selection of *A. pomorum*, we compared susceptibility of apple tree species using infestation rates of the domesticated apple, *Malus domestica* (Rosaceae: Pyreae), and the European crab apple, *M. sylvestris*. We evaluated the suitability of the two apple species for *A. pomorum* by quantifying the mass of weevil offspring. Because volatile organic compounds (VOCs) emitted from flower buds of domesticated apple have previously been suggested to mediate female weevil preference via olfactory cues, we conducted bioassay experiments with blossom buds of both apple species to explore the olfactory preference of adult weevils and, furthermore, identified the headspace VOCs of blossom buds of both apple species through GC-MS analysis. The infestation analysis showed that *A. pomorum* infested the native European crab apple stronger than the domesticated apple, which originated from Central Asia. The European crab apple also appeared to be better suited for weevil larval development than the domesticated apple, as weevils emerging from *M. sylvestris* had a higher body mass than those emerging from *M. domestica*. These field observations were supported by olfactory bioassays, which showed that *A. pomorum* significantly preferred the odor of *M. sylvestris* buds compared to the odor of *M. domestica* buds. The analysis of headspace VOCs indicated differences in the blossom bud volatiles separating several *M. domestica* individuals from *M. sylvestris* individuals. This knowledge might be employed in further studies to repel *A. pomorum* from *M. domestica* blossom buds.

Introduction

The apple blossom weevil, *Anthonomus pomorum* L. (Coleoptera: Curculionidae) is one of the economically most important insect pest herbivores of apple trees (Collatz & Dorn, 2013; Knuff et al., 2017). It is a widespread univoltine insect pest in European apple orchards directly reducing fruit harvest of domesticated apple, *Malus domestica* Borkh. (Rosaceae: Pyreae), by infesting the unopened blossom buds early in spring (Duan et al., 1998; Toepfer et al., 1999; Daniel et al., 2005; Žďárek et al., 2013). The apple blossom weevil used to be a minor pest in European apple orchards, as the use of broad-spectrum insecticides provided adequate control.

52   However, in recent decades the economic importance of this pest insect has increased (Toepfer et

53   al., 1999). It is now considered the most damaging pest weevil of apple throughout Europe

54   (Mody et al., 2015; Zabrodina et al., 2020). In some European countries, population densities of

55   the apple blossom weevil have exceeded economic thresholds due to changes in pest

56   management (Toepfer et al., 1999; Sipos & Marko, 2014). The percentage of blossoms infested

57   and destroyed by *A. pomorum* has reached 60 – 90 % as was reported for Lithuania (Toepfer et

58   al., 1999), or even up to 85 – 100 % in organic apple orchards in Slovenia (Bajec et al., 2013).

59   Zabrodina et al. (2020) call the apple blossom weevil "one of the most harmful insects in

60   horticulture" and report yield losses for Russia and the Ukraine of 50 – 100 %. The increasing

61   pest status of *A. pomorum* poses a serious threat to organic apple orchards and integrated pest

62   management (IPM) systems currently in use in European apple orchards (Miñarro & García,

63   2018; Shaw et al. 2021).

64   Although the biology and population ecology of *A. pomorum* is relatively well documented,

65   neither efficient monitoring nor management programs for this pest insect have yet been

66   developed, let alone implemented (Toepfer et al., 1999). There are two main monitoring

67   methods: limb jarring (which is inconvenient, Toepfer et al., 1999) and shelter traps (Hausmann

68   et al., 2004a; Hausmann et al., 2004b). Shelter traps are more preferred as they exploit the

69   weevils' preference for warm microclimates early in the year and may serve as a useful tool for

70   assessing the number of weevils that colonize the tree by crawling up the trunk (Hausmann et al.,

71   2004b). However, flight has been indicated as the most important mode of tree colonization in *A.*

72   *pomorum* (Toepfer et al., 2002). As flight-guiding pheromones are still unknown for this pest

73   insect (Dorn & Piñero, 2009), flight traps baited with attractive host-plant odors might constitute

74   an efficient monitoring tool (Natale et al., 2003). Flight traps baited with attractive odors may

75   present a useful component of novel strategies for an ecologically sustainable apple production

76   since they do not rely on broad-spectrum insecticides. To develop these strategies, it is of great

77   importance to gain more insight in the underlying mechanism(s) of host tree selection by *A.*

78   *pomorum* (Knuff et al., 2017).

79   Many studies have been conducted on the biology of *A. pomorum* (Čtvrtečka & Žďárek, 1992;

80   Duan et al., 1996; Duan et al., 1998; Toepfer et al., 1999; Kalinová et al., 2000; Toepfer et al.,

81   2000; Toepfer et al., 2002; Hausmann et al., 2004a; Hausmann et al., 2004c; Hausmann et al.,

82   2005; Piskorski & Dorn, 2010; Collatz & Dorn, 2013; Knuff et al., 2017), but still little is known

83   about the cues that female weevils use when colonizing apple orchards in early spring. Following

84   colonization from adjacent forests (Hausmann et al., 2004a; Dorn & Piñero, 2009), females lay

85   their eggs into closed blossom buds (Mody et al., 2015). Differences in infestation between apple

86   species or among cultivars of *M. domestica* are probably strongly mediated by preference

87   behavior of female weevils searching for suitable host trees for oviposition (Hogmire & Miller,

88   2005; Mody et al., 2015). Therefore, an identification of apple tree characteristics that guide

89   female *A. pomorum* in search of their host would be a first step for breeding less susceptible

90   apple cultivars (Knuff et al., 2017), or to develop effective and ecologically sustainable

91   monitoring and management tools. Because *A. pomorum* infests apple blossom buds at certain

92   developmental stages (Toepfer et al., 2002), the traits of blossom buds are particularly promising

93   for breeding apple cultivars that are less attractive and consequently less susceptible to the apple

94   blossom weevil (Mody et al., 2015) or to use these traits as a screening mechanism for newly

95   developed cultivars. To identify heritable traits of apple trees that might influence host selection

96   of female *A. pomorum*, it may be informative to study oviposition preferences for different

97   *Malus* species, as differences are assumedly more pronounced between species than among

98   cultivars of the same species (Knuff et al., 2017).

99   As different plant species or individuals vary in their suitability as host for the offspring of a

100   given herbivore, females should have evolved an ability to identify the host plant best suited for

101   their offspring (Jaenike, 1978; Thompson, 1988; Craig et al., 1989; Gripenberg et al., 2010).

102   Preference of oviposition in females determines host plant infestation by their offspring in many

103   insect herbivores (Gripenberg et al., 2010; Mody et al., 2015), leading to positive preference-

104   performance relationships between female preference and offspring performance (Mody et al.,

105   2015).

106   The apple blossom weevil, as stated above, is an important pest insect of domesticated apple

107   trees in Europe, but it is also likely to infest other species of the genus *Malus*, like the European

108   crab apple, *M. sylvestris* (L.) Mill. So far, there is only one published study on the ecology of the

109   apple blossom weevil on *M. sylvestris* trees (Knuff et al., 2017), further studies that assess the

110   specific entomofauna of this wild apple species are still lacking (Mody, 2013). Whereas the

111   origin of the domesticated apple lies in the Tian Shan region of Central Asia (Velasco et al.,

112   2010; Cornille et al., 2012), the European crab apple is the only wild apple species that is

113   indigenous to Central Europe (Robinson et al., 2001; Reim et al., 2013). It is a rare and

114   endangered species (Red List Bavaria: category 3) (LfU 2003) that grows in open forests, forest

115   edges and in hedgerows (Stephan et al., 2003; Aas, 2013; Knuff et al., 2017). However, *M.*

116   *sylvestris* easily hybridizes with *M. domestica* (Reim et al., 2013), and bidirectional gene flow

117   between the two apple species in Europe has resulted in the current *M. domestica* being

118   genetically more closely related to *M. sylvestris* than to its progenitors from Central Asia

119   (Cornille et al., 2012).

120   This study deals with the question of whether the apple blossom weevil shows specific host

121   selection patterns for the apple species *M. domestica* and *M. sylvestris*. A better understanding of

122   the observed patterns could lead to an identification of apple tree characteristics that may be used

123   for breeding less susceptible cultivars or, in the case of volatile organic compounds (VOCs)

124   emitted from the blossom buds, developing effective and sustainable monitoring tools. Plant

125   resistance to arthropods is often mediated by morphological and phenological plant traits (Smith,

126   2005; Miñarro & Dapena, 2008; Smith & Clement, 2012; Knuff et al., 2017), and most

127   knowledge on the expression of plant resistance is available for herbivores feeding on leaves

128   (Kessler & Baldwin, 2002; Dicke & Hilker, 2003; van Dam, 2009; Mody et al., 2015). Much less

129   is known about whether plant resistance is expressed similarly with regard to flower-feeders

130   (McCall & Irwin, 2006; Oguro & Sakai, 2014).

