

ORIGINAL CONTRIBUTION

Differential susceptibility and suitability of domestic and wild apple species for a florivorous weevil and its parasitoidsA. K. Knuff¹, E. Obermaier¹ & K. Mody²¹ Ecological-Botanical Garden, University of Bayreuth, Bayreuth, Germany² Ecological Networks, Department of Biology, Technical University Darmstadt, Darmstadt, Germany**Keywords**

Anthonomus pomorum, apple resistance to herbivores, *Bracon intercessor*, *Malus kirghisorum*, plant–herbivore–parasitoid interactions, *Pteromalus varians*, *Scambus pomorum*

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Received: March 2, 2016; accepted: May 26, 2016.

doi: 10.1111/jen.12341

Abstract

Crop plant domestication can change plant resistance to herbivores leading to differences in pest pressure experienced by crop plants and their wild relatives. To compare resistance to herbivores between domesticated and wild fruit trees, we quantified direct resistance and indirect resistance to a pest insect, the florivorous apple blossom weevil *Anthonomus pomorum* (Coleoptera: Curculionidae), for the cultivated apple *Malus domestica* and two wild apple species, the European crab apple *M. sylvestris* and the exotic *M. kirghisorum*. We measured weevil infestation and performance (weight, sex ratio), and weevil parasitism by parasitoid wasps for different cultivars of *M. domestica* and for the two wild apple species. To explain weevil and parasitoid responses to different apple species, we quantified tree characteristics including nitrogen content, size of flower buds, bark roughness, tree size, tree phenology and tree position. We found significant differences in susceptibility to weevil infestation between apple species, with lowest infestation (highest apple resistance) in *M. domestica* and highest infestation in *M. kirghisorum*. The suitability of apple species also varied significantly: weevils emerging from *M. sylvestris* were significantly lighter than those from *M. kirghisorum*. Parasitism of *A. pomorum* by different parasitoid species was significantly higher in *M. sylvestris* than in *M. domestica*. Infestation, weevil weight and parasitism were positively related to tree characteristics: infestation to bud nitrogen content and bark roughness, weevil size to nitrogen content and bud size, and parasitism to tree height and bud density. Our study revealed marked differences between apple species in susceptibility and suitability for the pest herbivore, but also for antagonistic parasitoids. Whereas direct resistance appeared to be higher in cultivated apple, indirect resistance via parasitoids was apparently higher in wild apple trees. Our findings suggest that wild and cultivated apple trees possess different resistance traits that may be combined to optimize resistance in commercial apple cultivars.

Introduction

The domestic apple *Malus domestica* Borkhausen belongs to the most important fruit crops worldwide (FAOStat, 2013). Evidently, considerable yield losses in such a high-value crop due to diseases or pests have to be prevented for economic reasons, which is mostly achieved by application of crop protection chemicals

in conventional farming systems (Cooper and Dobson 2007). However, the demand for alternative means of control is increasing for several reasons (Matson et al. 1997; Simon et al. 2010). These include negative effects of pesticides on human health, biodiversity and ecosystem processes (Tilman 1999; Alavanja et al. 2004) such as the reduction of biocontrol agents (Theiling and Croft 1988). Additionally, their

effectiveness is reduced due to development of pesticide resistance in pest species (Reyes et al. 2007; Grigg-McGuffin et al. 2015).

The use of resistant cultivars that are less susceptible to herbivores (direct resistance) or that are supporting biocontrol agents (indirect resistance) is a promising alternative approach for pest control (Wearing et al. 2003; Hogmire and Miller 2005; Smith and Clement 2012). Concerning common diseases such as scab (caused by *Venturia inaequalis*), fire blight (*Erwinia amylovora*) or mildew (*Podosphaera leucotricha*), cultivars expressing resistance have already been employed successfully, substantially reducing fungicide applications (Fischer and Fischer 2002). Considering resistance to herbivores, however, hardly any appropriate cultivars are commercially available (Hogmire and Miller 2005; Stoeckli et al. 2011), although several studies have addressed resistance of different apple cultivars to arthropod herbivores including the apple blossom weevil *Anthonomus pomorum* (Curculionidae) (Kalinová et al. 2000; Mody et al. 2015), several aphid species (Aphididae) (Miñarro and Dapena 2007; Stoeckli et al. 2008a) and the codling moth *Cydia pomonella* (Tortricidae) (Goonewardene et al. 1975, 1979; Hogmire and Miller 2005; Stoeckli et al. 2008b, 2009a). Furthermore, genes and quantitative trait loci (QTLs) for pest resistance in apple were identified for future marker-assisted breeding of resistant cultivars (Roche et al. 1997; Wearing et al. 2003; Bus et al. 2008; Stoeckli et al. 2008c, 2009a,b).

Breeding constraints are a major reason for the limited availability of herbivore-resistant tree cultivars. Firstly, host–herbivore relationships are complex and often still poorly understood, which challenges identification of heritable phenotypic traits that putatively drive resistance (Henery 2011). Secondly, a wide range of herbivore species is involved with specific requirements that would have to be combined to achieve general tree resistance (Hogmire and Miller 2005). And lastly, additional external factors including soil type and climate may affect tree resistance (Stoeckli et al. 2008a), rendering predictions concerning herbivore resistance difficult to achieve (Henery 2011). Despite these difficulties to breed herbivore-resistant cultivars, it is nevertheless of outstanding importance to better understand the underlying mechanisms driving tree resistance to herbivores to develop strategies for a more sustainable apple production.

To identify putative tree characteristics that may influence direct or indirect apple resistance to pest herbivores, the identification of herbivore preferences

for oviposition and differences in suitability among *Malus* species may be enlightening. The gained insights can be analysed in the context of the preference–performance hypothesis. This hypothesis is based on the assumption that larval development of herbivorous insects depends on the food quality of their host plant and that females should evolve an ability to identify and choose the best host for their offspring (Jaenike 1978; Thompson 1988; Gripenberg et al. 2010). In this context, studies on the apple blossom weevil *A. pomorum* may be particularly insightful as this herbivore is closely linked to apple trees. Therefore, it assumedly shows marked preference and performance responses to apple tree characteristics (Kalinová et al. 2000; Piskorski and Dorn 2010; Mody et al. 2015).

