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Title:

Aphid infestation induces plant-sex-specific changes in floral chemistry and pollinator behaviour in *Silene latifolia*

Runing Title:

Sex-specific floral responses to aphid infestation

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Highlight:

Aphid infestation alters multiple visual and chemical floral traits in a plant sex-specific manner, leading to reduced attractiveness to moth pollinators in female plants, but not in males.

Abstract:

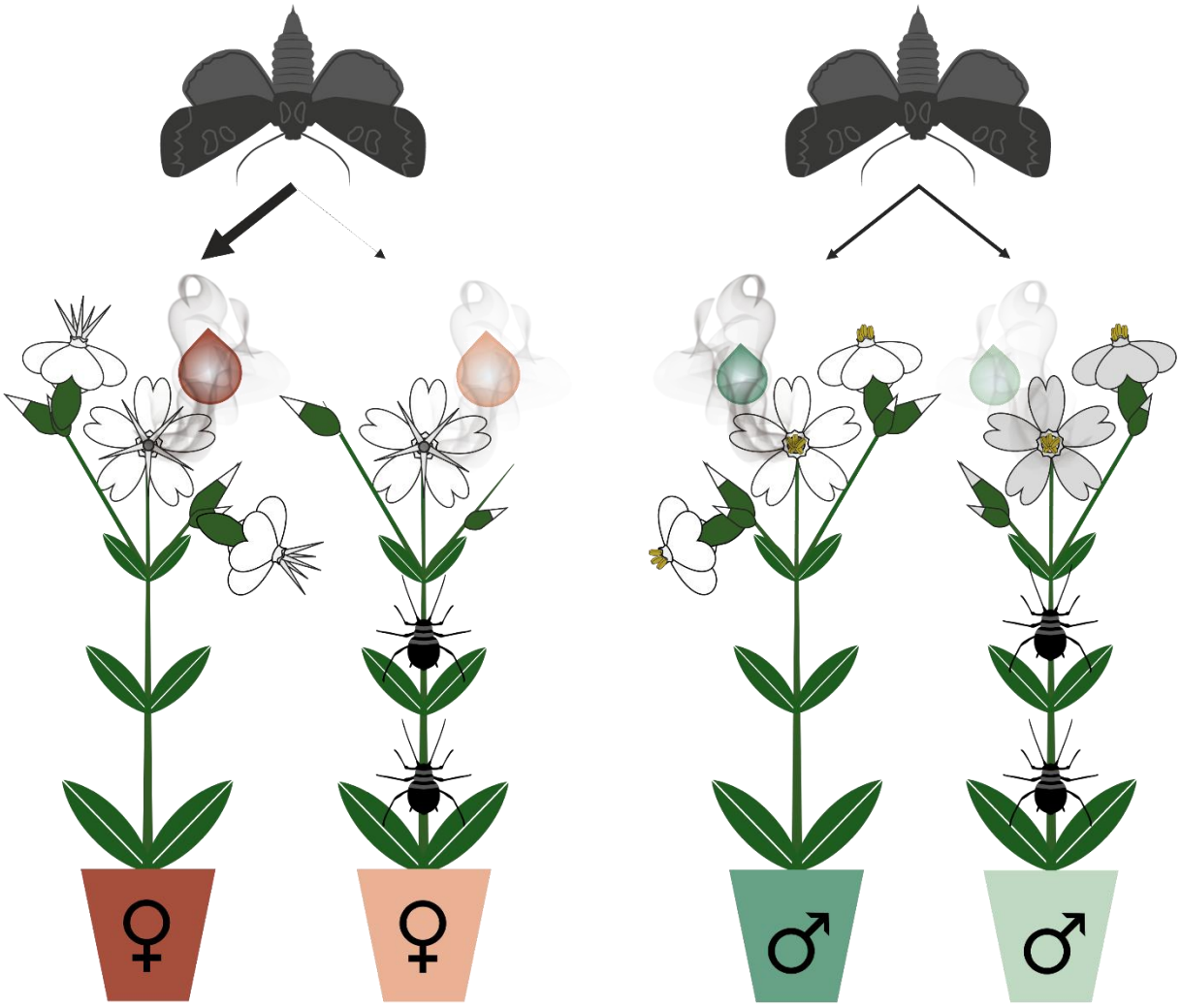
Pollinators consistently share the complex information and resource landscape of their host plants with herbivores. Yet, how sap feeders affect floral attractiveness to pollinators remains poorly understood, despite the critical role of this tripartite interaction in natural and agricultural ecosystems. These intricate interactions may be further enriched by pronounced sexual dimorphisms in dioecious species. In this study, we examined how infestation by the oligophagous aphid *Brachycaudus lychnidis* affects sex-specific interactions among the dioecious plant *Silene latifolia* and its specialist moth pollinator *Hadena bicruris*. We exposed male and female plants to aphid herbivory and evaluated its effects on floral traits (visual cues, floral scent, and nectar chemistry) and pollinator behaviour. Contrary to sexual selection theory, female plants were not less attractive than males under control conditions. Instead, our results suggest that females exhibit plasticity in floral trait expression depending on pollination success. Moreover, both sexes produced a diverse array of potentially bioactive nectar metabolites, with females showing higher abundances of many compounds that may influence female pollinator behaviour. Aphid infestation altered floral traits differently between sexes, with stronger declines in female floral attractiveness, suggesting sex-specific defence strategies and possible involvement of indirect defences in stabilizing this specialized plant–pollinator–herbivore system.

Keywords: *Brachycaudus*, dioecy, *Hadena bicruris*, nectar chemistry, nocturnal pollination, metabolomics, *Silene latifolia*, sexual dimorphism, plant-pollinator-herbivore interaction

Highlight, Abstract, Keywords

Graphical Abstract:

Plant-sex specific effect of aphid infestation on floral traits (number, size, colour, scent composition, nectar quantity and composition) and pollinator behaviour.



Abbreviations used in the main text:

- ANOVA: analysis of variance
- FC, FA, MC, MA: female control, female aphid infested, male control, male aphid infested
- GC-MS: gas chromatography-mass spectrometry
- (G)L(M)M: (generalized) linear (mixed effects) model
- NMDS: nonmetric multi-dimensional scaling
- UHPLC-ESI-qTof-MS/MS: quadrupole time of flight - electro spray ionization - tandem mass spectrometry
- PERMANOVA: permutational analysis of variance
- VOCs: Volatile organic compounds

1. Introduction

Understanding plant × pollinator × herbivore interactions and their component species' co-evolutionary dynamics has become a central goal in chemical ecology (Kessler and Chautá, 2020; De-la-Cruz *et al.*, 2022; Muola *et al.*, 2022). The complex plant traits mediating interactions with pollinators and herbivores have been shown to overlap significantly. While the ecological consequences of this shared information space are now well studied in systems involving chewing-biting herbivores, the effect of sap feeders on communication and resource exchange with pollinators is poorly understood, despite their critical role in the function of both, natural and agricultural ecosystems (Morales-Hojas, 2017; Noriega *et al.*, 2018).

Plants evolve diverse traits to attract pollinators and modulate their behaviour thereby maximizing their own fitness with minimal resource investment (Santamaría and Rodríguez-Gironés, 2015). Next to flowering phenology and visual signals (flower number, spatial arrangement, size, shape, hue, and colour luminance), floral chemistry plays a central role in ensuring these complex functions (Borghi *et al.*, 2017; Trunschke *et al.*, 2021; Wong *et al.*, 2023). The emission of floral volatile organic compounds (VOCs) can trigger oriented movement of the receiver towards the emitter, and elicit landing, feeding as well as reproductive behaviour (Muhlemann *et al.*, 2014). Such scent signals can be honest and guide visitors to a high-quality reward, or deceive them about the presence of food or mates, thereby exploiting their pollination service (Ito *et al.*, 2021; Peakall, 2023). Nectar - the primary nutritional reward provided to pollinators - is a complex secretion composed of sugars, amino acids, lipids, proteins and diverse secondary metabolites (Nicolson, 2022), and thus far more than a ready-to-use energy source for feeding animals. Plants may use nectar as a toolkit to manipulate the behaviour of their floral visitors. Secondary nectar metabolites can cause phagostimulation and satiety; improve learning capacity and memory; promote muscle performance, motivation and arousal; but also initiate sluggish and imprecise movements (Nepi *et al.*, 2018; Mustard, 2020; Barberis *et al.*, 2023). These very functions facilitate the exploitation of highly efficient pollination services while reducing energy investment in nectar sugars and amino acids, thus not specifically benefiting the floral visitors. Although we are becoming increasingly aware of this beautiful complexity, there are still few studies that take a holistic view of the pollination syndrome, considering the combined roles of visual traits, floral VOCs, and nectar metabolites.

Pollinators universally share the complex information and resource landscape of their host plants with herbivores. There is now evidence for interactions among traits and genes controlling plant reproduction and defence strategies, leading to complex reciprocal co-evolutionary feedbacks (Kessler and Chautá, 2020; De-la-Cruz *et al.*, 2022). Herbivores may

directly reduce plant attractiveness to pollinators through aggression or the consumption of floral tissues and vegetative structures necessary for their production (McCall and Irwin, 2006). Multiple floral traits have thus evolved to protect floral tissues from herbivory (Paul *et al.*, 2022). Constitutive and induced plant defences may not only alter a plant's suitability to herbivores, but also deter pollinators (Adler *et al.*, 2012) or reduce resource allocation to pollinator attraction (Stamp, 2003). Specifically chemical plant traits are considered to mediate multiple other antagonistic and synergistic pleiotropic effects on pollinator attraction and herbivore defence (Muola *et al.*, 2022). So far, insight into such complex tripartite interactions comes exclusively from chewing-biting herbivores. Aphids, which are among the most damaging plant pests (Dedryver *et al.*, 2010), however, have been largely overlooked. These exceptionally fast reproducing insects feed passively on the phloem sap of their hosts, thereby rapidly depleting them of essential nutrients, altering their source-sink relationships, and excreting phytotoxins as well as phytopathogenic viruses with their saliva (Nalam *et al.*, 2019; Twayana *et al.*, 2022). Plants in turn, actively defend against aphids by changing the composition of sugars and amino acids in the phloem sap or loading it with deterrents, toxins or anti-digestive proteins (Nalam *et al.*, 2019; Twayana *et al.*, 2022). Floral traits attracting pollinators are specifically prone to the direct and indirect effects of aphid infestation, as aphids often feed close to reproductive structures on flowering stems. This proximity suggests a high potential for interference with pollinator-mediated reproductive processes, warranting closer empirical attention.

The eco-evolutionary link between pollinator attraction and herbivore defence becomes particularly apparent in dioecious species (Johnson *et al.*, 2015). Plants that allocate male and female reproductive function to different individuals often exhibit complementary sexual divergence in both traits based on dramatic differences in resource demands for successful reproduction (Barrett and Hough, 2013; Johnson *et al.*, 2015). Male success is predicted to largely depend on efficient pollen transfer and thus pollinator attraction, while female success relies on pollination, as well as on the production and defence of seeds, and their dispersal (Moore and Pannell, 2011). Consequently, females are predicted to allocate fewer resources to floral attractiveness but exhibit more efficient herbivory defence and tolerance as compared to males (Avila-Sakar and Romanow, 2012; Barrett and Hough, 2013). As recent research has produced contradictory evidence for these theories (Cornelissen and Stiling, 2005; Tang-Martínez, 2012; Sargent and McKeough, 2022), a central question remains: What are the sex-specific ecological consequences of parallel interactions with pollinators and herbivores in dioecious plant species? Addressing this question is key to understanding how co-selection by pollinators and herbivores contributes to the evolution of divergent reproductive strategies in dioecious plants.

A system that is ideal to study these complex interplays consists of the dioecious plant *Silene latifolia* POIR. (Caryophyllaceae), its nocturnal pollinator *Hadena bicruris* HUFN. (Noctuidae), and the aphid *Brachycaudus lychnidis* L. (Aphididae). The species involved in this system engage in highly specialized relationships. Researchers have only begun to uncover the sophisticated functional traits that have co-evolved to stabilize the cost-benefit ratio of their individual bipartite interactions (Blair and Wolfe, 2004; Dötterl *et al.*, 2006; Labouche and Bernasconi, 2010; Schrieber *et al.*, 2021). Insight into the ecology of the tripartite interaction, however, is lacking. Here, we exposed female and male *S. latifolia* plants to control and *B. lychnidis* infestation treatments to assess flowering phenology, spatial flower arrangement, flower colour, the chemical composition of floral scent and nectar as well as nectar quantity, and attractiveness to *H. bicruris*. We specifically aimed at characterizing i) sexual dimorphisms in floral attractiveness; ii) the effect of aphid infestation on floral attractiveness to pollinators; and iii) sex-specific differences in the susceptibility to aphid infestation and the quality and magnitude of responses to aphid infestation.

2. Methods

2.1 Study organisms

Silene latifolia (white campion, *Figure 1A*) is a short-lived perennial plant within the Caryophyllaceae family, which is native to ruderal habitats across Eurasia and North Africa. The species belongs to the few angiosperms featuring a dioecious reproductive system and being simultaneously pollinated by specialized animals (Charlesworth, 1993; Käfer *et al.*, 2017). *Silene latifolia* plants exhibit a distinct moth pollination syndrome. Their white, large, and funnel-shaped flowers open at dusk to release a scent bouquet composed of more than 60 VOCs (Dötterl and Jürgens, 2005; Dötterl *et al.*, 2005) to advertise large amounts of nectar rewards (Tew *et al.*, 2021) with a mostly unknown chemical composition. Male and female *S. latifolia* are known to exhibit pronounced sexual dimorphisms in a broad set of floral traits (Gehring *et al.*, 2004; Dötterl *et al.*, 2005; Delph *et al.*, 2010; Schrieber *et al.*, 2021), which are largely in line with theory of sexual selection (Bateman, 1948).