131   As mentioned above, a particularly promising trait to mediate the preference of female *A.*

132   *pomorum* searching for suitable host trees, besides physical plant characteristics, is the spectrum

133   of VOCs emitted from the blossom buds (Kalinová et al., 2000; Piskorski & Dorn, 2010; Mody

134   et al., 2015). It has been shown through chemical analyses of plant headspace VOCs that were

135   combined with behavioral bioassays that host-plant odors play an important role in host location

136   for lepidopteran pest insects, like the oriental fruit moth *Cydia molesta* Busck (Natale et al.,

137   2003), or the codling moth *C. pomonella* (Vallat & Dorn, 2005; Piskorski & Dorn, 2010).

138   Studies also showed for many species of curculionids that they regularly rely on olfactory cues

139   for host detection, e. g. the pine weevil *Hylobius abietis* L. (Kännaste et al., 2009), the

140   strawberry blossom weevil *Anthonomus rubi* Herbst (Bichão et al., 2005), or the boll weevil

141   *Anthonomus grandis* Boheman (Minyard et al., 1969; Dickens, 1989), the latter two being close

142   relatives of the apple blossom weevil. However, despite an identified blend of VOCs released by

143   blossom buds of prebloom *M. domestica* trees (Piskorski & Dorn, 2010), knowledge on VOCs

144   from apple blossom buds released before bloom in the time of host-tree selection of apple

145 blossom weevils is still scarce. Further identification of such VOCs that control the oviposition

146 behavior of female *A. pomorum* may be of great practical significance aside from future apple

147 breeding programs: if effective attractants of the apple blossom weevil are found, synthetically

148 produced kairomones could be used in traps in apple orchards to monitor or even control weevil

149 populations (Kalinová et al., 2000; Piskorski & Dorn, 2010).

150 The research questions that are assessed in this study are therefore (i) does *A. pomorum* show

151 certain host selection patterns among the two *Malus* species, measured as infestation rate,

152 indicating an oviposition preference of females? (ii) Does performance of offspring, indicated by

153 weevil mass of newly hatched imagines, differ among the two *Malus* species, and if so, how does

154 it relate to preference of females? (iii) How do adult overwintered weevils behaviorally react to

155 the odor of blossom buds of the two apple species in olfactory bioassays? (iv) Does the spectrum

156 of VOCs emitted from the blossom buds differ among the two *Malus* species, and which

157 compounds can be identified through sampling of headspace VOCs?

158

Materials & Methods

Study sites

161 The study was conducted in 2017 in the Ecological-Botanical Garden of the University of

162 Bayreuth (49°55'N, 11°35'E, elevation: 355 m a. s. l.) and in the surroundings of Bayreuth. Two

163 apple species were investigated for their infestation by the apple blossom weevil *A. pomorum*:

164 the domesticated apple, *M. domestica*, and the European crap apple, *M. sylvestris*. Studied *M.*

165 *domestica* trees grew in the botanical garden on a meadow with scattered fruit trees in rows with

166 a spacing of 8 m x 6 m. The orchard contained 98 high stem apple trees of 81 different cultivars

167 and trees of several *Pyrus* and *Prunus* species planted in between the apple trees. The lower part

168 of the meadow was surrounded by a hedgerow in the south, grassland with some shrubs in the

169 west, beds with useful plants in the north, and further fruit trees in the east. For this study, ten *M.*

170 *domestica* trees of ten different cultivars planted between 1998 and 2008 were randomly selected

171 (Table S1) so that they were distributed over the whole area of the fruit orchard. Neither

172 pesticides nor fungicides were applied to the apple trees during the study period.

173 The *M. sylvestris* trees used in this study comprised two individuals (genotypes) that grew inside

174 the botanical garden, and eight individuals that grew in hedgerows, riverbanks, or forest margins

175 in the surroundings of Bayreuth (Table S1).

176   For the sampling of headspace VOCs, smaller potted trees of *M. domestica* and *M. sylvestris*

177   were used that we were able to move to the laboratory (Table S2).

178

179   **Infestation assessment and sampling of infested blossom buds**

180   Female weevils lay their eggs in blossom buds of certain developmental stages, namely the bud

181   stages 56 and 57 according to BBCH (Toepfer et al., 2002), shortly before the opening of the

182   flowers. Therefore, phenology of the blossom buds was assessed for both tree species following

183   BBCH (Meier, 2001; Meier et al., 2009) at weekly intervals. Infestation by *A. pomorum* was

184   assessed shortly after full flowering from mid-May to end of May by counting the number of

185   infested (recognizable as unopened blossom buds with brownish, dead petals forming a hollow

186   cavity, so-called "capped blossoms") and non-infested blossom buds of ten blossom clusters on

187   five branches, with two clusters per branch. Branches had been randomly selected in a way that

188   they were evenly distributed over the treetop (Knuff et al., 2017).

189   After infestation assessment, as many capped blossoms as possible were collected from each tree

190   (666 in total; per tree: 33.3 ± 15.8 [mean ± SD]) (Table 1). Capped blossoms were kept in plastic

191   containers with insect-proof gauze in the lids allowing for sufficient ventilation. The containers

192   were equipped with paper towels for moisture absorption and kept inside the laboratory at

193   approximately 22 °C until the insects had hatched.

194

195   **Weevil characteristics**

196   Plastic containers containing the capped blossoms were examined for the presence of freshly

197   emerged weevils at 24h-intervals. After emergence, weevils were immediately removed from the

198   containers and deep-frozen and stored in Eppendorf tubes at -16 °C (Mody et al., 2015; Knuff et

199   al., 2017). Emergence was monitored until no more insects had emerged for seven consecutive

200   days. The sex of emerged weevils was determined based on morphology of the last dorsal

201   abdominal plate (Duan et al., 1999). All weevils were then dried at 40 °C to mass constancy and

202   weighed (Ohaus Explorer EX423M high-precision scale, Ohaus Europe GmbH, Greifensee,

203   Switzerland). Weevil weight was calculated as the average weight of all individuals per tree and

204   sex to analyze the tree species' suitability for weevil development.

205

206   **Olfactory bioassays**

207   As many post-diapause *A. pomorum* imagines as possible were collected from *M. domestica*

208   trees by limb-jarring during their tree colonization period in the botanical garden, from mid-

209   March to mid-April. Weevils were kept in plastic vials (4.5 cm diameter, 10 cm height;

210   approximately 10 individuals per vial) for up to one week. They were kept under controlled

211   conditions in a climate chamber at 12/12 h day/night, 12°/8 °C, and 70 % relative humidity (RH).

212   Weevils were offered moist apple leaves ad libitum as food and shelter (Piskorski & Dorn,

213   2010).

214   Olfactory bioassays were performed as still-air dual-choice experiments in plastic Petri dishes as

215   described by Prokopy et al. (1995) (Figure S1). Two holes (10 mm diameter, 70 mm apart) were

216   bored through the lid of a Petri dish (14 cm diameter, 1.5 cm high). A polyethylene micropipet

217   tip (10 mm diameter at the base, 20 mm high, and 7 mm diameter at the top after cutting off the

218   tip) was fitted snugly into each hole so that the base was flush with the lid of the Petri dish and

219   the tip protruded above the lid. A 100-ml transparent polypropylene cup was centered over each

220   pipet tip, enclosing it (Figure S1). One or both of the cups contained a treatment in the form of

221   an apple twig of approximately 5 cm length carrying 5 ± 1 blossom buds that were in the suitable

222   stage for oviposition (Toepfer et al., 2002). The other cup contained either a twig of the second

223   apple species or an appropriate control in the form of a model of an apple twig made out of wire.

224   Cultivars of *M. domestica* or genotypes of *M. sylvestris* that were used in the form of apple twigs

225   for olfactory bioassays represented a subset of the individuals used for the infestation analysis,

226   namely the cultivars "Roter Boskoop", "Blauacher Wädenswil", and "James Grieve", as well as

227   the genotypes "Destuben", "Schlehenmühle", and "Lochau 2". To prevent the apple twigs from

228   drying out and subsequently changing their odor profile, each twig was placed in an Eppendorf

229   tube containing water. Thereby, the cut surface of each apple twig was covered with water to

230   avoid that the inflicted mechanical damage altered the composition of the volatile blend (Collatz

231   & Dorn, 2013). Conditions in the test chamber were kept constant at 16 °C and 70 % RH.

232   Prior to the start of bioassays, the weevils were deprived of food for 24 h and allowed to

233   acclimatize to the conditions in the test chamber for 90 min. At 3.5 h after the onset of

234   photophase, a single weevil was released on the bottom of each Petri dish, which was covered

235   with circular filter paper and tightly sealed with parafilm (Pechiney Plastic Packaging, Chicago,

236   IL). Bioassays were run for 180 min, after which the choice of each weevil was recorded (Collatz

237   & Dorn, 2013). Each weevil was only used once in bioassays. New apple twigs and a clean Petri

238   dish were used for each bioassay. The sex of weevils that were tested in bioassays was not

239   recorded, because odor preference had been shown to not differ between male and female apple

240   blossom weevils (Collatz & Dorn, 2013).