Anthonomus pomorum feeds on *Malus* spp. and *Pyrus* spp. (Troitzky 1928; Duan et al. 1998; Toepfer et al. 1999a), and it is quite common all over Europe (Čtvrtečka and Žďárek 1992; Daniel et al. 2005). It used to be a minor pest (Brown et al. 1993; Toepfer et al. 1999b), but with the application of more selective instead of broad-spectrum insecticides, populations can increase to damaging levels (Toepfer et al. 1999a,b; Sipos and Marko 2014), predominantly in years with high population densities and/or low bud set (Brown et al. 1993; Blommers 1994; Mody et al. 2011). The weevil causes damage in apple orchards because its larvae feed on the blossom buds (Gratwick 1992; Duan et al. 1998). Economic consequences can be disastrous (Toepfer et al. 1999a) because far more than half of the blossom buds can be destroyed, with reports varying between 60% and close to 100% (Babel 1931; Toepfer et al. 1999a; Bajec et al. 2013). Whereas numerous studies addressed different aspects of the biology of *A. pomorum* (Čtvrtečka and Žďárek 1992; Duan et al. 1996, 1998; Toepfer et al. 1999a,b, 2000, 2002; Kalinová et al. 2000; Hausmann et al. 2004a,b, 2005; Piskorski and Dorn 2010; Collatz and Dorn 2013), little is known about the criteria of host selection by females with regard to tree characteristics that contribute either directly to resistance to this herbivore via herbivore preference or performance (Mody et al. 2015), or indirectly via differential exposure to natural enemies such as parasitoids (Mody et al. 2011, 2012).

Besides *M. domestica*, *A. pomorum* likely infests also other species in the genus *Malus*, for example *M. sylvestris* (L.) Mill. and *M. kirghisorum* Al. Fed. & Fed. (syn. *M. sieversii* subsp. *kirghisorum* (Al. Fed. & Fed.) Likhonos) (Rheinheimer and Hassler 2013) although information on the herbivore fauna of those wild apple species is apparently lacking (A. Knuff,

personal observation; Mody 2013). *Malus kirghisorum* naturally occurs in the Tian Shan region in Central Asia (Forsline et al. 2003). It is considered a main progenitor of the cultivated apple that originates from the same region (Forsline et al. 2003; Velasco et al. 2010; Cornille et al. 2012) while *M. sylvestris* is indigenous to the Eurasian and sub-Mediterranean region (Robinson et al. 2001; Reim et al. 2012). It is a rare and vulnerable species (LfU 2003) that grows in open forest habitats and in hedgerows (Stephan et al. 2003; Aas 2013) and that easily hybridizes with *M. domestica* (Wagner et al. 2004; Velasco et al. 2010; Reim et al. 2013).

Considering apple resistance to *A. pomorum*, the present study addressed the following questions: (i) whether the apple species *M. domestica*, *M. sylvestris* and *M. kirghisorum* differ in susceptibility (infestation) to *A. pomorum*; (ii) whether observed infestation patterns can be related to tree characteristics that may act as resistance traits (Smith 2005; Miñarro and Dapena 2007); (iii) whether the different apple species varied in suitability to *A. pomorum*, which was measured by weevil performance (weight, sex ratio) and by its putative exposure to parasitoids (parasitism rate between apple species); and (iv) whether observed patterns of susceptibility and suitability follow the preference–performance hypothesis.

Materials and Methods

Study site

Our study was conducted in the Ecological-Botanical Garden of the University of Bayreuth (49°55'N, 11°35'E, elevation 355 m) and at field sites in the Bayreuth region in 2015. Three apple species were investigated for their infestation by *A. pomorum*: *M. domestica*, *M. sylvestris* and *M. kirghisorum*. Studied *M. domestica* trees grew in the botanical garden on an orchard meadow with scattered fruit trees in rows spaced at 8 × 6 m. The orchard contained 98 high-stem apple trees from 81 different cultivars and interspersed *Pyrus* and *Prunus* trees. Twenty *M. domestica* trees (grafted) belonging to 19 cultivars (Table S1) and ranging in age between 11 and 18 years were randomly selected by drawing lots. Six specimens of *M. sylvestris* trees (age approximately 20 years) were grown from seedlings in the botanical garden. Seven further individuals grew at field sites in the Bayreuth region in natural habitats including riverbanks, hedgerows and forest margins (Table S1). The four trees of *M. kirghisorum* (age approximately 30 years) were also grown from seedlings and integrated in the

planting of the botanical garden (Table S1). Neither pesticides nor fungicides were applied to trees in the botanical garden and at field sites.

Temperature was measured every hour at each site with thermobuttons (Maxim Integrated Products, Inc.: ThermoChron® iButton® DS1921G) from beginning of March until end of April during main weevil activity (Gratwick 1992; Duan et al. 1996). As weevil activity is reduced at temperatures below 5°C (Duan et al. 1996; Toepfer et al. 1999a), potential differences in temperature between sites might influence infestation and parasitization (Niedermayer et al. 2013).

Infestation assessment and sampling of infested flower buds

Infestation by *A. pomorum* was assessed shortly after full flowering from mid-May to end of May by counting the number of infested (recognizable by brownish, dried petals forming a hollow body, so-called 'capped blossoms') and not-infested blossoms of ten blossom clusters on five branches. Branches that were evenly distributed over the treetop had been randomly selected and marked before bud burst (Brown et al. 1993).

As many infested flower buds as possible were collected from all over the treetop (on average 38 buds, up to a maximum of 200 buds per tree) after infestation assessment (table 1). To ensure that larval development had already sufficiently progressed for full imago development in the laboratory at time of collection, the pupation rate was assessed. This was conducted at each sampling day for one studied tree by opening additional 20–30 buds, which were excluded from further analyses; 52–90% of the weevils had already reached the pupal stage. Capped blossoms were kept in transparent plastic containers with openings in the lids covered by insect-proof gauze allowing for ventilation. They were equipped with paper towels

Table 1 Numbers of collected blossoms and weighed weevils for each *Malus* species. Discrepancies between number of collected blossoms and total number of emerged weevils are due to parasitoids and weevils that did not complete their development

Tree species	Number of collected blossoms	Total number of emerged weevils (males/females)	Number of weighed weevils (males/females)
<i>M. domestica</i>	440	172/150	161/150
<i>M. sylvestris</i>	525	151/178	106/114
<i>M. kirghisorum</i>	242	94/89	73/69

for moisture absorption and kept inside the laboratory at approximately 22°C.