Hadena bicruris (campion owl, *Figure 1B*) - a crepuscular (active during twilight) moth within the Noctuidae family - is the most relevant pollinator species of *S. latifolia* in Eurasia (Jürgens *et al.*, 1996; van Putten *et al.*, 2007). Together both species form one of only ~13 worldwide known nursery pollination systems (Dufaÿ and Anstett, 2003). Female moths pollinate female plants while ovipositing on flower ovaries to provide their hatching larvae with nutritious seeds and shelter by developing fruits. The pollination services provided by male moths, together with the high egg and larval mortality caused by parasitoids and predators of *H. bicruris*, compensate for the costs of seed consumption (Labouche and Bernasconi, 2010; Villacañas de Castro and Hoffmeister, 2020). Together these factors shift the outcome of the interaction for the plant towards the latter end of a seed predation-pollination continuum. A substantial fraction of VOCs in the floral scent of *S. latifolia* triggers antennal and behavioural responses in *H. bicruris* (Dötterl *et al.*, 2006). While the function of floral scents in this intricate co-evolutionary relationship is well studied (Dötterl *et al.*, 2006), the chemistry of nectar - the only reward and main food source provided to adult moths - is largely unexplored.

A third species participating in this highly specialized system within Eurasia is the holocyclic aphid *Brachycaudus lychnidis (Figure 1C)*, which spends both the parthenogenetic and sexual part of its life cycle on *Silene*. The aphid feeds mainly on the phloem sap of flowering stems.

2.2 Plant and insect cultivation

Seeds from five *S. latifolia* mother plants were collected in a population in Wiesengrund, Germany (51°41'17.1"N, 14°35'45.8"E) and experimentally outcrossed for two generations under greenhouse conditions (see Schrieber *et al.*, 2019 for details). We cultivated 30 plants

per maternal family individually in 360 mL pots filled with organic potting soil (Floragard TKS® 2 Instant Plus, DE) in a greenhouse at 25 °C / 15 °C and 60 % RH in a 16 h day / 8 h night cycle. When plants started flowering after 6 weeks, we selected two males and two females from each of the five maternal families (N = 20). The selected plants were transferred into 8 L pots filled with the above-mentioned substrate and pruned to a height of 15 cm to induce over-compensatory growth. Please note that *S. latifolia* is adapted to grazing/mowing and thus cutting is near-natural (Jessen *et al.*, 2022). Moreover, cutting was necessary to induce the emergence of multiple shoots and sufficient flower production for the experiment. Plants were fertilized with 0.3 g Universol® Yellow (Everris-Headquarters, NL) dissolved in 200 mL of water every other week and prophylactically treated with sulphur vapor to prevent fungal infections as well as with biological pest control agents to prevent thrips and uncontrolled aphid infestation (agents: *Amblyseius cucumeris against thrips; Aphidius ervi, Aphidius colemani and Lysiphlebus testaceipes* against aphids Katz Biotech GmbH, DE). The preventive treatment with parasitoid wasps was stopped three weeks before controlled aphid infestation.

Adult *H. bicruris* moths were collected in different wild *S. latifolia* populations around Kiel, Germany (54°19′ N, 10°8′ O) in 2022 and kept together in ventilated, transparent 20 x 30 cm boxes equipped with egg cartons and tissue papers within a climate chamber (MLR-352H, PHC Corporation, Ōizumi-machi, JP) at 25 °C / 15 °C and 60 % RH in a 16 h day / 8 h night cycle. They were fed a honey-water solution (6 g organic flower honey, 14 mL distilled water) from artificial PET *S. latifolia* flowers. In addition, moths received one fresh male and female *S. latifolia* flower per day, as they did not reproduce without them. Individual flowers were harvested from a pool of food plants and presented in Eppendorf tubes filled with water. *Hadena bicruris* eggs, which were deposited all across the breeding boxes, were collected and placed on moist filter paper in individual ventilated plastic containers (50 mL). Upon hatching, larvae were provided with an artificial diet according to [43], which was additionally supplemented with 20 mL of *S. latifolia* seeds per 500 mL to increase acceptance. Pupated *H. bicruris* were moved to ventilated 50 mL containers filled with sterile sand and newly hatched moths were transferred into the above-mentioned boxes for adults.

Brachycaudus lychnidis aphids were collected and identified according to (influentialpoints.com, 2024) in spring 2024 in different wild *S. latifolia* populations around Kiel, Germany (54°19′ N, 10°8′ O). They were cultivated in net cages on a balanced mixture of male and female *S. latifolia* plants from our study population in a climate chamber (HGC 1514 Module 7, Weiss Umwelttechnik GmbH, DE) at 23 °C and 55 % RH, in a 16 h day / 8 h night cycle. Food plants were watered and exchanged on demand.

233 ## *2.3* Experimental setup and data acquisition

234 In mid-July 2024 at the age of 13 weeks, all selected plants were enclosed in 150 × 50 cm
235 organza mesh bags and one individual per sex (male, female) × maternal family (I-V)
236 combination was inoculated with 50 adult aphids *(Figure 2A, Supplementary Figure S1A-C)*.
237 All aphid colonies showed an initial decline in population size of max. 10 % and switched to
238 positive growth rates max. three days after the inoculation. Thus, our approach does not
239 account for potential plant sex-specific host choice, early phase plant defences preventing
240 colonization success, and indirect defence; but focuses on the effects of established aphid
241 colonies having optimal growth conditions. Data on plant stress indicators, pollinator choice,
242 visual flower traits, floral scent, and nectar *(Figure 2B-F)* were collected over a 40-day period
243 following aphid inoculation *(Figure 2G)*.

244 ### *2.3.1* Plant stress indicators

245 To quantify the disruption of physiological homeostasis under aphid infestation, we optically
246 assessed the chlorophyll concentration, and the absorbance by epidermally located UV-
247 absorbing compounds, mainly flavonoids using the LEAF-STATE-ANALYZER LSA-2050
248 (Heinz Walz GmbH, DE) *(Figure 2B&G)* for all plants. As reference, data provided with the
249 instrument for the lower leaf side of *Sedum telephium* was used. Data were acquired directly
250 before and 40 days after aphid inoculation between 11:00 am and 12:00 noon in five leaves
251 per plant individual. The selected leaves were evenly distributed along the vertical axis of the
252 plants and well exposed to light (i.e., not significantly shaded by other leaves) and the
253 measured area, located in the upper middle part of the lamina, was free of honeydew and
254 aphids, which typically feed on stems. Immediately after these measurements, we counted the
255 number of aphid individuals per size category (small: < 1.5 mm, medium: < 2 mm, large: > 2
256 mm) directly on the plant. Subsequently, the aphids were carefully washed off the plants with
257 warm tap water, collected on filter paper and dried for 24 h at 25 °C, then for 24 h at 65 °C,
258 and finally for 24 h at 105 °C to determine total aphid dry biomass per plant.

259 ### *2.3.2* Pollinator behaviour

260 Behavioural assays with *H. bicruris* were conducted under laboratory conditions 30-39 days
261 after aphid inoculation *(Figure 2C&G)* between 8:00 pm and 0:30 am - the peak activity time
262 of our captive moths. For the time of data acquisition in the choice assays, adult moths were
263 kept individually in ventilated, transparent boxes (15 × 12 × 11 cm) equipped with shelters and
264 a feeding station featuring a honey-water solution as described in *chapter 2.2*. For each assay,
265 the artificial diet was removed in the morning and replaced in the evening with two fully

developed (male: fully dehisced anthers, female: fully expanded and papillous stigma), freshly picked flowers from the experimental plants, which were carefully fixed in lidless Eppendorf tubes filled with tap water. The two flowers were positioned on opposite sides of the boxes for maximum spatial separation *(Supplementary Figure S1D)* to provide the moths with the choice among the following treatment groups: control females *versus* aphid infested females, control males *versus* aphid infested males, control females *versus* control males, aphid infested females *versus* aphid infested males. The moth individuals used in these behavioural tests (N = 11) differed in age and sex (5 females, 6 males; moth sex was accounted for in the statistical analysis) and were tested in multiple-choice trials using a fully randomized design with respect to plant treatment groups and individuals, completing a total of 66 trials. Each night, up to eight moths were used, with two observers monitoring four moths each. The boxes were evenly lit with dim red LED lamps to allow observation without confounding the trials (the visual system of moths cannot capture red light). Three behavioural parameters were quantified for each of the two flowers: (i) the number of visits, with a visit starting and ending with physical contact to the flower; (ii) the number of feeding attempts per flower, with an attempt starting/ending by unrolling/rolling up and inserting/removing the proboscis into/from the corolla tube; and (iii) the total time spent with any physical contact on the flower.

2.3.3 Visual flower traits

We measured a broad set of visual flower traits at the plant individual and single flower level throughout the experiment *(Figure 2D&G)*.

Flower colour was quantified using a digital image transformation method that accounts for natural light conditions and the visual system of the pollinator based on (Troscianko and Stevens, 2015). Images were acquired 21-25 days after aphid inoculation for three to six flowers (depending on availability) from all plant individuals. They were taken outdoors in an open area, free from shadows cast by trees or buildings, during the last hour of daylight on rain-free days to match the natural light conditions perceived by *Hadena* (Bopp and Gottsberger, 2004). A fully developed but fresh flower was plugged into a custom-made black ethylene vinyl acetate platform, which featured two reflectance standards (PTFB 10% and Spectralon 99%, Labsphere, UK) along with a size reference *(Supplementary Figure 1E)*. Images were captured using a modified Samsung NX1000 camera (Suwon, KOR) with its sensor filter removed, to achieve full-spectrum sensitivity (300 – 1000 nm). The camera was equipped with a UV-sensitive lens (Nikon EL 80 mm, JP). To capture both visible and ultraviolet (UV) light, we took one photo with a UV/IR blocking filter (UV/IR Cut, transmitting 400-700 nm, Baader Planetarium, DE) and another one with a UV pass plus IR blocking filter (U-filter,

transmitting 300-400 nm, Baader Planetarium, DE) using a custom-made filter slide mount. All photos were taken with an aperture of 5.6, and ISO of 800, whereas the shutter speed was adjusted to the available light conditions to receive well-exposed photos. Post-processing was carried out using the Multispectral Image Calibration and Analysis (MICA) Toolbox plugin (Troscianko and Stevens, 2015) within ImageJ 1.47 t (Rueden *et al.*, 2017). We selected the flower silhouette for colour analysis. The images were linearized to correct the camera's non-linear light response and standardized based on the two reflectance standards to compensate for variations in natural lighting. We fitted the multispectral images to a mathematical model incorporating (i) the spectral sensitivity of our Samsung NX1000-Nikkor EL 80 mm 300-700 nm camera (data derived from Troscianko and Stevens, 2015); (ii) the spectral sensitivities of the three photoreceptors in the moth compound eye (data derived from Johnsen *et al.,* 2006); and (iii) the spectral composition of sun light during dusk (data derived from Johnsen *et al.,* 2006). For further details see Schrieber *et al.* (2021). The outcomes of these analyses are the percentage of green, blue and UV-A dusk sun radiation reflected by the flower, which are perceivable by a moth's visual system.

Flower area was measured from the same images for three to six flowers per plant individual. We assessed the exact area covered by a single, well developed petal limb, the area covered by the exact flower silhouette and the area covered by the circular perimeter *(Figure 2D, 1 = flower perimeter, 2 = flower silhouette, 3 = petal silhouette)*. The circular perimeter reflects the flower's maximum extension, but not its visual impact on pollinators due to natural variation in the shape of single petals, while the area covered by the exact flower silhouette may represent what pollinators see but not the plant's investment into flowers (petal overlap), which can be captured by individual petal area.

The number of opened flowers and flower buds per plant individual were recorded at three time points during the experiment (10, 20 and 40 days after aphid inoculation, *Figure 2D&G*) and corrected for the flowers removed for other analyses. Directly after the removal of aphids (40 days after aphid inoculation), plant aboveground parts were cut off directly on top of the soil and separated into shoots (without leaves), and flowers. Plant parts were dried individually in paper bags for 24 h at 65 °C followed by an additional 24 h at 105 °C to determine dry biomass. During data acquisition for pollinator behaviour, flower colour, and nectar rewards, we collected the harvested flowers in plant individual-specific bags to assess their dry biomass as described above and added it to the total flower dry biomass.

332 ### 2.3.4 Flower scent

333 At day 27 after the aphid inoculation, floral VOC emission was collected for all experimental
334 plants during the peak emission in *S. latifolia* between 10:00 pm (sunset) and 1:00 am (Dötterl
335 *et al.*, 2005) using a mobile push-pull system as described in Berkum *et al.*, (2024) . Up to
336 three fresh and fully developed flowers per plant from different shoots were enclosed in 35 cm
337 × 31 cm PET bags (RUBIN® Bratschlauch, DE), which were closed at both ends with cable
338 ties *(Figure 2E)*. Compressed air entered the system after passing through an activated
339 charcoal filter on the lower side of the bag at a flow rate of 1 L min^{-1} and was pulled out at the
340 top through an adsorbent Poropak filter (Alltech, USA), using a vacuum at the rate of 0.6 L/min.
341 After VOC collection, the filters were eluted with 200 µL dichloromethane (Sigma Aldrich, DE)
342 containing 10 ng/µL of nonyl acetate (Sigma Aldrich, DE) as an internal standard. As the
343 headspace of treated plants also included aphids, we additionally determined which
344 compounds were exclusively released by *Brachycaudus*. We enclosed 60 aphids (20 small: <
345 1.5 mm, 20 medium: > 1.5 mm, 20 large: > 2 mm) from our laboratory population in the above-
346 described PET bags to collect and analyse their volatile emissions as described for the plants
347 with three replicates.