241   The behavioral response of apple blossom weevils to the odor of *M. domestica* twigs with

242   blossom buds was assessed against controls (n = 50), as well as the behavioral response to twigs

243   of *M. sylvestris* against controls (n = 50). Twigs of *M. domestica* and *M. sylvestris* were also

244   compared directly in bioassays, so that weevils could choose between the two host-plant species

245   (n = 50). To assure that the controls did not have any influence on the behavioral response of

246   weevils, neither attracting nor repellent, the controls were also tested against blanks (n = 30).

247

248   **Sampling of VOCs**

249   Headspace VOCs of apple blossom buds were sampled in late April, when blossom buds were in

250   bud stages 56 and 57 according to BBCH (Meier, 2001), suitable for oviposition by apple

251   blossom weevils (Toepfer et al., 2002). It has been found that apple blossom weevils show their

252   highest levels of flight activity at bud stages 56 and 57 and that oviposition is restricted to these

253   bud stages (Toepfer et al., 2002; Zabrodina et al., 2020). Ten potted individuals of six different

254   cultivars of *M. domestica* were sampled, as well as eight potted individuals of four different

255   genotypes of *M. sylvestris* (Table S2). Originally, ten individuals of each apple species were

256   sampled, but two *M. sylvestris* individuals had to be removed from the study due to being

257   identified as hybrids. Each tree was sampled three times on the same day without precipitation,

258   yielding 30 samples of *M. domestica* and 24 samples of *M. sylvestris*. The sampling of VOCs

259   was conducted in the laboratory at 18 °C, 60 % RH, using headspace sorptive extraction (SBSE)

260   (Bicchi et al., 2000). Twigs carrying 10 ± 1 blossom buds were enclosed in a PET oven bag

261   (Toppits Bratschlauch, Cofresco GmbH, Minden, Germany) together with a 10-mm Gerstel©

262   Twister (Gerstel, Mühlheim, Germany) that consists of a magnetic stir bar enclosed in glass and

263   coated with 0.5 mm polydimethylsiloxane (PDMS) as sorbent. Each Twister was held by a

264   magnet from the outside of the PET oven bag. Sampling of headspace VOCs lasted 6 h.

265

266   **Analysis of VOCs**

267   The VOCs were thermally desorbed from the PDMS twisters with a Gerstel© Twister

268   Desorption Unit (TDU), and the chemical composition of headspace VOCs was analyzed by GC-

269 MS (Agilent 7890A GC coupled with a 5975C mass spectrometer [MS]). Twisters were placed

270 in the TDU at an initial temperature of 30 °C, which was enhanced at a rate of 30 °C/min to 210

271 °C and held for 10 min. The N_2-cooled injection system had an initial temperature of -50 °C

272 during desorption. Splitless injection of the apple blossom bud volatiles into the GC was

273 performed by heating at a rate of 12 °C/s to 220 °C that was held for 5 min. Chemical separation

274 in the GC was achieved using a DB-5 ms column, 30 m × 250 μm × 0.25 μm (Agilent

275 Technologies). The oven was programmed from 40 °C (held for 5 min) to 260 °C at 5 °C/min. A

276 transfer line set at 280 °C led to the MS. The MS was operated in electron impact mode (70 eV).

277 Helium was used as carrier gas (constant flow 1 ml/min). The mass range was 35 to 400 m/z at a

278 scan time of 1.0 s. The MS source was set to 230 °C and the MS quad set to 150 °C. A standard

279 mix of C8–C20 alkanes was analyzed using the same GC–MS method to calculate the Kovats

280 Retention Indices of apple blossom bud volatiles. These were compared to known values as

281 reported on the NIST website (NIST Chemistry WebBook, 2002). Identification of the detected

282 compounds was based on their relative retention times and their mass spectra in comparison with

283 those observed for pure standard substances. The other compounds were tentatively identified by

284 comparison of mass spectra and retention indices (calculated according to van den Dool & Kratz

285 [1963]) with those recorded in the Adams and NIST mass spectral databases and the previously

286 published data (NIST Chemistry WebBook, 2002; Adams, 2014). The retention indices of

287 individual components were calculated using a series of n-alkenes (C8–C40). Compounds for

288 which proper identification was not possible were defined as "unknown" and included in the

289 analysis. Relative proportions of VOCs were calculated by setting the sum of all selected

290 compounds to 100%. Only compounds unique to plant samples and not found in controls, i.e.,

291 Twisters in empty enclosures, and that had signal-to-noise ratios higher than 10 were retained.

292

293 **Data analysis**

294 Proportion data including infestation rate and sex ratio of *A. pomorum* were modelled using

295 generalized linear models (GLM) with binomial errors by analysis of deviance (categorical

296 explanatory variable: effects of tree species). A quasibinomial error distribution was used if data

297 were overdispersed with residual deviance > 1.2 times greater than residual degrees of freedom

298 (Crawley, 2012).

299   The influence of tree species and weevil sex on weevil body mass was analyzed by non-

300   parametric Kruskal-Wallis test as normal distribution and homoscedasticity of the data could not

301   be confirmed.

302   Differences in weevil body mass between males and females were analyzed by non-parametric

303   Mann-Whitney U test as normal distribution of the data could not be confirmed.

304   The choice of weevils in bioassays was analyzed with one-sample Chi² tests, with counts of

305   individuals choosing apple twigs with blossom buds or the control respectively.

306   Differences in proportions of individual compounds among the different apple species obtained

307   by GC-MS analysis were compared by Mann–Whitney U tests corrected by Benjamini–

308   Hochberg. All data analyses were conducted with R, version 3.3.1 (R Core Team, 2016).

309   Additionally, the chemical dataset was analyzed by a principal component analysis (PCA) (Wold

310   et al., 1987) using the software SIMCA-P, version 14.1 (Umetrics AB, Umeå, Sweden). The

311   PCA was used to compare the volatile patterns of the different *M. sylvestris* genotypes and *M.*

312   *domestica* cultivars with respect to the relative quantities of their volatile compounds (relative to

313   the sum of quantities of all compounds). Data were log-transformed, mean-centered, and scaled

314   to unit variance before being subjected to the analysis. The results of a PCA are usually

315   discussed in terms of the loading plot, which describes the relationships between the variables

316   with regard to the PCs (Eriksson et al., 2001).

317

318   **Results**

319   **Infestation rate by *A. pomorum***

320   Infestation rate of blossom buds was significantly higher in *M. sylvestris* than in *M. domestica*

321   ($F_{1,18} = 6.02$, $p = 0.016$). Likewise, percentage infestation was higher in *M. sylvestris* (10.32 ±

322   3.91 % (SD) infested blossom buds per tree) than in *M. domestica* (5.97 ± 2.26 %) (Figure 1).

323   Infestation rate also varied remarkably between cultivars of *M. domestica* and genotypes of *M.*

324   *sylvestris* (Figure S2) but was not tested statistically as only one individual per cultivar/genotype

325   was assessed (47.2 ± 3.94 (mean ± SD) blossom buds per tree were examined). Percentage

326   infestation was lowest in the *M. domestica* cultivar "Blauacher Wädenswil" (2.1 % infested

327   blossom buds), followed by "Kaiser Wilhelm" (2.5 %). The highest percentage infestation was

328   recorded in two *M. sylvestris* genotypes: "M. syl. 4" (16.7 %) that grew inside the botanical

329   garden, and "Eimersmühle 2" (16.3 %) that grew outside the botanical garden in a riverbank
330   (Figure S2).

331

332   **Weevil body mass and sex ratio**

333   A total of 420 weevils emerged from the capped blossoms (*M. domestica*: 139, *M. sylvestris*:
334   281). An average of 22.1 ± 14.06 (mean ± SD) weevils emerged from each study tree. Tree
335   species and weevil sex both had a significant effect on weevil body mass (Kruskal-Wallis test;
336   tree species: $\chi^2 = 10.30$, p = 0.0013; weevil sex: $\chi^2 = 220.81$, p < 0.001). Weevil body mass
337   differed significantly between male and female weevils (Mann-Whitney U test; U = 75055, r =
338   0.40, p < 0.001), with females being on average 69.6 % heavier than males. The average body
339   mass of weevils emerging from *M. sylvestris* (mean ± sd; males: 0.8 ± 0.24 mg, females: 1.45 ±
340   0.36 mg) was 15.2 % higher than the mass of those emerging from *M. domestica* (males: 0.78 ±
341   0.25 mg, females: 1.23 ± 0.4 mg) (Figure 2).
342   Tree cultivar/genotype also had a significant effect on weevil mass ($F_{1,18} = 13.39$, p < 0.001).
343   The heaviest weevils were those emerging from *M. sylvestris* genotype "Lochau 4" (males: 1.12
344   ± 0.44 mg, females: 1.95 ± 0.22 mg) and *M. domestica* cultivar "James Grieve" (males: 1.45 ±
345   0.16 mg, females: 1.86 ± 0.17 mg). The lightest weevils emerged from *M. domestica* cultivars
346   "Kaiser Wilhelm" (males: 0.60 mg, females: 0.82 mg) and "Hauxapfel" (males: 0.64 ± 0.10 mg,
347   females: 0.88 ± 0.07 mg) (Figure S3).
348   Sex ratio was balanced. The percentage of females was higher in *M. sylvestris* (53 % of 281
349   weevils) than in *M. domestica* (47 % of 139 weevils) but no significant effect of tree species on
350   sex ratio was detected ($F_{1,17} = 1.46$, p = 0.23).