Weevil characteristics

After emergence, weevils and their hymenopteran parasitoids were deep-frozen and stored in Eppendorf tubes at -14°C. The sex of all properly emerged weevils was determined based on morphology of the last dorsal abdominal plate (Duan et al. 1999). All available beetles (maximum 20 individuals per tree and sex, see table 1) were dried at 40°C to mass constancy and weighed (Sartorius MC210P OCE high-precision scale).

Parasitism rate was determined as proportion of parasitoids from the total number of emerged and unhatched (obtained by dissecting blossoms after emergence period) weevils and hymenopteran parasitoids. Parasitoids belonged to three species in three families: *Scambus pomorum* Ratzeburg (Ichneumonidae), *Bracon intercessor* Nees (Braconidae) and *Pteromalus varians* Spinola (Pteromalidae). They parasitize the larvae of *A. pomorum* inside the capped blossoms (Zijp and Blommers 1992, 2002; Tóth et al. 2006; Mody et al. 2011; Žikić et al. 2012; Rheinheimer and Hassler 2013). For statistical purposes, no distinction was made between them.

Tree characteristics

Different tree characteristics were recorded to assess their influence on infestation rate, weight and sex ratio of the weevils and their parasitism rate. Tree height, DBH (diameter at breast height 130 cm above ground level) and canopy volume (estimated as a rotation ellipsoid with the height of the tree crown as major axis and its diameter as minor axis according to Hausmann et al. (2004a)) were measured to describe tree size (Table S1). Distance to hedgerows was recorded as hedgerows or forests may serve as overwintering sites for *A. pomorum* imagoes (Brown et al. 1993; Toepfer et al. 1999a; but see Hausmann et al. 2004a; Mody et al. 2011). Tree bark structure (bark roughness), which may represent shelter and hibernation sites for the weevils (Troitzky 1928; Toepfer et al. 2000), was recorded in three categories: (i) smooth bark, (ii) partially rough bark (mostly in the lower parts of the trunk) and (iii) rough bark (along the whole trunk). Bud density, which was suggested to influence host preference of *A. pomorum* by Brown et al. (1993) and Kalinová et al. (2000), was determined following Brown et al. (1993) as number of blossom clusters at short shoots per metre branch

multiplied by the average number of blossoms per cluster as derived from 10 clusters. Data from four branch sections totalling one metre length were averaged for each tree. Branching was assessed as number of long and short shoots per metre, averaged over the same four branch sections that were also examined for bud density. Bud size was measured as maximum bud width with a digital calliper. Buds were collected at BBCH stages 56 of 57 (Meier 2001) when they are suitable for oviposition by *A. pomorum* (Gratwick 1992) from trees of *M. domestica* and *M. sylvestris*. Values from lateral and central buds were averaged for each tree according to their occurrence on the respective tree. Data are missing for *M. kirghisorum* trees and three *M. sylvestris* trees. Additional flower buds at stages 56 of 57 were dried to mass constancy at 60°C and grinded in a mixer mill (Retsch, Type MM2, item number 20.713.0001) with 50 rev/s for 1 min to examine their nitrogen content (mg N/g dry weight) via elementary analysis (Flash EA 1112 ThermoQuest). Phenology of blossom buds was assessed for *M. domestica*, *M. kirghisorum* and *M. sylvestris* inside the botanical garden by determining the bud stages according to the BBCH scale (Meier 2001) at weekly intervals. Bud stages of *M. sylvestris* trees outside the botanical garden were controlled once when *M. sylvestris* trees inside the botanical garden had reached bud stages suitable for oviposition. For data analysis, bud stages at this date were considered.

Data analysis

To assess influence of tree species (categorical explanatory variable) on proportion data including infestation rate, sex ratio of *A. pomorum* and parasitism rate by hymenopteran parasitoids, we used analysis of deviance by generalized linear models (GLM) with binomial error distribution followed by Tukey's post hoc tests. A quasibinomial error-structure was chosen if data were overdispersed with residual deviance more than 1.2 times greater than residual degrees of freedom (Crawley 2008). The influence of tree species and weevil sex on weevil weight was analysed by two-way ANOVA as normal distribution and homoscedasticity of the data were confirmed. Weevil weight was the average weight of all individuals per tree and sex. Effects of bud density, bud size, branching, nitrogen content, bark structure, distance to nearest hedgerow, tree height, DBH and tree species (categorical and continuous explanatory variables) on infestation rate, sex ratio and parasitism rate were modelled by ANCOVA (GLM with binomial or quasibinomial error distribution) followed by Tukey's

post hoc tests. Effects of these variables on weevil mass were modelled using a linear model (ANCOVA). As several variables were correlated with each other (Table S2), they were not included simultaneously in the same maximal model for reasons of collinearity (Crawley 2008; Zuur et al. 2010). Variables were rather assigned to different groups that did not contain any correlating variables (group 1: tree species, nitrogen content, bud density, tree height and bud size; group 2: bark structure, branching, DBH and hedgerow distance). The two variable groups were fitted separately to each response variable, and the models were simplified until a minimal adequate model containing only significant variables was obtained. These significant variables from both groups were fitted to the response variable to obtain one comprehensive model. This procedure was applied to two data sets: the first data set contained all 36 trees without the parameter bud size. Bud size was included in the second data set, from which *M. kirghisorum* and three *M. sylvestris* trees were excluded, for which no data on bud size were available.

To assess the relationship between the variables in the minimal adequate comprehensive model and the respective response variable, different GLMs were applied. The effect of bud stage on infestation was tested by analysis of deviance followed by Tukey's post hoc test. Regression with binomial errors was conducted to analyse the effect of nitrogen content, bud density and tree height on infestation, sex ratio and parasitism rate. Correlation between bud size/nitrogen content and weevil weight was determined via nonparametric Spearman's rank correlation. Differences in tree height, bud density, nitrogen content and bud size among tree species were investigated by nonparametric

Kruskal–Wallis test followed by Dunn's post hoc test, if appropriate, or by Wilcoxon rank-sum test if only two groups were compared. Mean daily temperature was compared among different tree sites using Kruskal–Wallis test. Nonparametric tests were used when assumptions of parametric tests in terms of normal distribution and variance homogeneity were not met. All data analyses were conducted with R, version 3.1.1 (R Core Team 2016).

Results

Temperature characterization of sites

There were no differences in temperature among any tree sites neither inside nor outside the botanical garden (Kruskal–Wallis test; $\chi^2 = 11.02$, d.f. = 14, $P = 0.670$), with median temperature of daily mean ranging between 5.1°C and 6.5°C.