348 Samples were analysed on a gas chromatograph (TRACE GC1310, ThermoFisher Scientific,
349 DE) equipped with a TraceGOLD TG-5MS column (30 m × 0.25 mm × 0.25 µm + 5 m;
350 ThermoFisher Scientific, DE) and coupled with a mass spectrometer (TSQ Duo MS,
351 ThermoFisher Scientific, DE). A sample volume of 1 µL was injected with a split ratio of 1:10 at
352 250 °C and migrated with a helium-flow of 1 mL min^{-1}. The transfer line was set to 270 °C and
353 the ion source of the mass spectrometer was operated at 230 °C and 70 eV for ionization. The
354 temperature program started at 40 °C for 2 min, ramped to 180 °C at a rate of 6 °C min^{-1},
355 ramped to 300 °C at a rate of 60 °C min^{-1}, and finished with a 5 min hold time at 300 °C. Mass
356 spectra of separated compounds were acquired in full scan mode between 33 and 350 m/z.
357 An alkane standard mix (C_8-C_{20}, Sigma Aldrich, DE) was analysed under the same conditions
358 to calculate Kovats retention indices (RI) for the targeted compounds. The data processing
359 was performed with Chromeleon™ 7.3 (ThermoFisher Scientific, DE). The putative identity of
360 compounds was determined via comparisons of mass spectra and RI's with databases (NIST
361 Mass Spectral Search Program Version 3.0, (Smith *et al.*, 2004)) and (Dötterl *et al.*, 2005). The
362 identities were assigned according to the following criteria: match factor to the NIST databank
363 >750 and RI difference < 10. The base peak in the mass spectrum was used as quantitation
364 ion. Additionally, two secondary ions (confirmation ions) were selected per compound to
365 ensure correct peak assignment *(Supplementary Table S1A)*. The relative compound
366 abundance was quantified by dividing the peak area (based on individual quantitation ion) of
367 each volatile by the peak area of the internal standard nonyl acetate (quantitation ion 43,

368 confirmation ions 56, 70). To account for variation in flower number in the headspace, scent
369 abundance was normalized per flower. We selected all VOCs for the statistical analyses that
370 could not be clearly identified as contaminations.

371 ### 2.3.5 Nectar quantity and chemistry

372 We measured the nectar volume for the flowers that have been harvested for the colour and
373 size analyses, directly after they have been photographed. All nectar was extracted from the
374 base of the ovary / the base of the stamens and anthopophor into 1 µL microcapillary tubes
375 (Minicaps NA-HEP, Hirschmann Laborgeräte, DE). The length of the nectar column was
376 measured with a calliper to determine the exact volume.

377 Nectar chemistry was addressed based on a standardized nectar volume via Ultra High
378 Performance Liquid Chromatography - Quadrupole Time of Flight - Electro Spray Ionization -
379 Tandem Mass Spectrometry (UHPLC-ESI-qTof-MS/MS). For these analyses, we additionally
380 collected all nectar from three fresh, fully developed flowers per plant individual using 1 µL
381 microcapillary tubes on day 28 after aphid infestation. After determining the exact nectar
382 volume as described above, each microcapillary was rinsed with 30 µL of HPLC grade water
383 (Agilent, USA) into a 0.5 mL Eppendorf tube, in which all nectar from one plant individual was
384 pooled. The samples were then vortexed, snap-frozen in liquid N_2 and stored at -80 °C.
385 Samples underwent a modified simultaneous metabolite, protein, and lipid extraction
386 (SIMPLEX) as described in (Schrieber *et al.*, 2023) to obtain both, hydrophilic and lipophilic
387 nectar metabolites. After defrosting and vortexing, 0.1 mL of nectar solution was mixed with
388 0.5 mL of ultra-pure methanol (Carl Roth, GE) and 4 mL of methyl-tert-butylether (LC-grade,
389 Carl Roth, GE). Samples were incubated for 30 min at 25 rpm in an end-over-end shaker
390 before phase separation was induced by adding 0.5 mL of ultra-pure water (Carl Roth, GE).
391 After 10 min of incubation, samples were centrifuged at 4000 *g* and 4 °C for 10 min and the
392 upper lipophilic phase was collected. The lower hydrophilic phase was used as a template to
393 repeat the above-described protocol for the extraction of hydrophilic metabolites. Extracts were
394 re-suspended in 0.5 mL of either ultra-pure water and ultra-pure methanol (hydrophilic extracts:
395 50 / 50, v / v, Carl Roth, GE) or ultra-pure isopropanol and LC-grade chloroform (lipophilic
396 extracts: 3:1, v / v, Carl Roth, GE). The dilution of the resulting nectar stock solutions for
397 analyses via UPLC-qTof-MS/MS was then adjusted to differences in the volume of extracted
398 nectar among plant individuals. Hence, the volume of analysed nectar was standardized for
399 these analyses.

400 For UHPLC-ESI-qTof-MS/MS analyses, 2 µL of extract were injected at 30 °C into a UHPLC
401 (Nexara, Shimadzu, DE) containing a C_{18} Column (100 × 2.1 mm, 1.7 µm, Luna Omega Polar,

402 Phenomenx, DE). The solvent system was composed of (A) water with 0.1% formic acid, and
403 (B) acetonitrile with 0,1% formic acid (all LC-grade, Carl Roth, DE). The gradient program
404 started at a flow rate of 0.3 mL min^{-1} with 5 % B, was then raised over 20 min to 95 % B, and
405 finally kept at 95 % B for an additional 5 min. Afterwards, the column was re-equilibrated at 5%
406 B for 6 min. Chromatographically separated analytes entered a qTOF-MS/MS (x500R, Sciex,
407 DE) with the following parameters: ion source gas 45 psi (nitrogen), curtain gas 35 psi
408 (nitrogen), CAD gas 7 psi (nitrogen), electrospray ion source temperature 500 °C, ion spray
409 voltage 5500 V. The MS acquisitions parameters were: MS1 scans at 100-1000 m/z with a
410 data-dependent MS2 trigger having a collision energy (CID) of 40 eV and a scan range of 50-
411 1000 m/z. The acquired data were then converted to .mzML format and further processed in
412 mzMine (Schmid *et al.*, 2023). Metabolic features with a maximum m/z difference of 0.001 amu
413 and maximum retention time difference of 0.1 min were bucketed and treated as identical
414 features in the following analysis. For feature annotation, the acquired MS/MS spectra were
415 compared against public mass spectral libraries from Global Natural Products Social Network
416 (GNPS) (Wang *et al.*, 2016) and the MassBank (MassBank consortium and its contributors,
417 2024) using the NIST search algorithm (cosine similarity of 0.6) and the MassBank formula
418 (cosine similarity of 0.5). Features that remained unidentified were further annotated using
419 COSMIC and CSI:FingerID according to the SIRIUS software package (Hoffmann *et al.*, 2022).

420 ### *2.3.6* Statistical analyses

421 All statistical analyses were implemented in R 4.4.1 (R Development Core Team, 2024). Plant
422 stress indicators, spatial flower traits, flower colour traits, and floral rewards were analysed as
423 responses in (generalized) linear mixed effects models - (G)LMMs, which included the aphid
424 infestation treatment, plant sex and the interaction among both variables as predictors. Highly
425 correlated traits were combined into a single response and analysed within one model. These
426 models included the different types of summarized response traits as a factorial co-predictor,
427 for which all possible interactions with sex and aphid infestation treatment were tested
428 according to the scheme *response at plant individual level (level of summary) ~ cofactor:* leaf
429 chlorophyll content (on day 0 and 40 of the experiment) ~ time point; leaf epidermal light
430 absorption by flavonoids (on day 0 and 40 of the experiment) ~ time point; number aphids
431 (belonging to the size category small, medium and large) ~ aphid size category; flower number
432 (on day 10, 20 and 40 of the experiment) ~ time point; dry biomass (of a single flower, all
433 flowers, and flowering stems) ~ biomass component; display area (of a single petal limb, all
434 petal limbs, and the full corolla expansion) ~ flower component; flower light reflection (in the
435 green, blue and UV-A range) ~ colour quality. The remaining traits (nectar volume) were
436 acquired across several flowers per plant individual and analysed in this high resolution (i.e.,

not based on means). Consequently, all models had repeated measurements on the plant individual level and thus included a random effect for the plant individual. All models were checked for violations of their underlying assumptions using tests and plots from the R-package "DHARMa" (Hartig, 2022) and were adjusted with regard to error family, dispersion or zero-inflation, if necessary. An overview of all model parameters is provided in *Table 1*. Based on these models, we calculated type III ANOVA tables (based on Wald- χ^2 tests) while setting all contrasts to sum-zero coding using the R-package “car” (Fox and Weisberg, 2019). If factorial predictors were involved in significant interactions, we additionally calculated post-hoc contrasts among the estimated means of their levels within levels of other factors involved in the respective interaction (R-package “emmeans”; Lenth, 2024)).

The binary choice tests were analysed with two models testing (i) preferences for a specific aphid infestation treatment within sexes (assays: control female *versus* aphid-infested female, control male *versus* aphid-infested male); and (ii) preferences for a specific plant sex within aphid infestation treatments (assays: control female *versus* control male, aphid infested female *versus* aphid-infested male). Model i / ii included the behavioural parameter in control relative to aphid infested (i) or female relative to male (ii) flowers as binomial response and the flowers sexes (i) or the flowers aphid infestation treatments (ii) as predictors. Further predictors included in both models were the behavioural parameter type (visits, feeding attempts, time; all acquired in the same moth and trial), and its interaction with the previously mentioned model-specific predictor. In addition, both models included the sex of the moth as a cofactor for which only additive effects were tested, as sample sizes were not large enough to reliably test for all possible interactions. Moth individual and trial were set as random effects *(Table 1)*. Model checking, adjustment and downstream analyses with type III ANOVA were implemented as described above. Post-hoc contrast (R-package “emmeans”; Lenth, 2024) were used to test for a significant deviation of the binary outcome from the intercept of 0.5 (i.e., preference or avoidance) within the different plant sex × behavioural type combinations (i) or aphid infestation treatment × behavioural type combinations (ii).

The statistical approaches used for the analyses of the metabolome are identical for floral nectar and scent. In a first step, we performed a permutational analyses of variance (PERMANOVA) using the Bray-Curtis dissimilarities of the full metabolome datasets (n = 41 features in scent, n = 4,385 features in nectar assessed via UPLC-qTof-MS/MS) as the response distance matrix. We included aphid infestation treatment, sex, and their interactions as predictors and ran the models with 100,000 permutations (R-package “vegan” (Oksanen *et al.*, 2024)). As previously implemented tests for multivariate homogeneity of group dispersions in the distance matrices among all treatment levels yielded no deviation for all datasets, the predictor effects assessed via PERMANOVA are based on differences in group centroids

473 rather than differences in group dispersions (Anderson and Walsh, 2013). The multivariate
474 group separations among plant infestation treatments and sexes were illustrated with Non-
475 metric Multidimensional Scaling (NMDS) plots (R-package “vegan” (Oksanen *et al.*, 2024)). To
476 identify the compounds responsible for this separation, we used an approach that is
477 comparable to a volcano plot (Cui and Churchill, 2003) and simultaneously allows the statistical
478 assessment of interaction effects among plant sex and aphid infestation treatment. Instead of
479 applying two-sided significance tests (t or u-tests), we entered all metabolites as responses
480 into a linear model loop to test for the main and interactive effects of plant sex and aphid
481 infestation treatment. Each metabolite ran with three alternative models as untransformed,
482 squareroot-transfromed and log-transformed response. To check for violations of model
483 assumptions and select the best of the three alternative models for each metabolite
484 accordingly, we extracted the results from the Kolmogorov-Smirnov test for uniformity
485 (normality of residuals) and Levene’s test for homogeneity of variance, which are provided for
486 targeted model optimization in the R-package "DHARMa" (Hartig, 2022). If model assumptions
487 were violated in all three alternative models, the respective metabolite was not further
488 analysed. The best fit models for the remaining metabolites were further analysed with type III
489 ANOVAs based on F-tests (R-package “car” (Fox and Weisberg, 2019)) and the calculation of
490 post-hoc contrasts (R-package “emmeans” (Lenth, 2024)) as described in the previous section.
491 *P*-values were corrected for inflation of alpha errors by multiple testing (false discovery rate:
492 FDR) according to (Benjamini and Hochberg, 1995). If a metabolites p-value in a given group
493 comparison ($F_{C\&A}/M_{C\&A}$, $T_{F\&M}/C_{F\&M}$, FC/FT, MC/MT, FC/MC, FT/MT) was significant and the
494 corresponding $\log_2$-fold change in the intensity of this metabolite was either ≤ -1 (halving of
495 intensity in the first relative to the second listed group) or ≥ 1 (doubling of intensity in the first
496 relative to the second listed group) it was considered to be modulated.