351

352   **Olfactory bioassays**

353   Responsiveness, meaning the percentage of weevils that made a choice for either odor source,
354   was high throughout all experiments that involved a treatment in the form of apple twigs with
355   blossom buds (56-62 %). When no blossom buds were tested and weevils could only choose
356   between the wire control and a blank, the responsiveness was lowest (23.3 %) (Figure 3).
357   Weevils that were tested in bioassays for their preference for volatiles from apple blossom buds
358   versus controls, significantly preferred *M. sylvestris* ($\chi^2 = 6.53$; p = 0.011), but not *M. domestica*
359   over the controls ($\chi^2 = 0.57$; p = 0.45). Likewise, weevils that could choose directly between

360   odors from *M. domestica* and *M. sylvestris*, significantly preferred *M. sylvestris* (χ^2 = 3.90; p =

361   0.048). When the controls were tested against blanks to assure that they did neither have an

362   attracting nor a repellent effect on weevils, responsiveness was lowest, and weevils did not prefer

363   one over the other (χ^2 = 0.14; p = 0.71) (Figure 3).

364

365   **Analysis of headspace VOCs**

366   Most compounds were emitted in higher relative amounts from blossom buds of *M. domestica*

367   cultivars compared to *M. sylvestris* genotypes (Table 2). Acetophenone was the most emitted

368   compound from blossom buds of *M. domestica* (25.5 %) and *M. sylvestris* (24.0 %), followed by

369   pyridine (*M. domestica*: 12.2 %, *M. sylvestris*: 10.7 %). There were significant differences for

370   phenylacetonitrile (emitted only from *M. domestica*), germacrene D and (-)-bornyl acetate (and

371   five unidentified compounds) and tentative differences for α-cadinene and γ-elemene. Linalool,

372   cis-3-hexenyl isovalerate, and benzaldehyde were tentatively emitted in higher amounts from

373   blossom buds of *M. sylvestris* (Table 2).

374   When comparing the blossom bud volatiles of *M. domestica* cultivars "Jonagold" and "Topaz" to

375   those of *M. sylvestris* genotype "Eimersmühle 2" that showed highly different infestation rates in

376   the field (see Figure S2), linalool was emitted in 4-5-fold higher amounts from "Eimersmühle 2"

377   blossom buds (10.8 %) compared to "Jonagold" and "Topaz" (2.4 %) (Table S3). Also, 1-

378   pentadecene and γ-cadinene were emitted from *M. sylvestris* genotype "Eimersmühle 2" in

379   higher amounts (1.5 %) compared to other genotypes/cultivars. Comparisons between VOCs

380   emitted by blossom buds of similarly infested *M. domestica* cultivars "Jonagold"/"Topaz" and

381   *M. sylvestris* genotype "Lochau 1" did not reveal significant differences in their volatile bouquet

382   (Table S3). However, similarly infested *M. domestica* cultivars "Jonagold"/"Topaz" and *M.

383   sylvestris* genotype "Lochau 1" all emitted higher relative amounts of acetophenone, pyridine,

384   and nonane compared to the more infested "Eimersmühle 2" (Table S3).

385   The PCA (Figure 4) explained only a total of 28.2 % of the variance of the data with 17.0 % by

386   the first and 11.2 % by the second principal component. The score plot (Figure 4a) of the relative

387   amount of volatile compounds emitted by the two different apple species shows that the *M.

388   domestica* cultivars and the *M. sylvestris* genotypes cannot be separated with respect to the

389   relative amount of volatile compounds in total. While the *M. domestica* cultivar "Golden

390   Delicious" or the *M. sylvestris* genotype "Lochau 1" build clusters, others do not cluster together

391   based on their identified blossom bud volatiles. In the loading plot, variables not explaining the

392   separation of the clusters are generally located toward the zero origin and the more important

393   variables are located at the periphery of the plot (Figure 4b). The locations of sesquiterpenes like

394   α-cadinene, δ-cadinene, and germacrene D on the top of PC2 indicate important variables that

395   are separating "Golden Delicious" plants and other *M. domestica* cultivars from the cluster of *M.*

396   *sylvestris* genotypes, which are characterized by linalool emission.

397

Discussion

399   A few studies have been published so far that examine the infestation rate and resistance of

400   different cultivars of *M. domestica* to the apple blossom weevil *A. pomorum* (Kalinová et al.,

401   2000; Mody et al., 2011; Mody et al., 2015). However, to our knowledge, only one study has

402   previously addressed the susceptibility and suitability of different *Malus* species, including the

403   European crab apple *M. sylvestris*, to *A. pomorum* (Knuff et al. 2017). In terms of olfactory host

404   plant choice of *A. pomorum*, *M. sylvestris* as the only apple species native to central Europe has

405   not been investigated at all.

406   The results of our comparative study confirm the findings of Knuff et al. (2017) that female *A.*

407   *pomorum* show specific host selection patterns among *Malus* species, which is indicated by

408   significant differences in infestation rates between *M. domestica* and *M. sylvestris*. The European

409   crab apple showed significantly higher infestation by *A. pomorum* than the domesticated apple.

410   Differences in weevil mass confirm the previous findings that the two *Malus* species vary in their

411   suitability as hosts (Knuff et al. 2017). However, in contrast to the results of Knuff et al. (2017),

412   weevils emerging from *M. domestica* were significantly lighter than those originating from *M.*

413   *sylvestris*. These results imply that *M. domestica* blossom buds offer decreased suitability for *A.*

414   *pomorum*, meaning a higher antibiosis resistance (Mody et al. 2015) compared to *M. sylvestris*.

415   In addition, trees of *M. domestica* also proved less attractive to *A. pomorum*, meaning a higher

416   antixenosis resistance (Mody et al. 2015), as indicated by both less attractive odors of blossom

417   buds in bioassays and a lower infestation rate compared to *M. sylvestris*. The analysis of

418   headspace VOCs revealed differences in the blossom bud odors separating a group of *M.*

419   *sylvestris* genotypes from *M. domestica* and another group of *M. sylvestris* genotypes which

420   might be employed in further studies to repel *A. pomorum* from *M. domestica*.

421

Infestation rate

As stated above, there were significant differences in infestation rate by *A. pomorum* between *M. domestica* and *M. sylvestris*. Showing that crab apple trees were significantly more infested than *M. domestica* trees, our study provides new evidence in support of earlier observations by Knuff et al. (2017). Infestation rate also varied remarkably between cultivars of *M. domestica*, as was already reported by other studies (Kalinová et al., 2000; Mody et al., 2011; Mody et al., 2015). Overall percentage infestation of *M. domestica* was 5.97 %, indicating that weevil populations in the Ecological-Botanical Garden of the University of Bayreuth were below the economic threshold level set at 10-15 %, above which pest control is advised to prevent yield losses (Höhn & Stäubli, 1989). Trees of *M. sylvestris* showed an overall percentage infestation of 10.23 %. Although economic threshold levels do not apply for wild apple species, it is nevertheless interesting to notice that weevil populations on *M. sylvestris* trees reached the economic threshold level set for *M. domestica*.

The high infestation rate of *M. sylvestris* is remarkable, since wild apple individuals did not grow on a meadow surrounded by other apple trees like the *M. domestica* individuals used in this study. Except for the two individuals inside the botanical garden, *M. sylvestris* individuals grew in hedgerows, riverbanks and forest margins in the surroundings of Bayreuth where host density was much smaller compared to the apple orchard in the botanical garden. However, there are no indications that *A. pomorum* populations were generally lower inside the botanical garden than outside, as one of the *M. sylvestris* individuals inside the botanical garden was the most infested tree in this study ("M. syl. 4": 16.67 % infested blossom buds).

What also makes the high infestation rate of *M. sylvestris* remarkable is the notion that domesticated crop plants are generally assumed to be more heavily infested by pest insects than their wild relatives due to tradeoffs between growth and defense against herbivores (Strauss et al., 2002; Stoeckli et al., 2009; Kempel et al., 2011; Stoeckli et al., 2011; Huot et al., 2014). Implementation of effective defense mechanisms imposes a substantial demand for resources, which has been suggested to reduce growth (Hout et al., 2014). However, domesticated crop plants like *M. domestica* are bred for high yield and fruits that are larger and sweeter than those of their wild relatives, which requires an increased resource allocation to growth processes (Strauss et al., 2002; Kaplan et al., 2009; Stoeckli et al., 2011). This tradeoff mechanism usually reduces antiherbivore defense in domesticated crop plants making them more susceptible to

453   herbivores (Koricheva, 2002; Strauss et al., 2002; Kaplan et al., 2009; Kempel et al., 2011). This

454   mechanism was not observed in our study, as illustrated by the higher infestation rate of *M.*

455   *sylvestris* trees compared to *M. domestica*. But what could explain the higher susceptibility of *M.*

456   *sylvestris* trees to *A. pomorum*?