Characterization of *Malus* species

Trees of *M. domestica* were significantly smaller than trees of *M. sylvestris* and *M. kirghisorum* ($\chi^2 = 23.47$, d.f. = 2, $P < 0.001$; Dunn's post hoc $P < 0.05$) (Table S1). Bud density did not differ between any tree species ($\chi^2 = 1.89$, d.f. = 2, $P = 0.389$) (table 2). Nitrogen content of flower buds at BBCH stages 56 of 57 was significantly higher in *M. sylvestris* than in *M. domestica* and in *M. kirghisorum*, whereas there was no difference between the latter two ($\chi^2 = 19.06$, d.f. = 2, $P < 0.001$; Dunn's post hoc $P < 0.05$) (table 2). Bud size did not differ between the tested *M. domestica* (min–max; mean: 3.3–4.4 mm; 3.8 mm) and *M. sylvestris* trees (3.0–4.0 mm; 3.6 mm) (Wilcoxon rank-sum test; $W = 514$, $P = 0.075$) (table 2). The bud stages 56 of 57, which are suitable for oviposition, occurred on most *M. domestica* trees 1 week later than on *M. kirghisorum* and *M. sylvestris* trees (table 3).

Infestation rate by *A. pomorum*

Flower infestation by *A. pomorum* was significantly different between all three *Malus* species (analysis of deviance; $F_{2,36} = 14.80$, $P < 0.001$). Average infestation rate was lowest in *M. domestica* (min–max; mean: 0–13%; 5% of 4154 blossoms), intermediate in *M. sylvestris* (0–25%; 9%; 2389) and highest in *M. kirghisorum* (21–38%; 25%; 567) (fig. 1). No difference in infestation was detected between *M. sylvestris* trees growing inside or outside the botanical garden ($F_{1,12} = 1.13$, $P = 0.310$). Their average

Table 2 Characterization of studied *Malus* trees. Minimum–maximum/mean (median). Bud density was measured as mean number of blossoms per metre branch for each tree. n.a.: not available. Mean values marked with different superscript letters (a, b) indicate statistically significant differences. n.s.: not significant

Tree species	Bud density	Nitrogen content (mg/g dry weight)	Bud size (mm)
<i>M. domestica</i>	9–141/68.3 ^{n.s.} (65.5)	34.3–60.2/42.1 ^a (42.0)	3.3–4.4/3.8 ^{n.s.} (3.8)
<i>M. sylvestris</i>	8–117/62 ^{n.s.} (56)	40.6–51.1/46.9 ^b (48.0)	2.9–4.2/3.6 ^{n.s.} (3.7)
<i>M. kirghisorum</i>	38–55/46.5 ^{n.s.} (46.5)	38.8–44.1/40.6 ^a (39.7)	n.a.

Table 3 Phenology of flower buds. Bud stages were determined according to the BBCH scale (Meier 2001) at weekly intervals. Bud stages 56 of 57, which are suitable for oviposition, are highlighted in bold. *M. sylvestris* 7–13 are outside the botanical garden, and their bud stages were evaluated only once

Tree individual	Cultivar/species	Proportion of infested blossoms	Date			
			09.04	20.04	28.05	07.05
1	Brauner Matapfel	0.13	52	56	59	65
2	Finkenwerder Prinzenapfel	0.00	52	54	56	59
3	Gala	0.04	53	55	56	63
4	Gelber Edelapfel	0.06	52	54	56	57
5	Goldrenette Freiherr von Berlepsch	0.01	53	55	57	64
6	Grahams Jubiläumsapfel	0.02	52	55	57	63
7	Hibernal	0.02	53	56	59	64
8	Jakob Fischer	0.03	53	55	57	65
9	Jonagold 1	0.07	52	54	57	64
10	Jonagold 2	0.07	52	55	56	64
11	Jonathan Watson	0.07	52	55	57	62
12	Maunzenapfel	0.01	52	54	56	64
13	Öhringen	0.05	53	55	56	61
14	Roter Boskoop	0.05	53	56	59	65
15	Roter Eiserapfel	0.03	52	55	57	65
16	Schneiderapfel	0.04	53	55	57	65
17	Summerred	0.03	52	54	59	65
18	Topaz	0.05	53	55	57	64
19	Trierer Weinapfel	0.09	52	55	57	64
20	Weißer Winterglockenapfel	0.07	52	54	56	63
21	<i>M. sylvestris</i> 1	0.00	53	56	59	65
22	<i>M. sylvestris</i> 2	0.00	53	57	59	65
23	<i>M. sylvestris</i> 3	0.08	53	56	59	65
24	<i>M. sylvestris</i> 4	0.18	53	56	59	65
25	<i>M. sylvestris</i> 5	0.06	53	55	59	65
26	<i>M. sylvestris</i> 6	0.01	53	56	59	65
27	<i>M. sylvestris</i> 7	0.04	n.a.	56	n.a.	n.a.
28	<i>M. sylvestris</i> 8	0.13	n.a.	56	n.a.	n.a.
29	<i>M. sylvestris</i> 9	0.05	n.a.	57	n.a.	n.a.
30	<i>M. sylvestris</i> 10	0.16	n.a.	57	n.a.	n.a.
31	<i>M. sylvestris</i> 11	0.10	n.a.	57	n.a.	n.a.
32	<i>M. sylvestris</i> 12	0.25	n.a.	57	n.a.	n.a.
33	<i>M. sylvestris</i> 13	0.00	n.a.	57	n.a.	n.a.
34	<i>M. kirghisorum</i> 1	0.21	54	57	59	67
35	<i>M. kirghisorum</i> 2	0.28	54	57	59	67
36	<i>M. kirghisorum</i> 3	0.38	54	57	59	67
37	<i>M. kirghisorum</i> 4	0.22	54	57	60	67

n.a. = not available.

infestation was 6% and 11%, respectively. Infestation of apple blossoms was influenced by tree species ($F_{2,36} = 4.57$, $P = 0.019$), bud stage ($F_{3,36} = 8.85$, $P < 0.001$) and nitrogen content of blossom buds ($F_{1,36} = 4.76$, $P = 0.037$). Infestation was significantly higher on trees with flower buds at the latest stage 57 than at earlier stages 54 or 55 ($F_{3,36} = 7.09$, $P < 0.001$; Tukey's post hoc: 57–54: $P = 0.008$; 57–55:

$P < 0.001$). There was no difference in infestation between trees with bud stages 57 or 56, which are both suitable for oviposition (57–56: $P = 0.061$). When the effect of nitrogen content on infestation was examined separately, no effect was detectable (GLM with binomial errors; $F_{1,36} = 1.05$, $P = 0.313$). Bud density, tree height, DBH, distance to nearest hedgerow and branching did not show any effect.