3. Results

4.1 Plant stress indicators

After 40 days of inoculation with *B. lychnidis*, aphid load in terms of weight and number was equally high in female and male *S. latifolia* plants *(Table 1; weight: females = 0.60 +/- 0.12, males = 0.53 +/- 0.12, p>0.05,* F_{2DF}*= 0.42; number: females = 566 +/- 169, males = 484 +/- 146, p>0.05,* F_{2DF}*= 0.95)*. Leaf dry mass per plant individual, leaf chlorophyll content and absorption by epidermal flavonoids were not affected by the aphid infestation treatment but significantly higher in females than males in both, control and aphid infested plants *(Table 1, leaf number: females = 5.33 +/- 0.56, males = 3.50 +/- 0.56, p<0.05,* F_{2DF}*= 5.28; chlorophyll content: females = 27.5 +/- 1.37, males = 22.9 +/- 0.91, p<0.001,* χ^2_{2DF}*= 14.37; absorption by epidermal flavonoids: females = 0.70 +/- 0.03, males = 0.62 +/- 0.03, p< 0.05,* χ^2_{2DF}*= 4.80)*. Moreover, leaf chlorophyll contents decreased from 35.9 +/- 1.01 to 17.6 +/- 0.80 nmol cm^{-2} *(p<0.001,* χ^2_{2DF}*= 60.34)*, whereas absorption by epidermal flavonoids increased from 0.61 to 0.71 *(p<0.001,* χ^2_{2DF}*= 49.47)* from the beginning towards the end of the experiment, regardless of sex and aphid infestation treatment *(Table 1*, column cofactor*)*.

3.1 Effects of plant sex and aphid infestation on pollinator behaviour

A subset of our binary choice assays tested behavioural moth responses to single flowers from control versus aphid-infested plants, and whether these responses varied between male and female plants. We detected significant preferences for flowers from control plants over aphid-infested ones across all behavioural parameters in females, but not in males *(Figure 3A; z-ratio female plants C-A: 2.11* [visits], 2.29* [feeding attempts], 3.54*** [time spent]; z-ratio male plants C-A: 0.23*^{ns} *[visits], 0.05*^{ns} *[feeding attempts], 0.93*^{ns} *[time spent])*. Consequently, the effect of plant sex on preference for control plants was highly significant *(Table 1, p< 0.001,* χ^2_{2DF}*= 101.20)*. In addition, we detected a marginally significant main effect of pollinator sex *(Table 1, p<0.06,* χ^2_{2DF}*= 3.72)*, with only female but not male *H. bicruris* moths showing a significant preference for control over aphid-infested *S. latifolia* plants *(z-ratio female moth C-A: 2.32*, z-ratio male moth C-A: -0.03*^{ns}*)*.

Another subset of our binary choice assays tested whether moths prefer single flowers from one plant sex over the other and whether this preference is modified by the aphid infestation treatment. The statistical analysis yielded no significant preferences of the moths for either plant sex *(Figure 3B; z-ratio control plants F-M: -0.78*^{ns} *[visits], -0.76*^{ns} *[feeding attempts], -0.59*^{ns} *[time spent]; z-ratio aphid infested plants F-M: -0.45*^{ns} *[visits], -0.66*^{ns} *[feeding attempts],*

529 *-0.55^{ns} [time spent])*. Neither the aphid infestation treatment of the plants nor moth sex shaped
530 the choice of plant sexes *(Table 1)*.

531 **3.2 Effects of plant sex and aphid infestation on visual flower traits**

532 Flower number was significantly shaped by the three-way interaction sex × infestation
533 treatment × and infestation time *(Table 1, Figure 4A, $p< 0.05$, $\chi^2_{2DF}= 8.88$)*. Post-hoc
534 comparisons revealed that female plants suffered both earlier and stronger declines in flower
535 number upon aphid infestation as compared to males *(female t-ratio C-A: 2.05^{ns} [10 days],*
536 *4.25*** [20 days], 9.26*** [40 days]; male t-ratio C-A: 0.40^{ns} [10 days], 1.36^{ns} [20 days], 4.74****
537 *[40 days])*. Correspondingly, females produced significantly fewer flowers than males only in
538 aphid infested plants and the magnitude of this effect increased over time *(control t-ratio F-M:*
539 *-2.14^{ns} [10 days], -1.41^{ns} [20 days], -1.26^{ns} [40 days]; infestation t-ratio F-M: -3.84** [10 days],*
540 *-4.31*** [20 days], -5.31*** [40 days])*. Reproductive biomass depended on the three-way
541 interaction sex × infestation treatment × component *(Table 1, Figure 4B, $p< 0.001$, $\chi^2_{2DF}=$*
542 *16.86)*. Post-hoc comparisons revealed that the component single flower mass was not
543 affected by the infestation treatment *(t-ratio C-A: 1.17^{ns} [female], 1.14^{ns} [male])* but generally
544 higher in females than males *(t-ratio F-M: 6.22*** [control], 5.76*** [infested])*. In contrast, the
545 component total flower mass declined severely under aphid infestation, specifically in females
546 *(t-ratio C-A: 12.89*** [female], 7.26*** [male])* and was accordingly higher in females only in
547 controls but not under aphid infestation *(t-ratio F-M: 5.22*** [control], -0.41^{ns} [infested])*. The
548 mass component of flowering stems was not affected by aphid infestation *(t-ratio C-A: 2.73$^{n.s}$*
549 *[female], 0.86$^{n.s}$ [male*]), and plant sex *(t-ratio F-M: 2.56^{ns} [control], 0.68^{ns} [infested])*. Flower
550 light reflectance *(Table 1, Figure 4C)* was significantly shaped by the two two-way interactions
551 sex × aphid infestation treatment (*$p< 0.05$, $\chi^2_{1DF}= 4.91$)* and aphid infestation treatment × colour
552 type (*$p< 0.05$, $\chi^2_{2DF}= 8.27$*). Post-hoc comparisons showed that the aphid-infestation treatment
553 reduced floral light reflectance in the green and blue spectrum only in male, but not in female
554 plants *(female t-ratio C-A: 1.39^{ns} [green], 1.69$^{ns.}$ [blue], 0.11^{ns} [UV]; male t-ratio C-A: 3.00**
555 *[green], 3.22* [blue], 2.09^{ns} [UV]).* Flower area was neither affected by plant sex, aphid
556 infestation treatment or their interaction *(Table 1)*.

557 **3.1 Effects of plant sex and aphid infestation on the composition of floral scent**

558 We detected a total of 41 VOCs in the floral headspace of *S. latifolia*, with values normalized
559 per flower. Of these 35 could be putatively identified via matches of RIs and mass spectra with
560 databases *(Supplementary Table S1A)*. A large fraction of these VOCs have been previously

561 described in *S. latifolia* (Dötterl *et al.*, 2005; Mamadalieva *et al.*, 2014) and of these, 16 have
562 been shown to elicit antennal and/or behavioural responses in *H. bicruris* (Dötterl *et al.*, 2006).

563 The PERMANOVA detected a marginally significant main effect of aphid infestation treatment
564 on the composition of these floral VOCs *(Table 1, $p < 0.09$, $F_{1DF} = 2.41$, $R^2 = 0.13$)* but no
565 divergence among sexes or interactive effects. This is in accordance with both, the group
566 dispersion visualized in the NMDS ordination plot *(Figure 5A)* and the outcomes of the model
567 loop *(Figure 5B, Supplementary Table S1A)*. Without FDR correction, the loop identified eight
568 compounds shaped by the main effect of treatment with reduced abundance in aphid infested
569 as compared to control plants. These included five compounds known to elicit behavioural
570 responses in *H. bicruris* according to Dötterl *et al.* (2006) -benzaldehyde, trans-β-ocimene, lilac
571 aldehyde A, lilac aldehyde B+C, and lilac alcohol D- as well as two of their degradation
572 products, lilac alcohol formate A and lilac alcohol formate B. One compound which was not
573 tested for behavioural effects on *H. bicruris* so far - phenethyl benzoate - was significantly
574 modulated by the interaction plant sex × aphid infestation treatment and decreased under
575 aphid infestation in female plants but not in males. When accounting for inflation of alpha
576 errors, all these effects were non-significant.

577 **3.2 Effects of plant sex and aphid infestation on nectar traits**

578 Nectar volume was unaffected by the aphid infestation treatment but considerably shaped by
579 plant sex with female flowers featuring higher nectar amounts (*Table 1, Figure 6A, $p< 0.001$,*
580 *$\chi^2_{1DF}= 46.10$)*. The untargeted metabolome analyses of a standardized nectar volume per plant
581 via UPLC-qTof-MS/MS yielded abundance data for 4,385 features. Of these, 761 features were
582 tentatively identified via matches of their mass spectra with databases or via cheminformatics
583 according (Hoffmann *et al.*, 2022), and an additional 71 could be assigned to a compound
584 class *(Supplementary Table 1B)*. This is the first detailed description of nectar chemistry in the
585 ecological model system *S. latifolia.* The species' nectar contained various nutritious
586 metabolites, including sugars (D-glucose, D-fructose, sucrose, melezitose, stachyose,
587 nystose, mannobiose, palatinose), proteinogenic and non-proteinogenic amino acids (e.g.,
588 proline, glutamic acid, tyrosine ornithine) and numerous oligopeptides, nucleosides
589 (adenosine, adenosine monophosphate, a guanosine derivate), and multiple lipids (e.g., fatty
590 alkyls; fatty acids, amides and alcohols; ceramides; glycerophosphoethanolamines,
591 sphingolipids; prostaglandins). Its diverse secondary metabolites comprised vitamins (e.g.,
592 calcitriol, riboflavin), flavonoids (e.g., saponarin, vitexin-2"-O-rhamnoside), terpenoids (e.g.,
593 myrtenol, perillyl alcohol, raddeanin D, pisumoside A), as well as fatty amides and amines
594 (oleamide -a neuroactive compound [Mendelson and Basile, 2001] which has so far not been

described in nectar, tyramine, homospermidine). Most of these secondary compounds are likely derived from plant metabolism, but several have a known fungal or bacterial origin and thus likely derive from the nectar microbiome (e.g., oleandomycin, enniatin B, asperiamide B, N-acetylglucosamine [chitin monomer]).

The PERMANOVA detected a highly significant effect of plant sex *(Table 1, $p < 0.001$, $F_{1DF} = 10.65$, $R^2 = 0.39$)*, but no effects of infestation treatment or the interaction sex × infestation treatment on the composition of the metabolome in a standardized volume of nectar. The group dispersions visualized in ordination plots via NMDS illustrate a clear differentiation of the nectar metabolome among female and male plants and additionally indicate different responses to the aphid infestation treatment in males and females in some features *(Figure 6B)*. The results from the linear model loop further support this observation *(Figure 6C-F, Table 2, Supplementary Table S1B).* When accounting (not accounting) for multiple testing, a total of 219 (3) features had significantly and remarkably ($\log_2$ fold change >1) higher abundance in females than males, including six features that were absent in the latter. These did not include primary nutritional compounds such as sugars or free amino acids, but seven oligopeptides, nine fatty amides, six terpenoids (including three triterpenoid steroids), a glycerophosphate, a flavonoid and the microbial compound N-acetylglucosamine [chitin monomer]. The remaining discriminatory features, including those unique to female nectar, could not be identified *(Figure 6C, Table 2, Supplementary Table 1B)*. On the other hand, 45 (15) features had significant and remarkably ($\log_2$ fold change <-1) higher abundances in males than females with 20 of them being absent in the latter. These included the free proteinogenic amino acid tyrosine (unique), N-(3-Methylbutanoyl)valine (unique), two oligopeptides (one unique), a macrocyclic despipeptide of probable microbial origin (unique), two flavonoid glycosides (one unique), a glycerosphingolipid, and a guanosine derivate (unique, highly significant). The aphid infestation treatment had clearly weaker effects on the composition of plant nectar. Plants showed increased abundances of 0 (10) features including the tetrasaccharide stachyose; and decreased abundances of 0 (18) features including the amino acid phenylalanine, a disaccharidester, and the isoflavonoid genistin *(Figure 6D, Table 2, Supplementary Table S1B)*. The interaction plant sex × aphid infestation treatment shaped the abundance of 21 (7) features *(Figure 6E&F; please note that both plots display the same metabolic features with different group comparisons; Table 2; Supplementary Table S1B)*. These interactions had seven major effect directions, which are listed as labels at the top of both plots. The two most prominent patterns were: significantly and markedly higher abundances in females compared to males under control conditions, but not under aphid infestation for 16 (0) features, including a nucleoside analogue; and significantly and markedly reduced abundances under aphid infestation in females, but not in males in 1(6) features, none of which could be identified.

4. Discussion

Building on the pronounced sexual differentiation in floral traits of the dioecious plant *Silene latifolia*, we examined how controlled infestation by the oligophagous aphid *Brachycaudus lychnidis* affects interactions with the specialist moth pollinator *Hadena bicruris*. Contrary to classical expectations from sexual selection theory, female plants were not outcompeted by males in floral attractiveness under control conditions. Instead, they exhibited high plasticity in floral trait expression and produced diverse, potentially bioactive nectar metabolites, some of which may specifically target female pollinator behaviour. Under aphid infestation, however, female floral attractiveness declined more strongly than in males, suggesting sex-specific defence strategies and highlighting the potential role of indirect defences in stabilizing plant–pollinator–herbivore interactions.

4.1 Female *Silene latifolia* plants challenge expectations: no evidence of reduced floral attractiveness compared to males

Bateman's theory of sexual selection predicts that, relative to males, female reproductive success is more limited by available resources and therefore does not increase with the number of mates, leading to less attractive flowers that serve as a resource exploited and competed for by males (Bateman, 1948). In the present study, we found no evidence for these predictions.