457   Distance to hedgerows is apparently not a significant factor for the lower infestation rate among

458   *M. domestica* trees as the two *M. domestica* trees closest to the hedgerow in the south, namely

459   the individuals "Gala" and "Jonathan type 'Watson'" were not among the most infested *M.*

460   *domestica* trees. Temperature and site-dependent effects could mostly be ruled out since *M.*

461   *sylvestris* trees inside and outside of the botanical garden were both on average more infested

462   than the *M. domestica* trees inside the botanical garden. Therefore, it seems that *M. sylvestris*

463   trees possess certain traits that attract *A. pomorum* and guide females in search of their host plant.

464   Since *A. pomorum* is attracted by blossom buds, the traits of these buds have previously been

465   pointed out to be particularly promising for explaining differences in susceptibility to *A.*

466   *pomorum* (Mody et al., 2015).

467   The finding that tree species affected infestation rates indicates that there is at least one species-

468   dependent variable that has not been investigated (Knuff et al., 2017). This variable could be the

469   composition of headspace VOCs emitted by the blossom buds. VOCs have been described as a

470   promising trait mediating the preference of host-searching female weevils (Mody et al., 2015), as

471   they may provide leading cues for females in search of a suitable oviposition site (Piskorski &

472   Dorn, 2010; Collatz & Dorn, 2013; Mody et al., 2015).

473

474   **Weevil body mass and sex ratio**

475   Weevils emerging from *M. sylvestris* had a significantly higher body mass than those originating

476   from *M. domestica*, indicating a higher suitability of *M. sylvestris* for the weevil offspring.

477   According to the preference-performance hypothesis, this finding fits quite well to the higher

478   infestation rate of *M. sylvestris* observed in this study. Knuff et al. (2017), in contrast, did not

479   detect significant differences in body mass of weevil offspring originating from *M. sylvestris* or

480   *M. domestica*. In our study, significant differences in weevil mass were also identified between

481   male and female weevils for both tree species. Female weevils emerging from the blossom buds

482   had a higher body mass than males. This female-biased sexual size dimorphism (SSD) was

483   consistent with results of previous studies (Mody et al., 2015; Knuff et al., 2017). We also

484   detected significant differences in weevil mass between single cultivars of *M. domestica* or

485   genotypes of *M. sylvestris*, which is consistent with the findings of Mody et al. (2015) who

486   reported significant differences in body mass of weevils that originated from different cultivars

487   of *M. domestica*. This confirms the notion that cultivars of *M. domestica* that differ in their

488   nutritional composition also differ in their suitability for *A. pomorum* (Mody et al., 2015). The

489   same might be true for different genotypes of *M. sylvestris*.

490   Preference-performance relationships are regularly expected, although they are not necessarily

491   detected in nature (Mody et al., 2015), probably due to imperfect adaptations, imperfect

492   decisions and a prolonged decision time especially in polyphagous herbivore species (Bernays,

493   2001; Gripenberg et al., 2010; Mody et al., 2015). This study showed that *M. sylvestris* that is

494   native to Central Europe and offers a higher nitrogen content in its blossom buds compared to *M.*

495   *domestica* (Knuff et al., 2017), was significantly more infested by the apple blossom weevil,

496   indicating a higher preference of *A. pomorum* for this species. Furthermore, weevil offspring

497   developing within *M. sylvestris* blossom buds had a significantly higher body mass than weevils

498   developing within *M. domestica* blossom buds, indicating also a better performance of *A.*

499   *pomorum* larvae on *M. sylvestris*. Hence, this study confirms the findings of Mody et al. (2015)

500   that preference-performance relationships may play a role in infestation of *Malus* cultivars by the

501   oligophagous weevil *A. pomorum* and indicates that this is also true across different *Malus*

502   species.

503

504   **Olfactory orientation**

505   This study represents the first examination of the behavioral response of apple blossom weevils

506   to the headspace VOCs of *M. sylvestris* blossom buds. It has been shown in previous studies that

507   apple blossom weevils were attracted to the odor of *M. domestica* blossom buds, and therefore

508   most likely rely on olfactory cues to locate their host plant (Piskorski & Dorn, 2010; Collatz &

509   Dorn, 2013). However, behavioral response to the odor of blossom buds of wild apple species

510   has not been tested yet. Composition of headspace VOCs emitted by the blossom buds might

511   explain the higher infestation rate by *A. pomorum* of *M. sylvestris* in comparison to *M. domestica*

512   that was observed in this study.

513   The olfactory response of field-collected apple blossom weevils to headspace VOCs of both

514   *Malus* species showed that VOCs emitted from blossom buds of *M. sylvestris* were significantly

515   more attractive to *A. pomorum* than VOCs emitted from *M. domestica* blossom buds. This

516   finding was obtained when both *Malus* species were tested against each other in the same setup,

517   but also when blossom buds of each apple species were tested against the wire controls. When

518   controls were tested against blanks, results showed that controls had neither an attractant nor a

519   repellent effect on weevils. Responsiveness, meaning the percentage of weevils making a choice

520   in the dual-choice bioassays, was sufficiently high throughout the experiments, and at a similar

521   level to that reported for other herbivores responding to natural samples or synthetic blends of

522   host plant-derived odors (Prokopy et al., 1995; Piñero & Dorn, 2007; Collatz & Dorn, 2013).

523   VOCs emitted from prebloom *M. domestica* blossom buds that attracted *A. pomorum* have been

524   identified (Piskorski & Dorn, 2010), and bioassays with nature-identical synthetic compounds

525   have been successfully conducted (Collatz & Dorn, 2013). The finding of the present study, that

526   VOCs emitted from *M. sylvestris* blossom buds were significantly more attractive than those

527   emitted from *M. domestica*, might favor the successful use of *M. sylvestris*-derived synthetic

528   volatiles in monitoring and management of *A. pomorum*. It might also offer the opportunity to

529   use *M. sylvestris* as trap crop in apple plantations when establishing push-pull systems.

530

531   **Headspace VOCs**

532   Prior to the testing of *M. sylvestris*- or *M. domestica*-derived synthetic volatiles for monitoring or

533   management of *A. pomorum* populations in commercial apple orchards, VOCs have to be

534   identified that are responsible for the increased attractive effect of *M. sylvestris* blossom buds or

535   the decreased attractivity of *M. domestica* ones. Although a complex blend of volatiles

536   comprised of 16 VOCs from *M. domestica* blossom buds emitted in a suitable stage for

537   oviposition by *A. pomorum* has already been described (Piskorski & Dorn, 2010), knowledge on

538   VOCs from apple blossom buds released before bloom is still scarce. Therefore, the chemical

539   spectrum of VOCs from *M. sylvestris* blossom buds released before bloom has been analyzed

540   here for the first time. We are aware that the results of the VOC analysis cannot readily be

541   employed to explain the results from the field trials as the potted trees were much younger than

542   the study trees in the botanical garden and in field sites which could influence their VOC profile.

543   The fact that some cultivars/genotypes were used in the sampling of VOCs that have not been

544   used for the field trials might also weaken the connection between the results of the VOC

545 analysis and the field results. However, the results do serve as an indication of which VOCs

546 might be of interest and further study is required.

547 It has been shown for many curculionids that they regularly rely on olfactory cues for host

548 detection, among them close relatives of the apple blossom weevil, e. g. the strawberry blossom

549 weevil *Anthonomus rubi* Herbst (Bichão et al. 2005), or the boll weevil *Anthonomus grandis*

550 Boheman (Minyard et al., 1969; Dickens, 1989). As *A. grandis* can become a devastating pest of

551 cotton, it is by far the best-studied species of the genus *Anthonomus* (Collatz & Dorn, 2013).

552 Minyard et al. (1969) reported attractant effects of cotton-square VOCs in bioassays conducted

553 with *A. grandis*, as well as attractant effects of several single compounds identified in the blend,

554 including the ubiquitous plant volatile β-caryophyllene.

555 Furthermore, it has recently been shown for two other species of curculionids, the plum curculio

556 *Conotrachelus nenuphar* Herbst and the vine weevil *Otiorhynchus sulcatus* Fabricius, that traps,

557 which were moderately effective became more powerful in attracting the target weevils after the

558 addition of host-plant-derived VOCs (Leskey et al., 2005; van Tol et al., 2012). A similar effect

559 was reported for *A. grandis* when components of host-plant odor were added to traps baited with

560 aggregation pheromones (Dickens, 1989). However, as pheromones of *A. pomorum* are unknown

561 so far (Dorn & Piñero, 2009), host plant-derived VOCs are of increased relevance for the

562 development of monitoring or management tools for this herbivore. Olfactory attraction to odor

563 traps baited with a synthetic blend of host plant-derived VOCs holds the potential to substantially

564 increase the number of captured individuals and might lead to an improved monitoring system

565 (Collatz & Dorn, 2013).