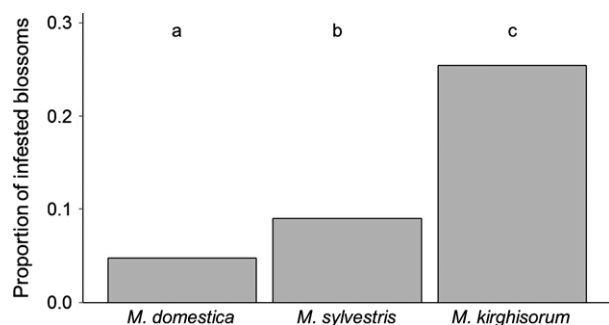


Fig. 1 Proportion of blossoms infested by *A. pomorum* in three apple species. Bars marked with different lowercase letters (a, b, c) indicate statistically significant differences (analysis of deviance with proportion data; Tukey's post hoc $P < 0.05$). Number of sampled trees: *M. domestica*: 20, *M. sylvestris*: 13, *M. kirghisorum*: 4. Number of sampled blossoms: *M. domestica*: 4154, *M. sylvestris*: 2389, *M. kirghisorum*: 567.

In a model that included bud size, only nitrogen content had an effect on infestation (ANCOVA; $F_{1,29} = 6.68$, $P = 0.015$), whereas bud density, tree height, DBH, hedgerow distance, branching, tree species, bark structure and bud size had no effect. There was a positive relationship between nitrogen content and infestation when analysed separately (GLM with binomial errors; $F_{1,28} = 4.52$, $P = 0.043$).

Performance of *A. pomorum*

Weevil weights differed between males and females (two-way ANOVA; $F_{1,55} = 5.79$, $P = 0.020$) with females being on average heavier than males (*M. kirghisorum*: females 0–8%, on average 3% heavier than males; *M. sylvestris*: 1–13%, mean 6%; *M. domestica*: 3–22%, mean 7%). Weevil weights also varied between tree species (two-way ANOVA; $F_{2,55} = 4.27$, $P = 0.019$), namely between *M. kirghisorum* (mean \pm sd: females: 1.04 mg \pm 0.08; males: 1.01 mg \pm 0.05) and *M. sylvestris* (females: 0.95 mg \pm 0.08; males: 0.89 mg \pm 0.08) (Tukey's post hoc; $P = 0.027$). Differences in weight of weevils emerging from *M. domestica* (females: 1.02 mg \pm 0.13; males: 0.95 mg \pm 0.10) were not significant (Tukey's post hoc; *M. domestica*–*M. sylvestris*: $P = 0.057$, *M. domestica*–*M. kirghisorum*: $P = 0.495$) (fig. 2). There was no significant interaction between tree species and weevil sex (two-way ANOVA; $F_{2,55} = 0.14$, $P = 0.867$).

In the model considering tree species, weevil sex, nitrogen content, bud density, bud size, tree height, DBH, bark structure, branching and hedgerow distance as additional explanatory variables, weevil sex and bud size did have an effect on weevil weight (ANCOVA; $F_{2,44} = 8.11$, $P = 0.001$, adjusted $R^2 = 0.236$;

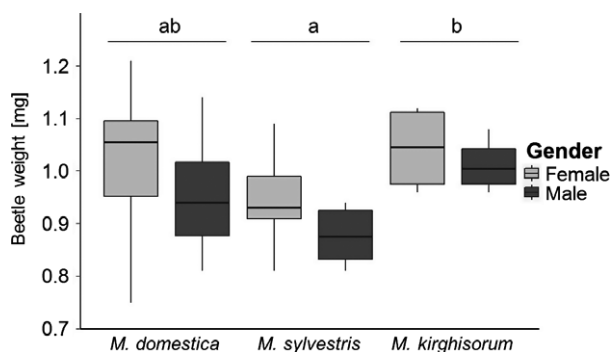


Fig. 2 Weight (mg) of male and female *A. pomorum* originating from different apple species. Boxes marked with different lowercase letters (a, b) indicate statistically significant differences in beetle weight between tree species (two-way ANOVA; Tukey's post hoc $P < 0.05$). Number of sampled trees: *M. domestica*: 18, *M. sylvestris*: 10, *M. kirghisorum*: 4. Number of sampled beetles: *M. domestica*: 161 males, 150 females; *M. sylvestris*: 106 males, 114 females; *M. kirghisorum*: 73 males, 69 females.

sex: $P = 0.021$; bud size: $P = 0.003$). Bud size was positively correlated with weevil weight (Spearman's rank order correlation; $\rho = 0.43$, $P = 0.003$). When bud size was removed from the model, nitrogen content, additionally to weevil sex, became significant (ANCOVA; $F_{2,58} = 6.91$, $P = 0.002$, adjusted $R^2 = 0.165$; sex: $P = 0.015$; nitrogen content: $P = 0.007$). Weevil weight decreased with rising nitrogen content (Spearman's rank correlation; $\rho = -0.31$, $P = 0.017$).

Sex ratio was balanced. Male proportion to total beetle number was highest in *M. domestica* (54% of 311 beetles), followed by *M. kirghisorum* (51% of 142 beetles) and *M. sylvestris* (46% of 220 beetles), without effect of tree species on sex ratio (analysis of deviance; $F_{2,31} = 3.14$, $P = 0.058$). Of all other tested variables (tree species, nitrogen content, bud density, tree height, bud size, bark structure, branching, DBH and hedgerow distance), only nitrogen content had an effect on sex ratio (ANCOVA; model including bud size: $F_{1,24} = 5.86$, $P = 0.024$; model excluding bud size: $F_{1,31} = 4.71$, $P = 0.038$). When the effect of nitrogen content on sex ratio was examined separately, a negative relationship between those two variables was detected (GLM with binomial errors; $F_{1,31} = 4.71$, $P = 0.038$). Accordingly, lowest male proportion was found in *M. sylvestris*, the tree species with highest nitrogen content.