Hadena bicruris moths did not generally prefer single flowers of either sex (*Figure 4B*, *Table 1* - pollinator choice F-M). Female and male plants exhibited no general differences in flower numbers (*Figure 4A* - control conditions), flower sizes (*Table 1* - petal area), and flower colours (*Figure 4C*). Female flowers even featured higher nectar volumes as compared to males (*Figure 6A*) and major nectar sugar constituents showed no sex-specific differences (*Figure 6C*, *Table 3*, *Supplementary Table S1B*). Instead, we observed higher abundances of over 200 other primary and secondary metabolites in females than males – some of which likely shape the nutritional and pharmacological value of nectar for moth pollinators. These included seven oligopeptides, partially enriched in branched-chain amino acids, which may serve as a rapid source of NADH, $FADH_2$, and acetyl-CoA for ATP production, supporting energy demands during flight or allocation to egg production in female moths (Levin *et al.*, 2017; Cavigliasso *et al.*, 2023); nine fatty amides potentially serving as energy stores, metabolic fuel or behavioural modulators (Ranger *et al.*, 2005; Yoshinaga, 2016; Levin *et al.*, 2017); six terpenoids (of which two were steroidal glycosides) that may provide selective feeding or oviposition cues (González-Coloma *et al.*, 2011; Boncan *et al.*, 2020); a glycerophospholipid that may support the maintenance and generation of somatic tissue due to its central role in rapid membrane

synthesis (Toprak *et al.*, 2020); and a flavonoid which may act as health promotor in moth pollinators (Palmer-Young *et al.*, 2019). The function and further identification of the numerous female biased metabolites will be the subject of future studies. As only female plants bear the cost of the interaction with *H. bicruris* (fruit predation), and only female moths impose this cost, there is great potential for the discovery of nectar metabolites interfering with reproduction in female moths. Male *S. latifolia* plants, on the other hand, exhibited higher abundances of over 40 compounds, including several from the same chemical classes and with similar putative functions as those dominant in female nectar (BCAA-rich oligopeptides, flavonoid glycosides, membrane building lipids such as ceramides and glycosphingolipids). Most strikingly, tyrosine and a derivate of guanosine were unique to male nectar. Both compounds can be considered essential for the nutrition of moth pollinators that rely exclusively on nectar as a nitrogen source and may enhance its taste and memorability accordingly (Nepi, 2014). In addition, tyrosine is a precursor for the synthesis of the neurotransmitters tyramine and dopamine, which shape numerous insect behavioural traits like e.g., motivation and learning (Barberis *et al.*, 2023). To the best of our knowledge, our study reports one of the most extensive cases of sexual differentiation in the composition of nectar for a dioecious plant to date (see also (Nepi *et al.*, 2001; Dötterl *et al.*, 2014)). Since *H. bicruris* showed no preference for either plant sex at the single-flower level (*Figure 4B*, *Table 1* - pollinator choice F-M), we conclude that these striking differences complement each other functionally to stabilize a dioecious system relying on frequent visits to both sexes, with plants promoting this by offering essential nectar resources in only one sex.

Overall, our study offers a more nuanced view of female floral traits than predicted by Bateman's principle. A potential criticism is that females in our greenhouse setting did not exhibit lower floral attractiveness as they were not pollinated and hence did not invest in seeds, which aligns with long-term personal observations of visual flower traits in *S. latifolia*. However, this very aspect underscores the limitations of Bateman's theory as it supports that females exhibit adaptive plasticity in floral traits, with attractiveness decreasing only as pollination success increases. High attractiveness of unpollinated female plants may promote female mate competition, post-pollination mate choice in females [84], multiple paternity in fruits, and ultimately greater offspring diversity and fitness [85]. This would be in line with the theory of flexible sex role behaviour [83], which -similar to Bateman's principle- originates from zoology and may be applicable to understanding reproductive strategies in dioecious plants. The substantial among-population variation in sexual dimorphism observed in *S. latifolia* in previous studies (Schrieber *et al.*, 2017, 2019, 2021) further contradicts the assumption of a fixed evolutionary trajectory, instead pointing toward locally driven and context-dependent trait evolution.

4.2 Aphid infestation reduces female floral attractiveness via sex-specific trait changes

In accordance with our hypothesis, infestation with *B. lychnidis* aphids affected a wide range of traits in *S. latifolia* flowers, as well as the behavioural responses of *H. bicruris* moth pollinators. Although most of the observed effects clearly depended on plant sex, we begin with a general discussion of aphid-induced changes, as these have, to our knowledge, not been previously reported.

Aphid infestation reduced flower numbers (more strongly in females than males, *Figure 4A*), and reduced flower light reflection in the blue and green range (only in males, *Figure 4C*). These adverse effects likely result from both resource depletion and the reallocation of resources toward plant defence (Twayana *et al.*, 2022), and may substantially limit plant visibility to pollinators (Barbot *et al.*, 2022). Aphid infestation also moderately reduced the emission of five key volatile organic compounds (VOCs) known to elicit antennal and feeding responses in *H. bicruris* (Dötterl *et al.*, 2006), at the level of individual flowers (*Figure 5B*, *Table 2*). Nectar volume was not lower in aphid-infested plants (*Figure 6A*), likely because they produced fewer flowers and could consequently invest more nectar in each one. While the major sugar constituents remained largely unaffected (*Figure 6D*, *Table 3*), stachyose levels increased in both sexes, suggesting that plants load their phloem with this poorly digestible tetrasaccharide to impair aphid performance (Twayana *et al.*, 2022), while simultaneously ensuring pollination by enzymatically breaking it down into simpler sugars such as fructose, glucose, or sucrose in nectar. Conversely, levels of phenylalanine, a nucleoside analogue, a phenolic acid glycoside, and an isoflavonoid were reduced. These changes may result either directly from phloem-feeding-induced resource depletion or reflect a passive carry-over of defensive phloem sap modifications targeting aphids (Twayana *et al.*, 2022). Although most of these alterations affected both sexes and would be expected to generally reduce pollinator attraction, this was observed only in one sex. It is important to note that our choice assays did not account for the markedly reduced flower numbers at the whole-plant level, which likely amplify the effects of floral chemical changes and thus have a stronger negative impact on pollinator attraction.

At the single-flower level, *H. bicruris* preferred flowers from control plants over aphid-infested ones only in *S. latifolia* females, but not males (*Figure 3*A). These preferences were also dependent on moth sex, being primarily exhibited by female *H. bicruris* (*Table 1*). This behaviour coincided with a reduction of the floral VOC phenylethyl benzoate - a compound not previously described in *S. latifolia* but found in other moth-pollinated species [84] - which was observed only in aphid-infested female plants, but not in males (*Figure 5B*, *Table 2*).

735 Additionally, six unidentified metabolic features in the nectar of *S. latifolia* were reduced by
736 aphid infestation only in female plants (*Figure 6F*, *Table 3*). Four of them simultaneously had
737 higher abundances in female than male plants only under control but not under aphid
738 infestation conditions (*Supplementary table S1B*). Although these effects were only observed
739 before FDR correction, the corresponding effect direction in pollinator behaviour suggests
740 biological relevance, which warrants intensive future investigation. The fact that they do not
741 belong to the major nutritional nectar constituents (sugars, amino acids) again highlights the
742 potential to discover novel secondary nectar metabolites shaping plant-pollinator interactions
743 in the *Silene-Hadena* system. It is highly likely that one or several of the highlighted compounds
744 act as selective feeding cues in female *S. latifolia* for female *H. bicruris*.

745 In summary, our findings support that male plants have more efficient plastic responses to
746 maintain their floral attractiveness under severe herbivore pressure. This is rather difficult to
747 explain with higher resource demands for reproduction in females, as our setup prevented
748 pollination and fruit set. Instead, it points to different defence strategies in males and females,
749 which cannot be fully disentangled with our setup. Our approach bypassed aphid host
750 preferences; direct plant defences preventing the early establishment of aphids, the attraction
751 of aphid parasitoids and predators, and it did not account for resource limitation and
752 competition among sexes that all occur in the field. Indeed, female plants in some dioecious
753 species have been shown to be less frequently attacked by herbivores (Sargent and
754 McKeough, 2022), to investigate more into direct defence (Sargent and McKeough, 2022) and
755 potentially also into indirect defences (Aranda-Rickert *et al.*, 2021). In fact it has been shown
756 that *S. latifolia* females in Europe are clearly less frequently attacked by aphids than males
757 (Wolfe *et al.*, 2004). Hence, they are unlikely to experience aphid infestation as strong as in
758 our experiment under more natural conditions, which may be one explanation for their higher
759 susceptibility. Yet, the cause for lower aphid abundances on female plants remains to be
760 investigated in the future. Sex-specific indirect defences against aphids appear to be a
761 particularly promising research route here. The seed-predating larvae of *H. bicruris* are
762 attacked by a broad range of parasitoids and predators (Villacañas de Castro and Hoffmeister,
763 2020) that -if the plant is involved at all- should be attracted by females only. There is ample
764 evidence that the evolution of indirect defences against different enemy species and guilds is
765 tightly coupled via shared plant signalling and VOC synthesis pathways or the attraction of
766 generalist predators (Kessler and Baldwin, 2001; Heil and McKey, 2003; Rasmann *et al.*,
767 2005). Finally, since female plants are often reported to exploit resources more rapidly than
768 males (Jiang *et al.*, 2016; Bürli *et al.*, 2022), which is indicated for *S. latifolia* in our study
769 through higher leaf biomass and greater chlorophyll content in females (*Table 1*), competition

among sexes in resource limited environments may substantially shift the sex-specific susceptibility patterns.

5. Conclusion and outlook

Our study provides evidence for multidirectional sexual differentiation of visual and chemical flower traits and their responses to aphid infestation in a dioecious plant species. It supports that the expression of sexual dimorphisms in floral attractiveness can be highly plastic depending on the presence of both, pollinators and herbivores, thereby opening exciting avenues for future research. Controlled experiments manipulating the presence of both, pollinators and aphids, ideally under field conditions reflecting natural conditions, while integrating data collection on plant, pollinator and aphid reproductive success with quantitative leaf and flower chemistry are a next step in disentangling the sex-specific co-evolution in this tripartite system. In such future studies, it might be more purposeful to focus on the complementarity of female and male defence and floral attractiveness traits in stabilizing a dioecious reproductive system that is dependent on a nursery pollinator and involves several other parties (aphids, aphid and caterpillar parasitoids and predators), instead of seeking for the better defended or more attractive sex.

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7. Supplementary data

Supplementary Table S1: Summary of floral metabolites in *Silene latifolia*: functional annotation and responses to sex and aphid infestation

Supplementary Figure S1: Representative images from the experimental procedure

8. Author contributions:

"Conceptualization, K.S.; Methodology, K.S., T.S., E.K, T.D., S.U., and W.B., Validation, K.S., T.S., E.K, and W.B.; Formal Analysis, K.S.; Investigation, K.Z., E.K., T.D., and T.S.; Resources, A.E.; S.U., T.S., E.K, T.D. and W.B.; Data Curation, K.S.; Writing – Original Draft Preparation, K.S. and K.Z.; Writing – Review & Editing, K.S., E.K, W.B., S.U., H.B., T.D., T.S; Visualization, K.S.; Supervision, K.S., S.U.; Project Administration, K.S.; Funding Acquisition, K.S.

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11. Data availability

All data / code supporting this article will be deposited in dryad / zenedo and made publicly available upon editorial acceptance of the manuscript

12. References

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Tables

Table 1: *Overview of statistical analyses investigating the effects of aphid infestation treatment (atm) with Brachycaudus lychnidis and Silene latifolia plant sex on plant stress indicators, behaviour of the pollinator Hadena bicruris and plant traits involved in communication and resource exchange with pollinators.*

	(1) Sex	(2) Atm	(3) Cofactor†	(4) Sex × Atm	(5) Sex × Cofactor†	(6) Atm × Cofactor†	(7) Sex × Amt. × Cofactor†	(8) Sex moth	Random effects
Aphid number None, GLMM, NB, none	0.328	-	**0.000**	**-**	-	0.207	-	-	Plant individual
Aphid weight None, LM, G, none	0.535	-	-	**-**	-	-	-	-	-
Leaf biomass None, LM, G, none	**0.035**	0.516	-	0.397	-	-	-	-	-
Chlorophyll Log, LMM, G, D~(1-7)	**0.000**	0.774	**0.000**	0.206	0.530	0.495	0.938	-	Plant individual
Absorption by Flavonoids None, LMM, G, none	**0.028**	0.637	**0.000**	0.167	0.421-	0.539	0.448	-	Plant individual
Pollinator choice C-A None, GLMM, B, none	**0.000**	††	**0.000**	††	0.266	††	††	0.052	Moth individual, trial
Pollinator choice F-M None, GLMM, BB, none	††	0.909	0.954	††	††	0.962	††	0.862	Moth individual, trial
Flower number None, GLMM, P, Z~ I D~(1)	**0.000**[ii]	**0.082**[ii]	**0.000**[ii]	0.238[ii]	0.757[ii]	**0.000**[ii]	**0.012**	**-**	Plant individual
Flower biomass None, LMM, G, D~(3)	**0.000**[ii]	**0.001**[ii]	**0.000**[ii]	0.123[ii]	**0.000**[ii]	**0.000**[ii]	**0.000**	**-**	Plant individual
Flower colour None, LMM, G, D~(1,3,5)	0.394[ii]	**0.000**[ii]	**0.000**[ii]	**0.027**	0.977	**0.016**	0.6908	**-**	Plant individual
Flower area None, GLMM, NB, D~(1-7)	0.348	0.092	**0.000**	0.645	0.124	0.942	0.53	**-**	Plant individual
Scent metabolome PERMANOVA (Bray-Curtis)	0.550	0.081	-	0.477	-	-	-	-	-
Nectar volume None, LMM, G, none	**0.000**	0.554	-	0.225	-	-	-	-	Plant individual
Nectar metabolome (LC) PERMANOVA (Bray-Curtis)	**0.000**	0.204	-	0.308	-	-	-	-	-

The table provides an overview of the model structures with regard to the included responses (column 1), predictors and random effects (row 1); as well as further model characteristics such as response transformations (log = natural logarithm, none = none), model types (GLMM, LMM, LM, PERMANOVA), the assumed error families (B = binomial, BB = betabinomial, G = Gaussian, NB = overdispersed Poisson with quadratic parametrization, P = Poisson), and additional zero-inflation / dispersion formulas (Z / D~ = account for access of zeroes in Poisson data / account for predictor-specific variance inhomogeneity, none = none). Moreover, the table provides an overview of p-values from type III ANOVAs (based on Wald χ^2-test for (G)LMMs,

F-tests for LMs, and permutation statistics with pseudo-F estimation for PERMANOVA). Significant predictor terms are highlighted with bold lettering. Main effects and lower order interaction terms involved in significant (higher order) interactions are marked with "ii". Effects that were not tested are marked with "-". Please note that (†) the identity of cofactors for each response is described in detail in chapter 3.3.6; and (††) that in pollinator behaviour models, the actual test whether pollinators prefer control (C) over aphid infested (A) plants or female (F) over male (M) plants is a post-hoc on the intercept of the binomial model, see chapter 3.2 for details.