566 Since headspace VOCs have been shown to differ among cultivars of *M. domestica* (Kalinová et

567 al., 2000; Hern & Dorn, 2003), they can be expected to differ even more among *Malus* species

568 and may provide leading cues for females in search of a suitable oviposition site (Piskorski &

569 Dorn, 2010; Collatz & Dorn, 2013; Mody et al., 2015).

570 The present study is the first that compares headspace VOCs of blossom buds of different *Malus*

571 species before bloom and presents the first report of headspace VOCs of *M. sylvestris* sampled at

572 early phenological stages. This enables a comparison between headspace VOCs of *M. sylvestris*

573 and *M. domestica*. The collection of headspace VOCs from both apple species using stir bar

574 sorptive extraction (SBSE) with Twisters, followed by thermal desorption and GC-MS analysis,

575 allowed tentative identification of a total of 47 volatile compounds, while 30 compounds

576 remained unidentified. Methodological differences likely account for a deviation from
577 compounds identified by Piskorski & Dorn (2010) in *M. domestica*. For example, in the present
578 study we sampled more buds at later bud stages and at higher temperatures with a different
579 technique and adsorbent.
580 The principal component analysis (PCA) revealed no clear separation of *M. domestica* cultivars
581 and *M. sylvestris* genotypes based on blossom bud volatiles (Figure 4). While plants from a few
582 genotypes and cultivars cluster together with respect to their chemical profiles, the high variation
583 of the composition of emitted compounds within other genotypes and cultivars conceals a clear
584 pattern. Another reason for the variation might be slight differences in the phenology of the
585 blossom buds. The chromatographic analysis of bud emanations from different phenological
586 stages in the same cultivar revealed both quantitative and qualitative differences (Kalinova et al.,
587 2000).
588 Acetophenone, the most-emitted compound of both *Malus* species, was also found in blossom
589 buds or flowers of the wild Italian red apple (Fraternale et al., 2014), strawberries (Mozūraitis et
590 al., 2020), or red clover (Buttery et al., 1984). Acetophenone has a repellent effect on
591 woodwasps, possibly preventing them from ovipositing in Mongolian pine infested by non-
592 symbiotic fungi (Wang et al., 2019). Acetophenone can repel *Drosophila suzukii* from
593 raspberries (Renkema & Smith, 2020) and can even have insecticidal effects (Dettner et al.,
594 1992). Pyridine, the second most-emitted plant volatile from the blossom buds has rarely been
595 detected from plant odors, but it was reported for grape leaves (Giacomuzzi et al., 2017),
596 bearberry plants (Radulović et al., 2010), and floral volatiles of *Eriotheca longitubulosa*
597 (MacFarlane et al., 2003) and is known to attract flies and thrips (Lyczko et al., 2021).
598 Most compounds were emitted in higher relative amounts from *M. domestica*. Phenylacetonitrile
599 was emitted only from *M. domestica* and there were significant differences for germacrene D, (-
600)-bornyl acetate and tentative differences for α-cadinene and γ-elemene. Phenylacetonitrile is
601 known from apple tree volatiles induced by caterpillar feeding and attracting conspecific
602 herbivores and a generalist predator (El-Sayed et al., 2018). The sesquiterpenes germacrene D, α-
603 cadinene, and γ-elemene emitted from *M. domestica* flower buds are known from emissions of
604 different plants and can repel or kill insects at higher concentrations (Benelli et al., 2018; Tholl,
605 2015). Germacrene D and (-)-bornyl acetate are known from apple tree flower emissions and
606 elicit responses in herbivores (Bengtson et al., 2001; Buchbauer et al., 1993; Yaqin & Shixiang,

607 2021). Linalool was tentatively emitted in higher relative amounts from blossom buds of all

608 genotypes of *M. sylvestris* and 4-5 times more from genotype "Eimersmühle 2" (10.8 %). It

609 might have been even more detectable since the relative amounts of most other compounds were

610 reduced. Linalool is known to be a main volatile (43 %) of flower buds of red apples attracting

611 pollinators (Fraternale et al., 2014). Linalool is also present in higher amounts in flower buds of

612 cultivar "Royal Gala" apples, possibly providing protection of the reproductive organ against

613 oxidative stress and pathogenic microbes (Nieuwenhuizen et al., 2013). According to

614 Nieuwenhuizen et al. (2013) its production may thus be maintained under a positive selective

615 pressure in wild apples, while the small number of terpenes found in modern cultivars may be

616 related to commercial breeding strategies.

617

Conclusions

619 This study confirmed the hypothesis that *A. pomorum*, an economically relevant pest insect in

620 European apple orchards, shows specific host selection patterns among *Malus* species.

621 Infestation rate of *A. pomorum* was significantly higher in the wild European crab apple *M.*

622 *sylvestris*, indicating a preference of female weevils searching for a suitable host tree for

623 oviposition. Furthermore, a higher average mass of weevils originating from *M. sylvestris*

624 implies a higher suitability of the native *M. sylvestris* for the apple blossom weevil. These two

625 findings combined strongly support the idea that preference-performance relationships might

626 play a role for *A. pomorum*.

627 Olfactory bioassays conducted with blossom buds of both *Malus* species showed the weevils'

628 preference for the complex odor of *M. sylvestris* blossom buds over VOCs emitted from blossom

629 buds of *M. domestica*. This finding may explain the higher infestation rate of *M. sylvestris* trees

630 in the field and may therefore represent the missing variable accounting for an increased

631 susceptibility of *M. sylvestris* trees compared to *M. domestica*.

632 Therefore, an analysis of headspace VOCs emitted from blossom buds of both *Malus* species

633 before bloom was conducted for *M. sylvestris* for the first time and candidate compounds for

634 further study have been identified.

635 Olfactory attraction of apple blossom weevils in combination with other tools holds the potential

636 to substantially increase the number of *A. pomorum* captured in shelter traps and might therefore

637 constitute an improved monitoring system. Since pheromones are still unknown for this

638   economically relevant pest insect (Dorn & Piñero, 2009), host plant-derived VOCs of *M.*

639   *sylvestris* and *M. domestica* blossom buds represent promising cues in a multisensorial

640   monitoring tool.

641

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649

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Figure 1

Infestation rates (%) of blossom buds of *Malus domestica* and *M. sylvestris*.

Different lowercase letters (a, b) indicate statistically significant differences (analysis of deviance; $F_{1,18}$ = 6.02, p = 0.016). Number of sampled trees: *M. domestica*: 10, *M. sylvestris*: 10. Number of infested/sampled buds: *M. domestica*: 28/469 (5.97 ± 2.26% (mean ± SD) infested), *M. sylvestris*: 49/475 (10.32 ± 3.91% infested).