Parasitism rate of *A. pomorum*

Three species of parasitoids from three different hymenopteran families emerged from 171 of the 1207

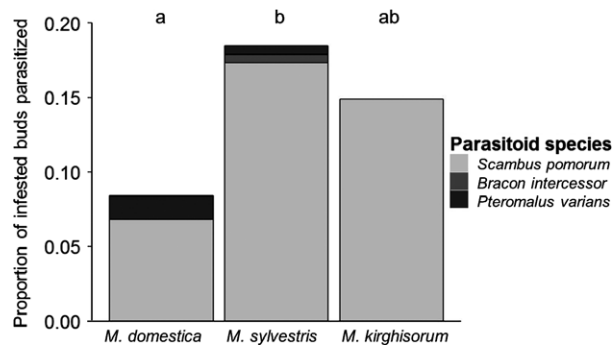


Fig. 3 Proportion of emerged parasitoids to the total number of blossoms that were infested by *A. pomorum*. Bars marked with different lowercase letters (a, b) indicate statistically significant differences (analysis of deviance with proportion data; Tukey's post hoc $P < 0.05$). Total number of parasitoids: *M. domestica*: 37, *M. sylvestris*: 98, *M. kirghisorum*: 36. Number of collected infested blossoms: *M. domestica*: 440, *M. sylvestris*: 525, *M. kirghisorum*: 242.

collected capped blossoms (total parasitism rate = 14%). *S. pomorum* was the most abundant species with 157 individuals (91.8%), followed by *P. varians* with 10 individuals (6.8%) and *B. intercessor* with four individuals (2.3%). All three species were found on *M. sylvestris*, both on trees inside and outside the botanical garden. On *M. domestica*, *S. pomorum* and *P. varians* were found, and on *M. kirghisorum*, only *S. pomorum* was found.

The parasitism rate (all species of parasitoids combined) of *A. pomorum* varied significantly between *M. domestica* and *M. sylvestris* (analysis of deviance; $F_{2,31} = 6.01$, $P = 0.006$). It was lowest in *M. domestica* (min–max; mean: 0–9%; 8% of 440 collected infested blossoms), intermediate in *M. kirghisorum* (9–20%; 15% of 242 collected infested blossoms) and highest in *M. sylvestris* (0–24%; 19% of 525 collected infested blossoms) (fig. 3). No difference in parasitism rate was detected between *M. sylvestris* trees growing inside (24%) or outside (15%) the botanical garden, whereas they both differed significantly from *M. domestica* trees (analysis of deviance; $F_{2,27} = 8.36$, $P = 0.002$; Tukey's post hoc; *M. domestica*–*M. sylvestris* inside: $P < 0.001$, *M. domestica*–*M. sylvestris* outside: $P = 0.050$, *M. sylvestris* inside–outside: $P = 0.155$).

In a model considering tree species, bud size, bud density, nitrogen content, tree height, bark structure, branching, DBH and hedgerow distance as additional variables, tree height (ANCOVA; model including bud size: $F_{1,24} = 16.29$, $P < 0.001$; model excluding bud size: $F_{1,31} = 8.63$, $P = 0.006$) and bud density (model including bud size: $F_{1,24} = 9.43$, $P = 0.006$; model excluding bud size: $F_{1,31} = 6.42$, $P = 0.017$) were found to have an effect on parasitism rate, no matter

if bud size was included in the model. Effect of tree species on parasitism rate was only detected in the model where bud size was included ($F_{1,24} = 6.75$, $P = 0.017$). Separate analyses of the significant variables revealed that tree height and parasitism rate were positively related (GLM with binomial errors; model including bud size: $F_{1,24} = 10.67$, $P = 0.003$; model excluding bud size: $F_{1,31} = 6.93$, $P = 0.013$) whereas there was only a slight but not significant positive relationship between bud density and parasitism (GLM with binomial errors; model including bud size: $F_{1,24} = 1.75$, $P = 0.200$; model excluding bud size: $F_{1,31} = 0.90$, $P = 0.351$).

Discussion

The present study is the first comparative analysis of susceptibility and suitability of three *Malus* species, the cultivated apple *M. domestica*, the indigenous European crab apple *M. sylvestris* and the exotic *M. kirghisorum*, for the florivorous apple blossom weevil *A. pomorum* and some of its parasitoid natural enemies. The studied herbivore is of economic importance in commercial apple orchards but so far there only exist studies on its relation to *M. domestica* (e.g. Brown et al. 1993; Toepfer et al. 1999a; Mody et al. 2015) but not to other *Malus* species.

The results from our study indicate that *Malus* species differ in their direct resistance and indirect resistance to *A. pomorum*. Infestation by *A. pomorum* was lowest in *M. domestica* and highest in *M. kirghisorum*. Furthermore, differences in weevil weight and parasitism rate revealed that the studied *Malus* species vary in their suitability as hosts. Weevils emerging from *M. sylvestris* were significantly lighter than those originating from *M. kirghisorum*, and parasitism rate was markedly higher in *M. sylvestris* than in *M. domestica*.

Infestation rate by *A. pomorum*

Several factors may have contributed to the observed significant differences in infestation by *A. pomorum* between the three *Malus* species. As mean daily temperature was comparable for all tree sites inside and outside the botanical garden, activity of the temperature-dependent weevil (Toepfer et al. 2002; Hausmann et al. 2005) and thusly differences in infestation were probably not affected by temperature.

Infestation levels of *M. domestica* cultivars of 5% are in accordance with reports from some organic and integrated apple orchards. Generally, highly variable infestation rates have been reported: 5.4–15.3%

(Mody et al. 2011), 5–50% (Hausmann et al. 2004c), 0.25–2.4% (Mody et al. 2015), 0–45% (Brown et al. 1993) and 85–100% (Bajec et al. 2013).

In contrast to *M. domestica*, information on infestation by *A. pomorum* is lacking for *M. sylvestris* and *M. kirghisorum*. In fact, it has only been assumed that other *Malus* species also serve as hosts for the weevil due to their close relationship to *M. domestica* (Troitzky 1928; Rheinheimer and Hassler 2013). Thus, the present study provides this evidence for the two studied wild apple species for the first time.