Tables

Table 2: *Overview of metabolites highlighted in Figure 5B.*

ID Figure 5	Effect(s)	Effect direction(s)	Molecular formula	Compund class	Compound identity	ID S1
1	None	-	C7H8O2	Benzenoids	2-Methoxy phenol	C15
2	None	-	C8H10O	Benzenoids	2-Phenylethanol	C19
3	Atm	↓	C7H6O	Benzenoids	Benzaldehyde	C07
4	None	-	C8H8O2	Benzenoids	Methyl benzoate	C16
5	None	-	C8H8O3	Benzenoids	Methyl salicylate	C24
6	None	-	C8H8O	Benzenoids	Phenylacetaldehyde	C11
7	None	-	C8H14O2	Fatty acid derivatives	cis-3-Hexenyl acetate	C09
8	None	-	C9H16O2	Mono-terpenoids	Lilac alcohol A	C25
9	None	-	C9H16O2	Mono-terpenoids	Lilac alcohol B + C	C26
10	Atm	↓	C9H16O2	Mono-terpenoids	Lilac alcohol D	C27
11	Atm	↓	C10H16O3	Mono-terpenoids	Lilac alcohol formate A	C34_35
12	Atm	↓	C10H16O3	Mono-terpenoids	Lilac alcohol formate B	C36
13	Atm	↓	C9H14O2	Mono-terpenoids	Lilac aldehyde A	C21
14	Atm	↓	C9H14O2	Mono-terpenoids	Lilac aldehyde B+C	C22
15	None	-	C9H14O2	Mono-terpenoids	Lilac aldehyde D	C23
16	Atm	↓	C10H16	Mono-terpenoids	trans-ß-Ocimene	C12
17	None	-	C5H11NO	N-bearing compounds	anti-3-Methyl-butyl-aldoxime	C06
18	None	-	C5H11NO	N-bearing compounds	syn-3-Methyl-butyl aldoxime	C04
19	Sex*Atm	F↓M-	C15H14O2	Phenyl-propanoids	Benzoic acid, 2-phenylethyl ester	C40
20	Atm	↓	-	-	-	C20

Metabolites highlighted with IDs in Figure 5 were significantly (uncorrected p-value < 0.05) and remarkably (log_2 fold change <-1 or >1) modulated by aphid infestation treatment (atm; ↓ = reduced, ↑ = increased, - = unaffected by aphid infestation*) and sex (F=female, M=male) and/or have been shown to elicit behavioural or antennal responses in the pollinator Hadena bicruris according to* (Dötterl *et al.*, 2006) *(all except Figure ID 19 and 20). Metabolites are listed with their molecular formula, compound class, putative identity, and ID in the full dataset Supplementary Table S1A.*

Tables

1029 ***Table 3:*** *Overview of metabolites highlighted in Figure 6C-F.*

ID Fig 6	Effect direction sex	Effect direction atm	Compund class	Compound identity	ID S1
1	-	-	Carbohydrates & conjugates	D-Glucose	ID0000
2	-	-	Carbohydrates & conjugates	D-Fructose	ID110_125_129
3	-	-	Carbohydrates & conjugates	Sucrose	ID60_138
4	-	-	Carbohydrates & conjugates	Nystose	ID0080
5	-	-	Carbohydrates & conjugates	Melezitose	ID71_142
6	-	-	Carbohydrates & conjugates	Palatinose	ID0117
7	-	↑	Carbohydrates & conjugates	Stachyose	ID0140
8	-	-	Carbohydrates & conjugates	2α-Mannobiose	ID0136
9	F>M	-	Carbohydrates & conjugates	N-Acetylglucosamine	ID0162
10	-	↓	Carbohydrates & conjugates	†Disaccharide glycosylglycerol ester	ID0115
11	**C:F<M, A:F=M**	**F↔, M↓**	**Carbohydrates & conjugates**	**† Nucleoside analogue**	**ID0054**
12	**C:F>M, A:F=M**	**F↔, M↔**	**Carbohydrates & conjugates**	**†Nucleoside analogue**	**ID0111**
13	-	↓	Carbohydrates & conjugates	†Phenolic acid glycoside	ID0033
14	-	↓	Carbohydrates & conjugates	Geinistin	ID0455
15	-	↓	Carbohydrates & conjugates	-	ID0050
16	-	↓	Amino acids, peptides, analogues	DL-Phenylalanine	ID0194
17	**F<M**	**-**	**Amino acids, peptides, analogues**	**L-Tyrosine**	**ID0179**
18	**F<M**	**-**	**Amino acids, peptides, analogues**	**N-(3-Methylbutanoyl)valine**	**ID0164**
19	**F>M**	**-**	**Amino acids, peptides, analogues**	**H-Ser-Leu$_5$-OH**	**ID1690**
20	**F<M**	**-**	**Amino acids, peptides, analogues**	**L-PheNH$_2$,Lys-Leu-Val-Phe**	**ID2889**
21	**F>M**	**-**	**Amino acids, peptides, analogues**	**Unknown peptide**	**ID1655**
22	**F>M**	**-**	**Amino acids, peptides, analogues**	**Unknown peptide**	**ID1700**
23	**F<M**	**-**	**Amino acids, peptides, analogues**	**Unknown peptide**	**ID0047**
24	**F<M**	**-**	**Amino acids, peptides, analogues**	**†Macrocyclic despipeptide**	**ID1489**
25	**F>M**	**-**	**Amino acids, peptides, analogues**	**† Amino alcohol diester**	**ID1870**
26	-	-	Amino acids, peptides, analogues	Trigonelline cation	ID0146
27	-	-	Phenethylamines	Tyramine	ID0181
28	-	-	Quaternary ammonium salts	Choline cation	ID0041
29	-	-	Purines and purine derivatives	Adenosine	ID0185
30	**F<M**	**-**	**Purines and purine derivatives**	**† Guanosine derivate**	**ID0165**
31	**F>M**	**-**	**Glycerophosphates**	**† PG(O-20:1/17:1)**	**ID2141**
32	**C:F>>M, A:F>M**	**F↔, M↔**	**Glycerophosphoethanolamines**	**† Alkyl-PE(C15:0)**	**ID1683**
33	**F<M**	**-**	**Glycosphingolipids**	**† β-GalCer (C20mu:0)**	**ID2724**
34	**C:F=M, A:F<M**	**F↔, M↔**	**Phosphatidylethanolamine**	**† PE(16:0/18:sie2)**	**ID3072**
35	F<M	-	Ceramides	† Bis(bishexyloxybiphenyl)amine	ID4068
36	**C:F<M, A:F=M**	**F↔, M↔**	**Ceramides**	**Palmitoleoyl ethanolamide**	**ID0990**
37	-	-	Fatty amides	Oleamide	ID2445
38	**F<M**	**-**	**Fatty amides**	**† GABA derivate**	**ID2723**
39	**F>M**	**-**	**Fatty amides**	**† Aminoglycerol-Derivate**	**ID1842**
40	**F>M**	**-**	**Fatty amides**	**Fumaric acid diester**	**ID1952**
41	**F>M**	**-**	**Fatty amides**	**†Glyco-hydroxy fatty amide**	**ID1787**
42	**F>M**	**-**	**Fatty amides**	**-**	**ID1633**
43	**F>M**	**-**	**Polyol**	**† Trimethyltrihydroxyundecanyl**	**ID1664**
44	F<M	-	Flavonoid glycosides	† Spiroflavonoid glycoside	ID0293
45	**F<M**	**-**	**Flavonoid glycosides**	**Vitexin-7-Rutinoside**	**ID0294**
46	**F>M**	**-**	**Flavonoid glycosides**	**-**	**ID1611**
47	**F>M**	**-**	**Terpene glycosides**	**-**	**ID2302**
48	**F>M**	**-**	**Triterpenoids**	**† Triterpenoid steroidal glycosides**	**ID2436**
49	**F>M**	**-**	**Triterpenoids**	**† Triterpenoid steroidal glycosides**	**ID2372**

Metabolites highlighted with IDs in Figure 6C-F were saccharides (primary nutritional compounds), well-documented behavioural modulators, and/or putatively identified

compounds that were significantly (FDR corrected [bold lettering] / uncorrected p-value <0.05) and remarkably (log_2 fold change <-1 or >1) modulated by plant sex (F=female, M=male, entries only in column 2), aphid infestation treatment (atm; ↓ = reduced, ↑ = increased under aphid infestation*, entries only in column 3) or the interaction plant sex × aphid infestation treatment (entries in column 2 and 3). Metabolites are listed with their compound class, putative identity, and ID in the full dataset Supplementary Table S1B. †: IUPAC names for the regarding metabolites are replaced with simplified names for compact presentation and fully listed S1B.*

Figures

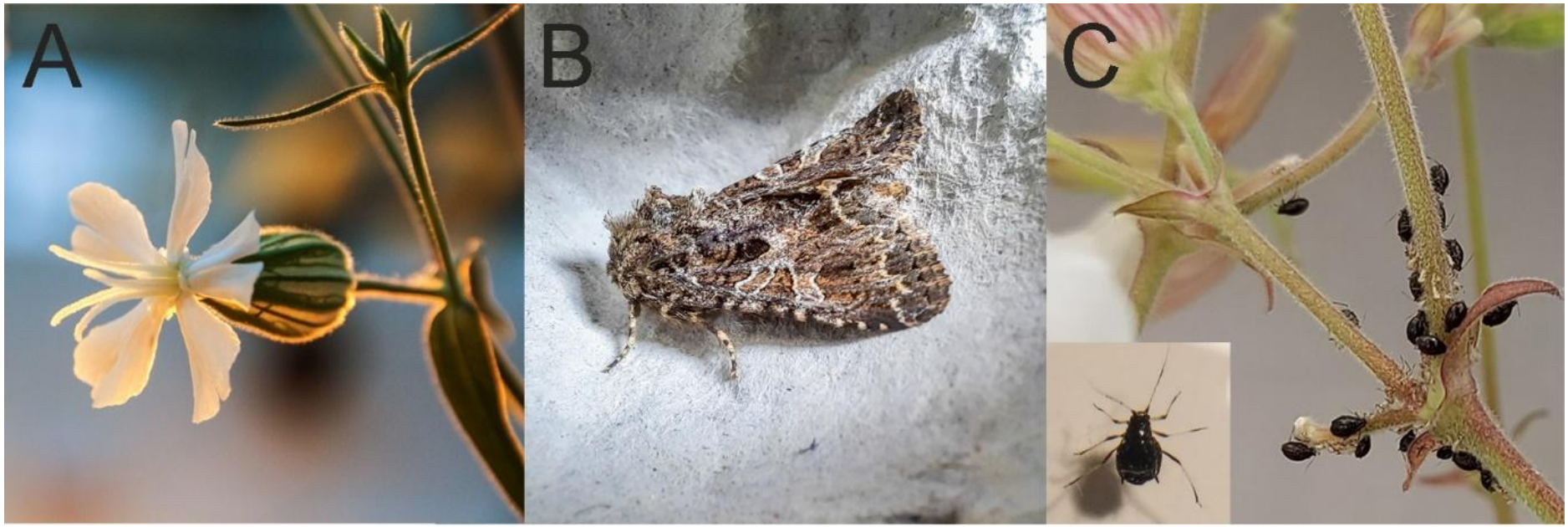


Figure 1: *Plant-pollinator-herbivore study system. (A) Silene latifolia (female), (B) Hadena bicruris (male), (C)* adult *Brachycaudus lychnidis individual and colony feeding on flowering stems of S. latifolia.*