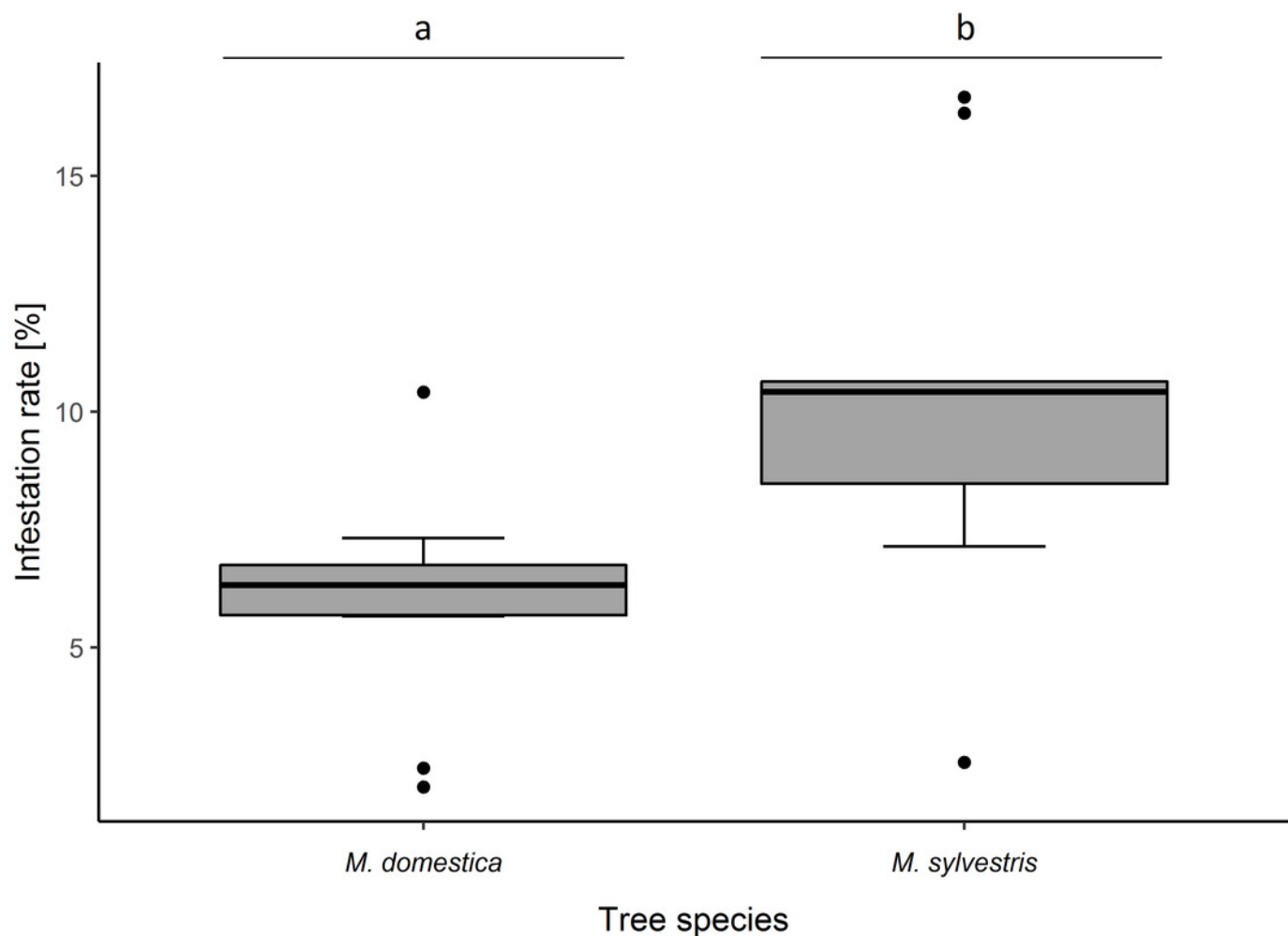



Figure 2

Dry body mass (mg) of male and female *Anthonomus pomorum* emerging from *Malus domestica* and *M. sylvestris*.

Different lowercase letters (a, b) indicate statistically significant differences (Kruskal-Wallis test; χ^2 = 10.30, p = 0.0013). Number of sampled weevils: *M. domestica*: 139 (males: 74, females: 65), *M. sylvestris*: 281 (males: 132, females: 149).

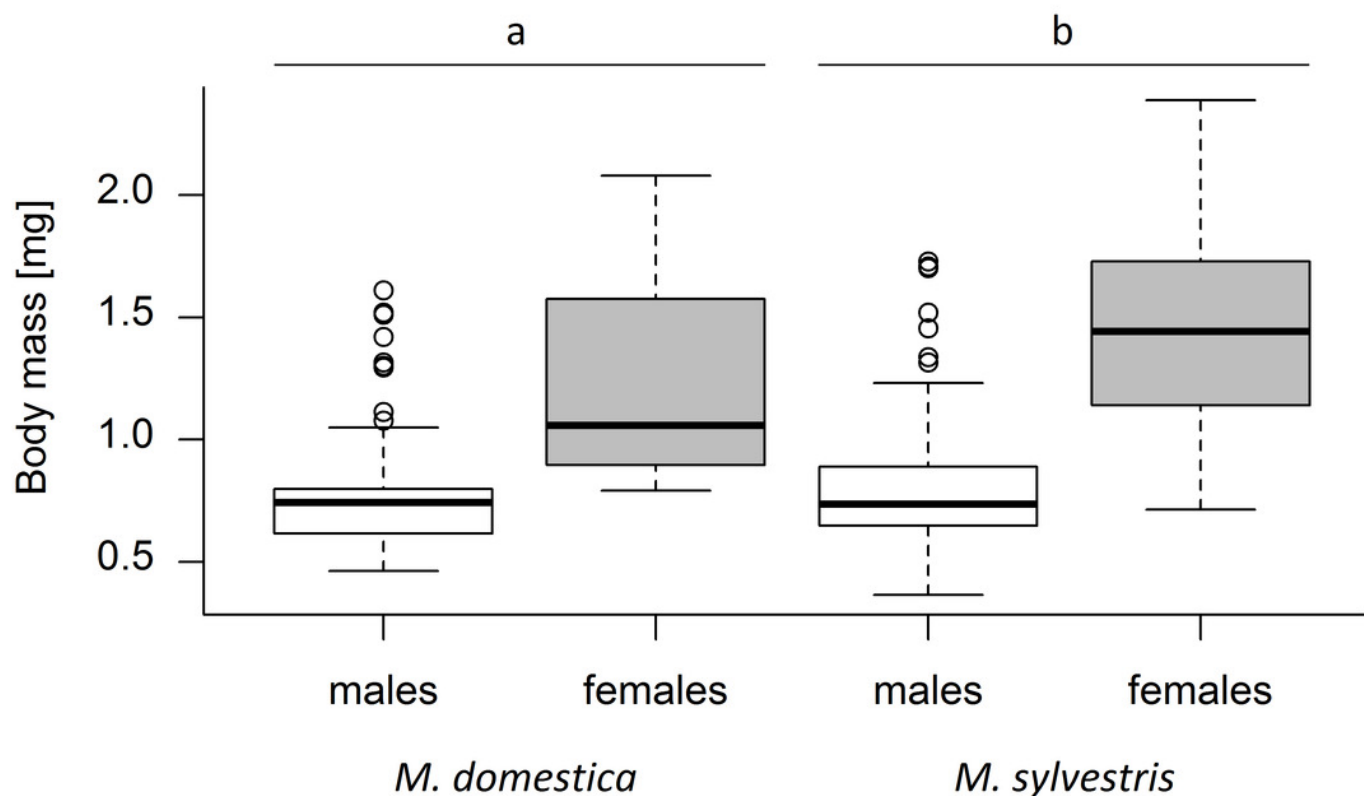



Figure 3

Response of field-collected *Anthonomus pomorum* in still-air, dual-choice olfactory bioassays to the odor of blossom buds of *Malus domestica* and *M. sylvestris.*

Both apple species were tested against each other, against controls as well as controls vs. blank. Sample sizes: n = 50, except for "controls vs. blank": n = 30. χ^2 test: * p < 0.05.

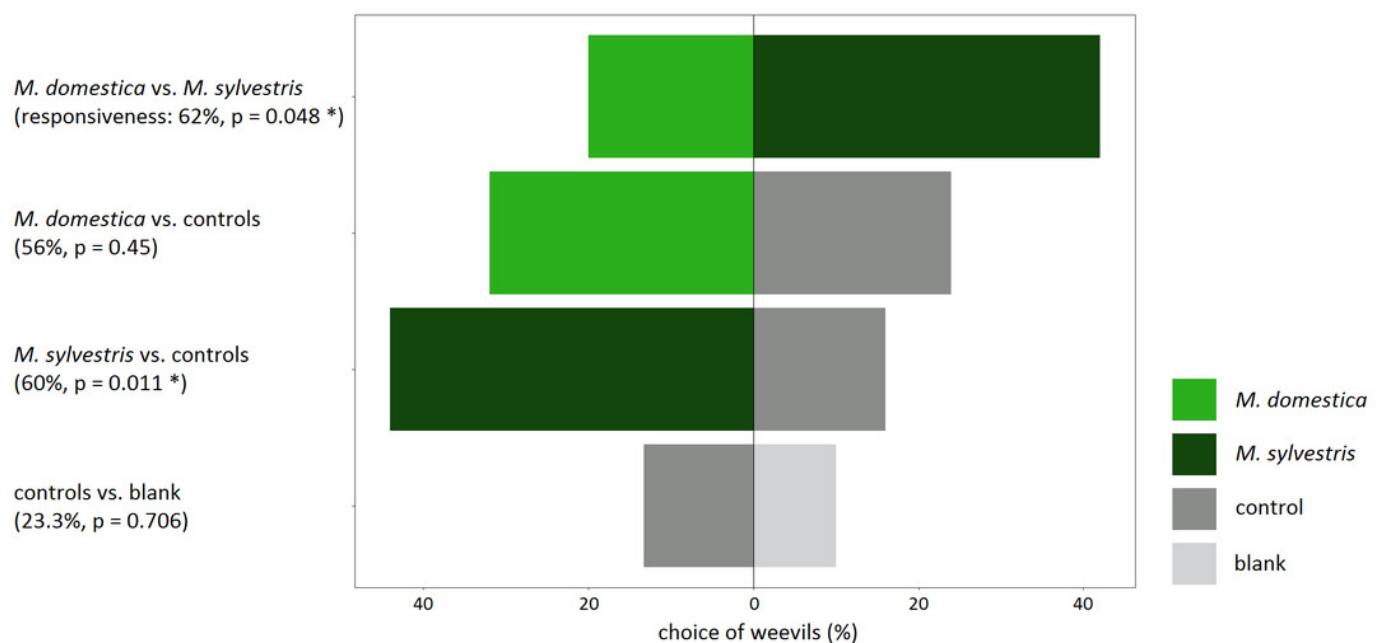
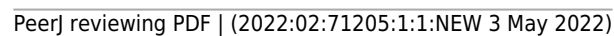



Figure 4

Principal components analysis (PCA) of the headspace VOCs emitted from blossom buds of *Malus domestica* and *M. sylvestris.*

(a) Score plot of the relative amounts of VOCs emitted from blossom buds of *M. domestica* (green) and *M. sylvestris* (blue). (b) Loading plot of the relative amount of VOCs emitted from blossom buds of *M. domestica* and *M. sylvestris.*

a

b

Numbers of infested "capped blossoms" that were collected from *Malus domestica* and *M. sylvestris* trees and numbers of weevils that emerged from the "capped blossoms".