Previous studies already reported that the degree of infestation by *A. pomorum* varies between different cultivars of *M. domestica* (Kalinová et al. 2000; Mody et al. 2011, 2015). Our study now revealed that the overall infestation of the different studied cultivars was lower than infestation of the two studied wild apple species. This finding contradicts the general notion that crop plants are usually more severely infested than their wild relatives because of possible trade-offs between plant growth and defence (Matyssek et al. 2002; Gayler et al. 2004; Stoeckli et al. 2011; Horgan 2012). *M. domestica* was bred for high yield and larger, more tasteful fruits compared to wild apples (Kaplan et al. 2009; Stoeckli et al. 2011; Horgan 2012). This may require an elevated resource allocation to growth processes, which is assumed to be at the expense of antiherbivore defensive tree characteristics (mechanical structures and/or metabolic compounds) resulting in higher susceptibility to herbivores (Koricheva 2002; Gayler et al. 2004; Kaplan et al. 2009).

As studies addressing the balance between growth and defence have not always found evidence for the described trade-off (Koricheva 2002; Gayler et al. 2004), supplementary hypotheses on the role of environmental effects have been developed (Gayler et al. 2004). Yet, Koricheva (2002) states that the lack of ability to detect trade-offs is rather due to methodological reasons than to biological mechanisms. Thus, what could be alternative explanations for the observed infestation pattern?

Site might be relevant as weevil abundance might differ inside and outside the botanical garden and infestation of *M. sylvestris* was, although not significantly, lower inside than outside. Yet, *M. kirghisorum*, the trees with highest infestation also grew inside the botanical garden, rendering site-dependent effects improbable. The neighbourhood of apple trees, however, might also have played a role. Dominance of heterospecific neighbour trees – as was the case for *M. sylvestris* and *M. kirghisorum* growing in mixed vegetation patches in comparison to *M. domestica* growing

accompanied by other fruit trees – may have led to increased associational susceptibility via host dilution effects (Otway et al. 2005; Plath et al. 2012).

Our analyses revealed that tree phenology (bud stage at a given time), nitrogen content of buds during oviposition and tree species were affecting infestation. In general, faster developing trees were more heavily infested than trees whose buds were still at an earlier stage. The heavier infested wild *Malus* species already had buds at stages 56 or 57 while at the same time buds of most *M. domestica* trees were still at stages 54 or 55. Yet, those few *M. domestica* trees which had developed as fast as the wild species were not necessarily those with highest infestation compared to other *M. domestica* trees (table 3). Thus, as the present study only lasted 1 year, the influence of phenology on weevil infestation remains ambiguous. Nitrogen content is regularly assumed to affect herbivore infestation of plants (Mattson 1980; White 1984). However, a positive relationship between nitrogen content and infestation was only detected when data from *M. domestica* and *M. sylvestris* were considered (model including bud size). The lack of a relationship between those variables when data from *M. kirghisorum* were included (model without bud size) indicates that the effect is not unequivocal. This interpretation is supported by the observation that both *M. kirghisorum* and *M. domestica* contained significantly less nitrogen in their buds than *M. sylvestris*, but that they were the tree species with the highest and lowest infestation, respectively. The finding that tree species affected infestation indicates that there was at least one species-dependent variable missing in the model. The succession in which the *Malus* species reached the appropriate bud stage for infestation was complementary to their infestation level. Buds of *M. kirghisorum* and *M. sylvestris* showed the fastest development and had higher infestation whereas *M. domestica* reached the same stage 1 week later and had the lowest infestation. This relation between phenology and infestation was also suggested by Toepfer et al. (2002). The end of hibernation of *A. pomorum* is not synchronized with development of its host, which may result in higher infestations of those trees that exhibit suitable bud stages at the time of main weevil activity independently of their suitability for larval development (Toepfer et al. 2002).

Performance of *A. pomorum*

Weevils emerging from *M. kirghisorum* were significantly heavier than those originating from *M. sylvestris*. Mody et al. (2015) have already reported

differences in weevil weight among different cultivars of *M. domestica*. Average weight of weevils from one cultivar (Rewena; females approximately 1.05 mg, males approximately 0.9 mg) was similar to weevil weight on *M. domestica* measured in the present study (mean weevil weight of females: $1.02 \text{ mg} \pm 0.13$; males: $0.95 \text{ mg} \pm 0.10$), but mean weights across all trees differed substantially between both studies. Even the heaviest weevils in this study, which emerged from *M. kirghisorum* (females $1.04 \text{ mg} \pm 0.08$; males $1.01 \text{ mg} \pm 0.05$), were still notably lighter compared to the average weights reported by Mody et al. (2015) (females: $1.27 \text{ mg} \pm 0.02$; males: $1.15 \text{ mg} \pm 0.02$).

The weevils measured in the present study all came from unmanaged trees whereas Mody et al. (2015) sampled a managed orchard. This suggests that besides cultivar-dependent differences, weevil performance (as indicated by weight) may also differ between managed and unmanaged apple trees. This might be due to general tree vigour (and nutritional quality) as trees in managed orchards are fertilized and pruned to optimize productivity whereas the growth of the examined *M. domestica* trees was not supported by any means.

For each apple species, females were heavier than males. This female-biased sexual size dimorphism was also found by Mody et al. (2015) for weevils emerging from different cultivars of *M. domestica*. Total differences in weight between females and males from different *Malus* species in the present study (females 3–7% heavier than males) were not as pronounced as reported from different *M. domestica* cultivars (6.4–17.5%). However, when only the weights of weevils originating from *M. domestica* trees were considered, variation in weight differences (3–22%) was even broader than reported by Mody et al. (2015).

We found a relationship between weevil weight and bud size or, if bud size was not considered, with bud nitrogen content. Weevil weight rose with bud size, which may reflect resource quantity. As larvae of *A. pomorum* are completely confined to the blossom bud in which they hatch, their final body mass depends on the resources provided by this bud. As bud size was not different between *M. domestica* and *M. sylvestris* trees, it cannot be the decisive factor that is responsible for differences in weevil weight among tree species.

Although a positive association between nitrogen content and herbivore performance is reported for many insects (Mattson 1980; White 1984; Obermaier and Zwölfer 1999), we found the opposite relationship with the lightest weevils emerging from

M. sylvestris, the species with highest nitrogen content in the blossom buds. Concordantly, lowest nitrogen content was found in *M. kirghisorum*, from where the heaviest weevils originated. A negative relationship between abundance or performance of herbivorous insects and plant nitrogen has already been recorded for other herbivores including weevils (Hancock et al. 2013), aphids (Johnson 2008), a butterfly species (Fischer and Fiedler 2000) and grasshoppers (Joern and Behmer 1998; Cease et al. 2012). As Fischer and Fiedler (2000) and Joern and Behmer (1998) suggest, the weevil larvae could have suffered from physiological problems due to an unbalanced nutrient uptake at higher nitrogen contents, which might have resulted in lower weight of the imagoes.