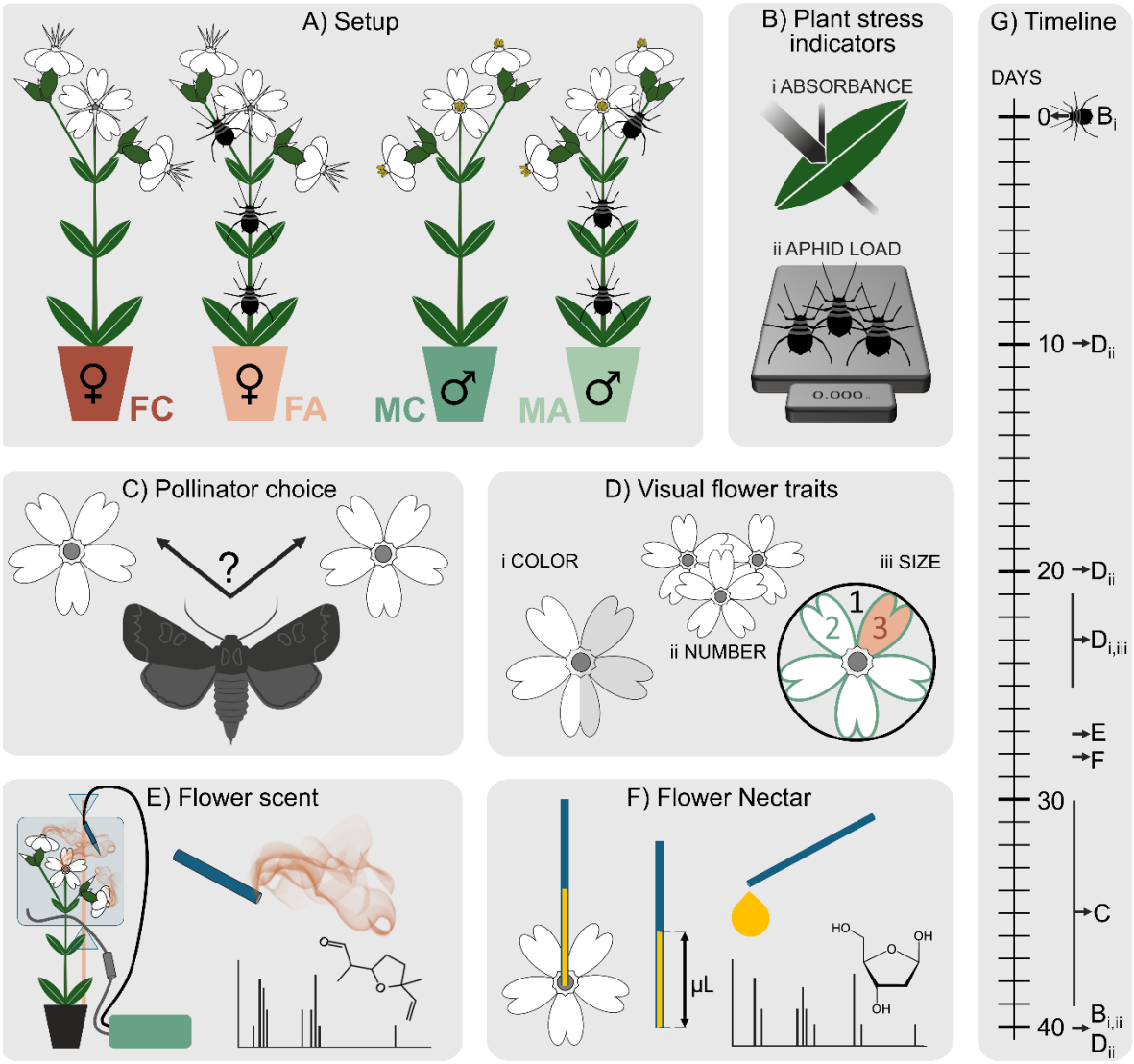


Figure 2: Overview of (A) the experimental setup (abbreviations: FC = control female, FA = aphid infested female, MC = control male, MA = aphid infested male); the data acquisition for (B) the plant stress indicators (i) absorbance by chlorophyll and epidermally located flavonoids and (ii) aphid load in terms of weight and number, (C) pollinator behaviour, (D) visual flower traits related to (i) color, (ii) quantity and (iii) display area, (E) floral scent, and (F) flower nectar; as well as (G) the time line for data acquisition after inoculation of plants with aphids.

Figures

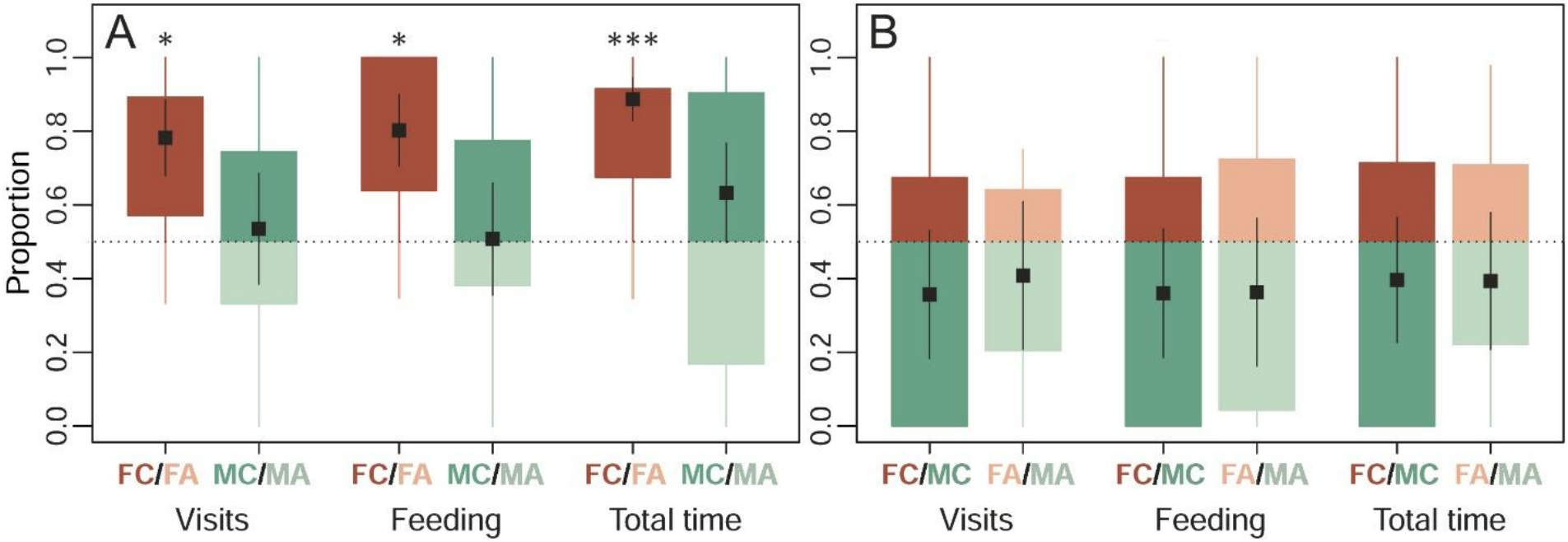


*Figure 3: Effects of Silene latifolia plant sex and infestation by Brachycaudus lychnidis on the behaviour of the moth pollinator Hadena bicruris in terms of flower visits, feeding attempts and the total time spent on a flower. (A) Illustrates choice tests for control (C, deep coloured) versus aphid infested (A, light coloured) female (F, red) and male (M, turquoise) plants (FC/FA: n=11, MC/MA: n=17). (B) Illustrates choice tests for female (red) versus male (turquoise) control (C, dark coloured) and aphid infested (A, light coloured) plants (FC/MC: n=19, FA/MA: n=9). Graphs combine Box-Whisker plots of raw data with the marginal means +/- standard error (black squares) estimated for all groups by the (G)LMMs. Asterisks indicate significant deviations of the binary outcome from 0.5 (i.e., significant preference > 0.5 > aversion; significance levels: ***p<0.001, **p<0.01, *p<0.05).*

Figures

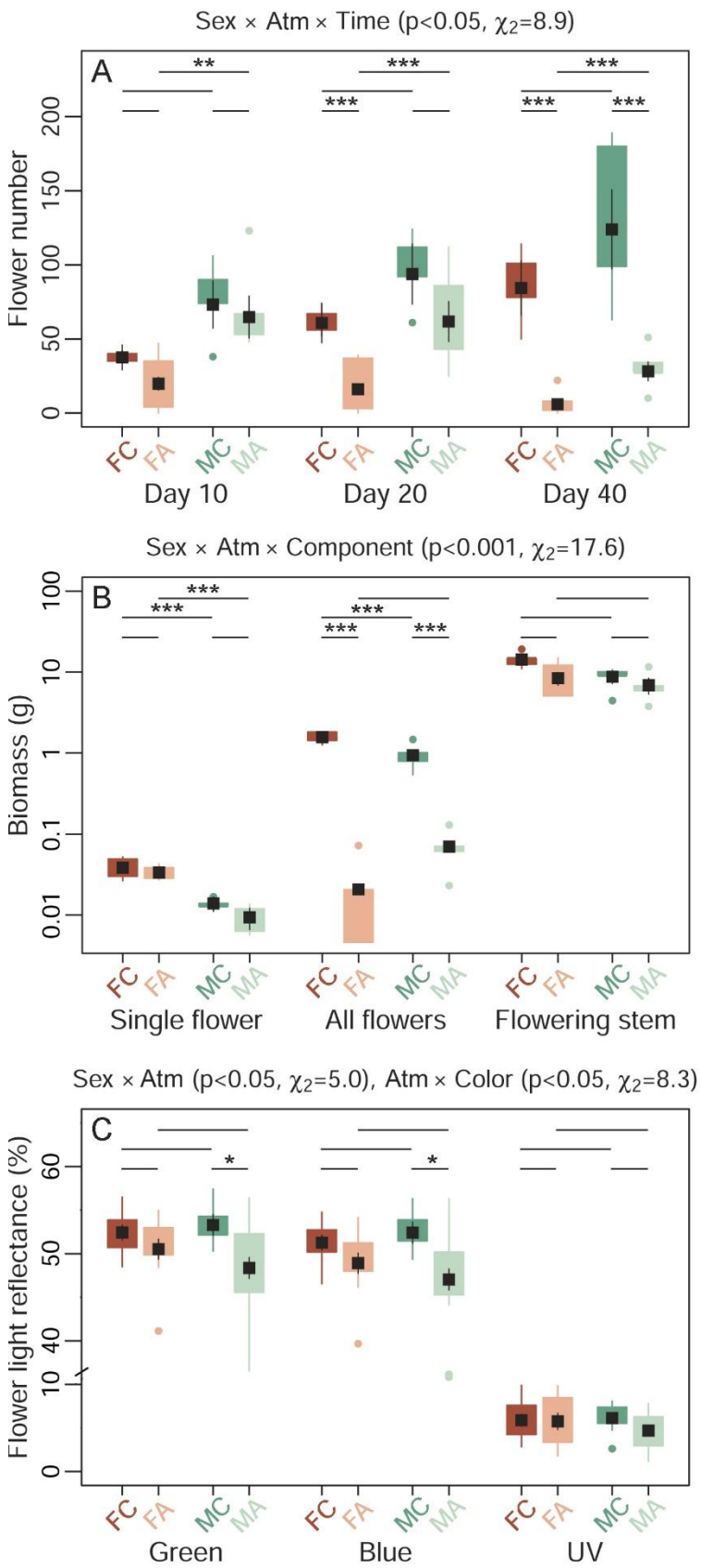


Figure 4: *Combined effects of Silene latifolia plant sex and Brachycaudus lychnidis aphid infestation treatment on (A) flower numbers, (B) reproductive biomasses, and (C) flower colours. The graphs combine Box-Whisker plots of raw data for female control (FC, dark red, n = $5_{(A,B)}/14_{(C)}$), female infested (FA, light red, n = $5_{(A,B)}/7_{(C)}$), male control (MC, dark turquoise, n = $5_{(A,B)}/13_{(C)}$), and male infested (MA, light turquoise, n = $5_{(A,B)}/13_{(C)}$) plants with the marginal means +/- standard error (black squares) for these groups estimated by the (G)LMMs. Significant effects detected via type III ANOVAs based on Wald χ^2 tests are denoted at the top of the plot, while the results of relevant post-hoc comparisons are denoted within the plots (significance levels: ***p<0.001, **p<0.01, *p<0.05).*