Numbers of "capped blossoms" per tree: 33.3 ± 15.8 (mean ± SD). Discrepancies between number of collected blossoms and total number of emerged weevils are due to parasitoids and weevils that could not complete their development.

Tree species	Number of collected "capped blossoms"	Total number of emerged weevils (males/females)
M. domestica	282	139 (74/65)
M. sylvestris	384	281 (132/149)

Variation of the phytochemical compositions (%) of blossom bud headspace VOCs among *Malus sylvestris* and *M. domestica*.

N	Components	GR[a]	RI[b]	Malus sylvestris		Malus domestica		
				mean	SD	mean	SD	P
1	Pyridine	AR	<800	9.02	10.82	12.85	12.24	n.s. (0.09)
2	Nonane	A	900	2.81	5.05	3.75	4.79	n.s. (0.08)
3	α-Pinene	MT	931	1.04	2.71	1.43	2.44	n.s.
4	β-Myrcene	MT	990	0.73	1.29	0.98	1.22	n.s.
5	Hexanoic acid	FA	992	0.40	0.82	0.27	0.51	n.s.
6	U1		993	0.07	0.22	0.20	0.33	n.s.
7	U2		999	0.87	2.39	0.16	0.31	n.s.
8	α-Phellandrene	MT	1000	0.07	0.22	0.41	0.91	n.s.
9	(Z)-3-Hexen-1-yl acetate	E	1005	2.57	2.98	1.82	2.66	n.s.
10	p-Cymene	ARMT	1021	0.20	0.56	0.34	0.56	n.s.
11	D-Limonene	MT	1024	0.28	0.78	0.39	0.67	n.s.
12	2-Ethyl-1-hexanol	OH	1028	0.48	0.61	0.54	0.48	n.s.
13	Propyl tiglate	E	1034	1.14	1.44	1.42	1.42	n.s.
14	(E)- β-Ocimene	MT	1047	0.84	1.21	1.87	3.53	n.s.
15	γ-Terpinene	MT	1056	0.11	0.41	0.17	0.34	n.s.
16	Acetophenone	AR	1061	18.11	7.92	19.19	7.10	*
17	Linalool	OMT	1099	5.64	5.68	2.61	1.87	n.s.
18	Nonanal	Al	1100	1.25	1.06	1.01	0.58	n.s.
19	2-Phenylethanol	AR	1110	0.20	0.38	0.33	0.44	n.s.
20	(E)-4,8-Dimethylnona-1,3,7-triene	HT	1116	0.52	0.96	0.61	1.49	n.s.
21	Phenylacetonitrile	AR	1134	0.00	0.00	0.18	0.49	n.s.
22	3,3-Dimethylheptanoic acid	FA	1148	0.33	0.53	0.72	1.19	n.s.
23	p-Cymen-8-ol	MT	1182	0.26	0.34	0.23	0.40	n.s.
24	Methyl Salicylate	AR	1190	0.46	0.57	0.52	0.71	n.s.
25	Hexyl butanoate	E	1192	0.14	0.24	0.27	0.35	n.s.
26	Ethyl octanoate	E	1197	0.05	0.14	0.11	0.18	n.s.
27	Dodecane	Al	1200	0.32	0.51	0.25	0.33	n.s.
28	Decanal	Al	1204	2.27	2.39	1.65	1.36	n.s.
29	β-Phenoxyethanol	OH	1218	2.47	2.92	1.63	1.31	n.s.
30	cis-3-Hexenyl isovalerate	E	1232	0.08	0.22	0.04	0.14	n.s.
31	U3		1271	0.50	0.29	0.37	0.28	n.s.
32	U4		1276	0.09	0.20	0.02	0.10	n.s.
33	U5		1280	0.31	0.50	0.60	0.79	*
34	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-	E	1283	0.08	0.25	0.02	0.10	n.s.
35	Tridecane	A	1300	2.32	2.73	2.75	3.39	n.s.
36	U7		1302	0.12	0.26	0.09	0.32	n.s.
37	U8		1305	0.29	0.71	0.32	0.65	n.s.
38	U9		1313	0.09	0.28	0.18	0.55	n.s.
39	U10		1319	0.13	0.42	0.22	0.47	n.s.
40	U11		1323	0.08	0.20	0.14	0.37	n.s.
41	U12		1327	0.88	0.84	1.10	1.55	n.s.
42	γ-Elemene	ST	1335	0.51	1.15	0.58	1.40	n.s.
43	U13		1342	0.02	0.09	0.09	0.17	n.s.
44	U14		1343	0.39	0.95	0.49	1.07	n.s.
45	U15		1346	0.28	0.91	0.38	0.98	n.s.
46	U16		1354	0.12	0.25	0.24	0.58	n.s.
47	U17		1361	6.02	10.40	7.20	10.93	*
48	U18		1364	0.32	1.10	0.25	0.65	n.s.
49	3-Methyl-tridecane	A	1370	0.11	0.25	0.07	0.22	n.s.
50	ß-Bourbonene	ST	1384	0.77	1.75	0.40	0.68	n.s.
51	U19		1387	1.65	2.75	1.69	2.17	*
52	U20		1394	1.06	1.94	0.95	1.32	n.s.
53	Dodecanal	Al	1407	3.72	4.13	1.46	1.28	n.s.
54	U21		1426	0.05	0.19	0.30	0.91	n.s.
55	ß-Copaene	ST	1446	0.93	2.27	0.33	0.52	n.s.
56	U22		1448	0.90	0.96	0.69	0.81	n.s.

2 [a] GR = group of chemical compounds (A alkane; AR aromatic compound; Al aldehyde; ARMT aromatic monoterpene; E ester; FA fatty acid
3 conjugate; K ketone; MMT monocyclic monoterpenoid; MT monoterpene; OH alcohol; OMT oxygenated monoterpene; ST sesquiterpenoid; HT
4 homoterpene)

5 [b] RI = retention index (DB5- fused silica capillary column 30 m× 0.25 mm i.d., 0.25 μm film thickness) experimentally determined using a
6 homologue series of n-alkanes.

N	Components	RI^a	RI^b	*Malus sylvestris*		*Malus domestica*		
				mean	SD	mean	SD	P
57	Geranyl Acetone	K	1450	0.88	1.78	0.60	1.11	n.s.
58	U23		1451	0.43	0.90	0.86	1.45	n.s.
59	p-Benzoquinone, 2,6-di-tert-butyl-	MMT	1464	0.61	0.54	0.38	0.33	n.s.
60	U24		1474	1.79	6.50	0.64	1.65	n.s.
61	U25		1474	0.45	1.20	0.83	1.67	n.s.
62	γ-Muurolene	ST	1475	0.74	2.89	0.17	0.25	n.s.
63	U26		1478	0.47	0.52	0.37	0.56	n.s.
64	Germacrene D	ST	1479	2.73	4.87	1.91	2.36	n.s.
65	1-Pentadecene	AE	1491	0.59	1.60	0.47	0.86	n.s.
66	Bicyclogermacrene	ST	1495	0.36	0.80	0.49	1.45	n.s.
67	U27		1495	1.40	3.06	1.30	2.10	n.s.
68	Pentadecane	A	1500	0.94	1.24	0.87	0.73	n.s.
69	α-Farnesene	ST	1508	6.16	7.05	6.39	11.54	n.s.
70	γ-Cadinene	ST	1512	0.19	0.38	0.21	0.34	n.s.
71	δ-Cadinene	ST	1523	0.27	0.44	0.30	0.35	n.s.
72	α-Cadinene	ST	1536	0.85	1.54	1.09	2.08	n.s.
73	U28		1541	1.69	3.81	1.71	2.74	*
74	U29		1549	0.41	1.40	0.43	0.91	n.s.
75	U30	K	1552	0.55	1.78	0.63	1.15	*

9 ^{a}GR = group of chemical compounds (A alkane; AE alkene; K ketone; MMT monocyclic monoterpenoid; ST sesquiterpenoid)

10 ^{b}RI = retention index (DB5- fused silica capillary column 30 m × 0.25 mm i.d., 0.25 μm film thickness) experimentally determined using a
11 homologue series of n-alkanes