Parasitism rate of *A. pomorum*

Three hymenopteran parasitoids were detected in flower buds infested by *A. pomorum*, the ichneumonid *S. pomorum*, the pteromalid *P. varians* and the braconid *B. intercessor*. The first two species are reported as parasitoids of *A. pomorum* on *M. domestica* by several other studies (Cross et al. 1999 and references therein, Mody et al. 2011), whereas another *Bracon* species (*B. variator* NEEB) was reported from northern Switzerland (Mody et al. 2011).

All three parasitoid species detected in the present study were found on *M. sylvestris*, both on trees inside and outside the botanical garden. *M. domestica* and *M. kirghisorum*, which grew inside the botanical garden, only harboured two or one of the parasitoid species, respectively. Similarly, parasitism rate of *A. pomorum* on *M. domestica* was lower compared to *M. sylvestris* (8% on *M. domestica* compared to 18% on *M. sylvestris* inside and outside the botanical garden). This difference was even more pronounced when only the trees from inside the botanical garden were considered (8% on *M. domestica*, 24% on *M. sylvestris*). Thus, levels of parasitism varied markedly between tree species that both grew in the botanical garden where comparable parasitoid abundances can be expected.

Recorded levels of parasitism were lower in this study than in comparable studies. They ranged from 0.8% to 50% (Cross et al. 1999) and from below 5% to 65% (Mody et al. 2011) as examined on *M. domestica* for parasitism by *S. pomorum*. Parasitism was positively related to tree height, and there was also a positive, yet insignificant, correlation between bud density and parasitism. The smallest trees were those of *M. domestica*, on which the parasitism rate of *A. pomorum* was lower as compared to *M. sylvestris* and

M. kirghisorum. This contradicts the findings of Cloyd and Sadof (2000) who found that the attack rate by a parasitoid decreased with plant size and height of a herbaceous plant as parasitoids can detect their hosts more easily in architecturally less complex canopies (Geitzenauer and Bernays 1996; Casa and Djemai 2002; Obermaier et al. 2008; Randlkofer et al. 2009). Another factor that affects parasitism levels is the density of the respective host (Cloyd and Sadof 2000; Hancock et al. 2013). In these studies, hosts were attacked at higher rates by their parasitoids when host density was higher. This was not explicitly analysed in our study, but considering that *M. domestica* trees were least infested by *A. pomorum*, it can be assumed that lower host density might have contributed to the observed low parasitism rate of *A. pomorum* on *M. domestica* cultivars.

Relationship between preference and performance of *A. pomorum*

Following the preference–performance hypothesis, females of phytophagous species should select suitable hosts for offspring development, especially when the larvae are sessile and cannot switch to better feeding sites, which is the case for *A. pomorum* (Thompson 1988; Gripenberg et al. 2010). Positive preference–performance relationships should be reflected by highest infestation of hosts that are most suitable for larval development (Gripenberg et al. 2010). Considering this hypothesis, our study yielded mixed results. In accordance with the preference–performance hypothesis, the heaviest weevils emerged from *M. kirghisorum*, the most heavily infested tree species. In contrast, the lightest weevils did not emerge from *M. domestica*, the trees with lowest infestation, but from *M. sylvestris*. However, female choice of oviposition sites does not solely depend on the nutritional quality of potential hosts, but other factors including natural enemies (Björkman et al. 1997), competitors (Wise and Weinberg 2002) or mutualists (Atsatt 1981) may also affect female preference.

The low explanatory power of preference–performance relationships for *A. pomorum* may have different reasons. Selection of high quality hosts may be of minor importance for *A. pomorum* as it distributes its eggs individually and thus reduces the value of individual host selection decisions (Mangel 1987; Hopper 1999). In addition, female *A. pomorum* are quite immobile (Toepfer et al. 1999a), which limits their ability to visit several potential hosts before oviposition, possibly resulting in increased infestation of trees that are nearest to overwintering sites, independently

of their suitability. In accordance with this, Brown et al. (1993) and Toepfer et al. (1999a) found that trees at the margins of orchards, which were close to forests or hedgerows where the weevils had overwintered, were most heavily infested (but see Hausmann et al. (2004a) and Mody et al. (2011)). We could not detect similar ‘edge-effects’ in our study which is possibly due to the (semi-)natural surroundings of the examined trees. The weevils might find overwintering sites in the immediate vicinity of the trees which renders weevil colonization from the margins unnecessary.

Conclusion

The findings from our study provide a basis for further investigations on resistance to herbivores of different apple species and cultivars. We compared for the first time the preference and performance of an economically important herbivore of apple (*A. pomorum*) for three *Malus* species (the cultivated apple *M. domestica*, the indigenous European *M. sylvestris* and the exotic *M. kirghisorum*) in order to gain insights into mechanisms underlying apple resistance to herbivores. Our study revealed marked differences between apple species in susceptibility and suitability for the pest herbivore, but also for antagonistic parasitoids. Whereas direct resistance appeared to be higher in cultivated apple, indirect resistance via parasitoids was apparently higher in wild apple trees. Our findings suggest that wild and cultivated apple trees possess different resistance traits that may be combined to optimize resistance in commercial apple cultivars. An enhanced understanding of the observed differences between apple species and its potential use for breeding of resistant apple cultivars will require further research on larger, spatially replicated samples of different *Malus* species and cultivars.

Acknowledgements

We thank Steffen York, Andy Fichte and Christian Popp for their valuable help during fieldwork, the team of gardeners in the botanical garden, especially Guido Arneth, Claus Rupprich, Helmut Zapf and Georg Seidler, for their support, Martin Feulner for showing us field sites with *Malus sylvestris*, Heike Feldhaar, Gunter Ilgen and Gerhard Huber for support with laboratory analyses, Helmut Zwölfer, Matthias Riedel and Michael Haas for identification of the parasitoids and Gregor Aas for helpful comments on the study design. We thank the biodiversity project for

conservation of fruit crops of the government of Upper Franconia for their financial support.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Information on apple trees sampled for infestation by *Anthonomus pomorum*.

Table S2. Correlation matrix between explanatory variables.