Figures

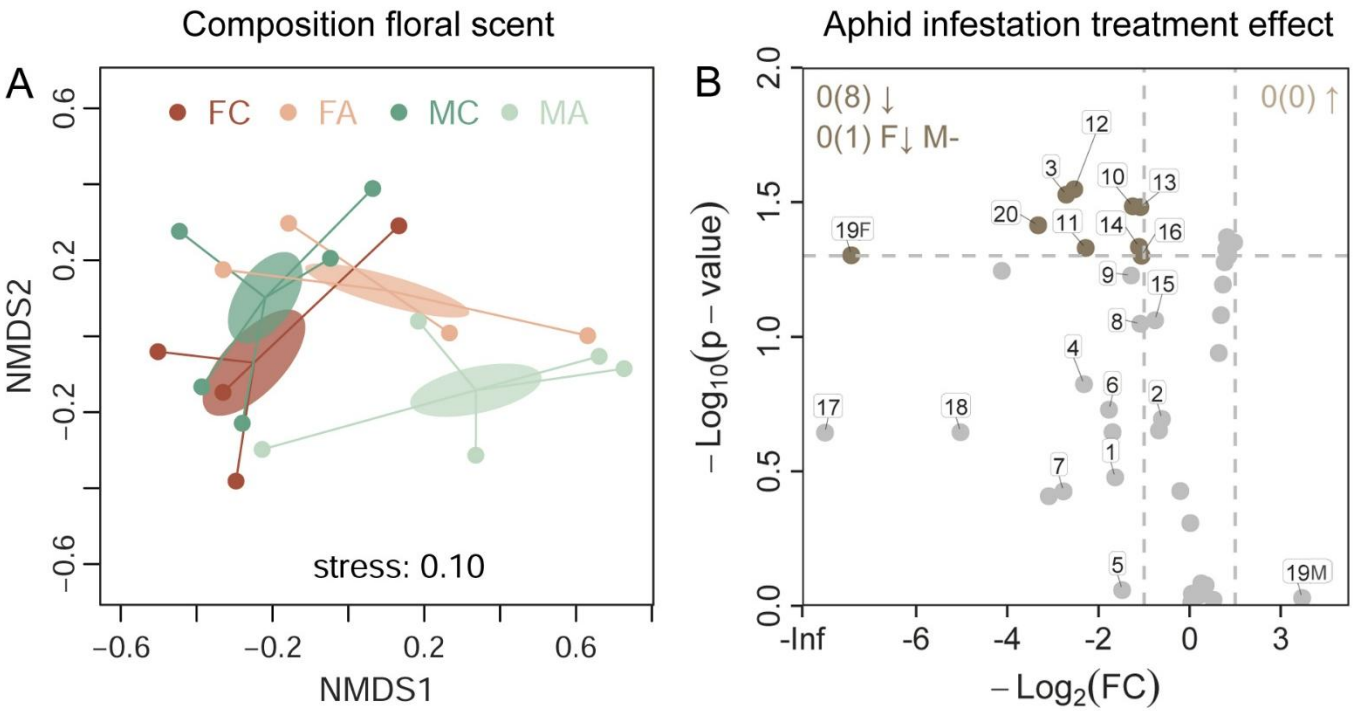
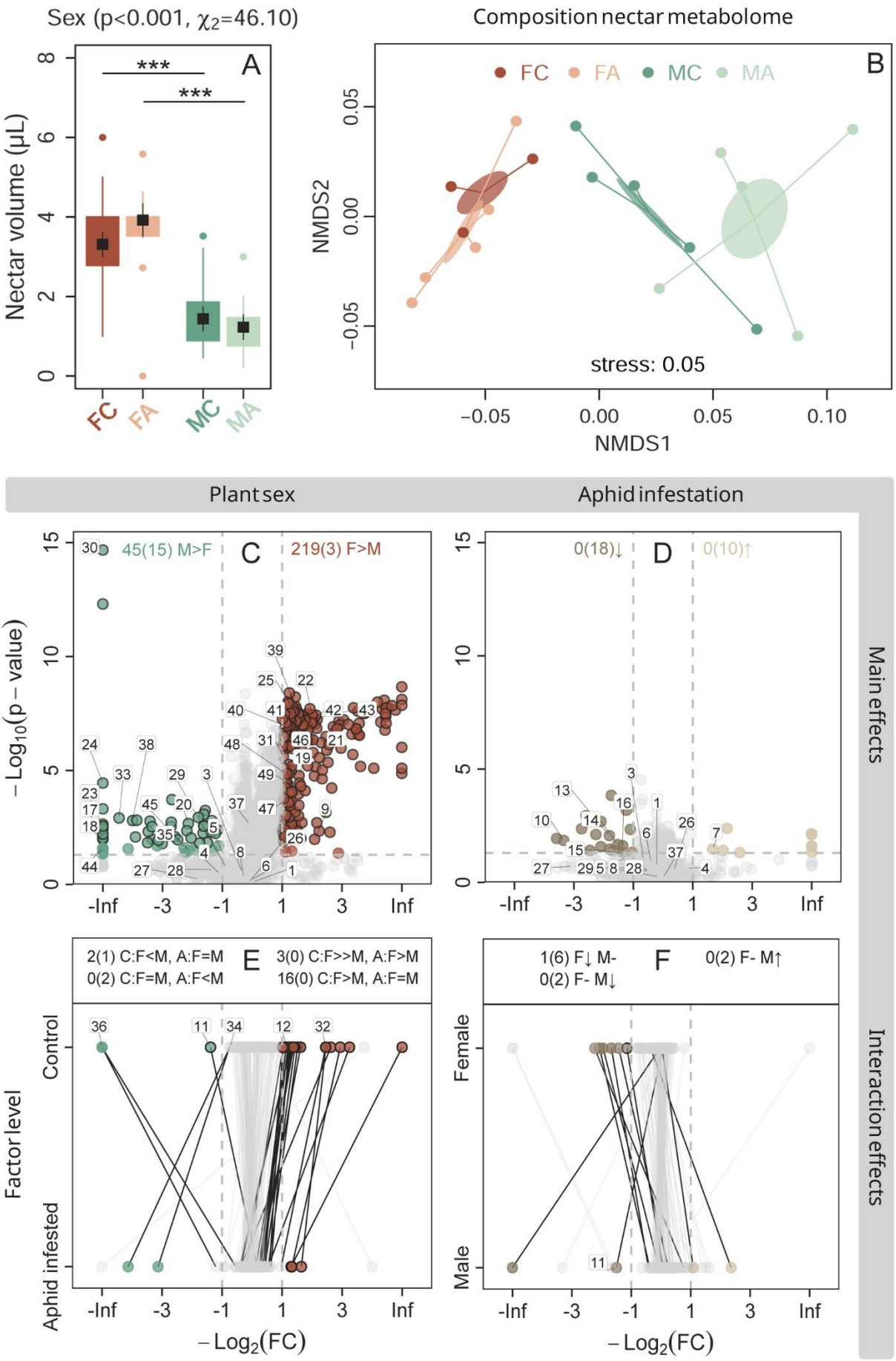


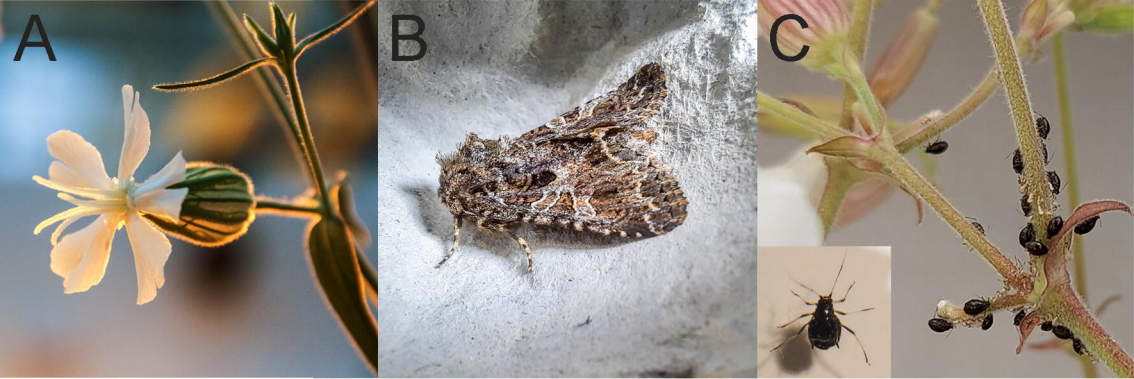
Figure 5: *Combined effects of Brachycaudus lychnidis aphid infestation treatment and plant sex on floral headspace VOC composition in Silene latifolia. (A) The NMDS ordination for female control (FC, dark red, n = 4), female infested (FA, light red, n = 4), male control (MC, dark turquoise, n = 5), and male infested (MA, light turquoise, n = 5) plants shows plant individual scores (points), distances to group centroids (spiders), and their standard error (ellipses). (B) Volcano plot summarizing the results of model loops, i.e., the main and interactive effects of plant sex and aphid infestation treatment on the abundance of individual metabolites. Metabolic features modulated by aphid infestation are highlighted (log_2 fold change <-1 or >1 and uncorrected [after FDR correction all effects non-significant] p-value <0.05 = coloured) with their effect direction (increased | decreased under aphid infestation (↑ | ↓) = brown | beige). A single metabolic feature (ID 18) was shaped by the interaction aphid infestation treatment × plant sex and is illustrated with one point for female (F) and male (M) plants. Numbered metabolic features correspond to Table 2. For further details see Supplementary Table S1A.*

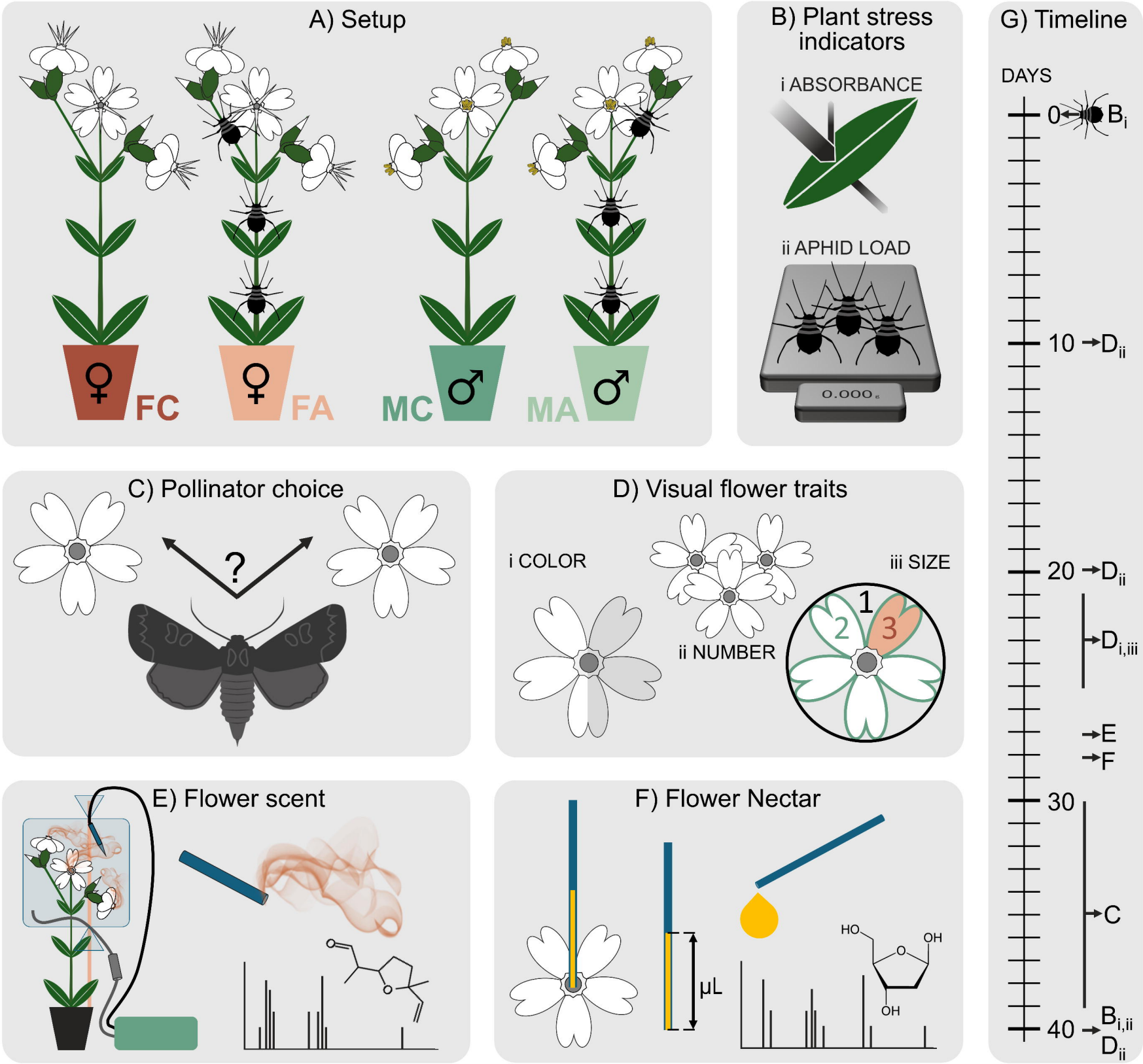
Figures

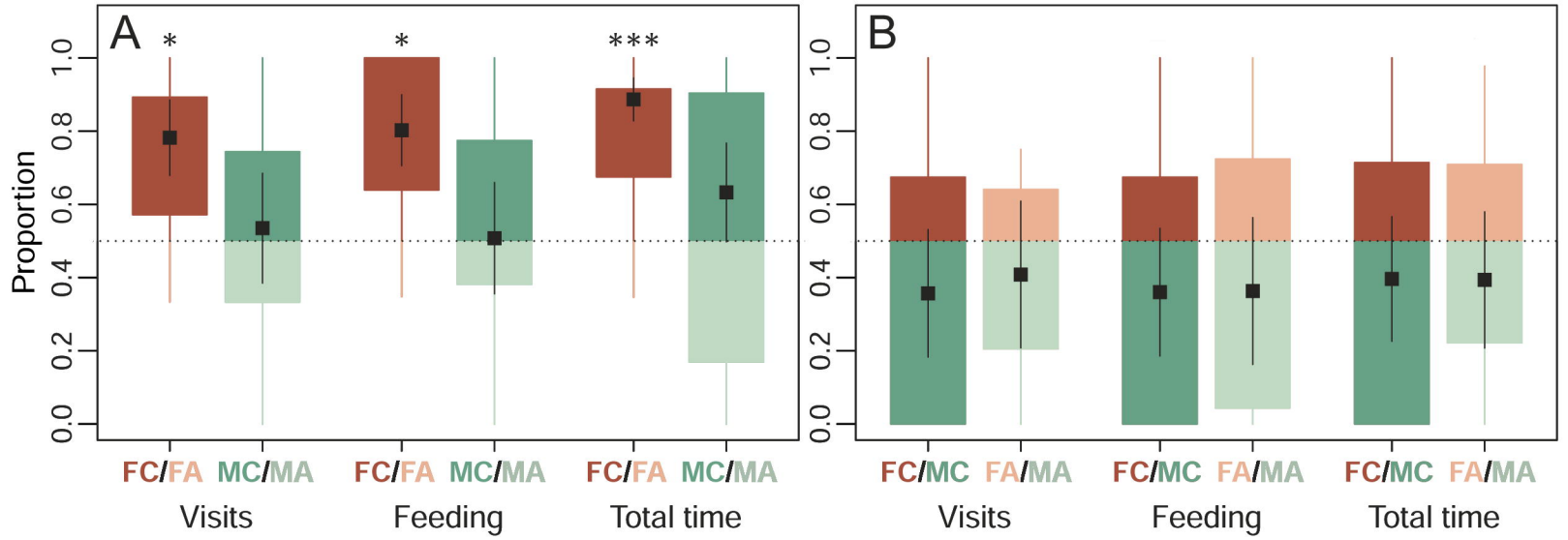


1057

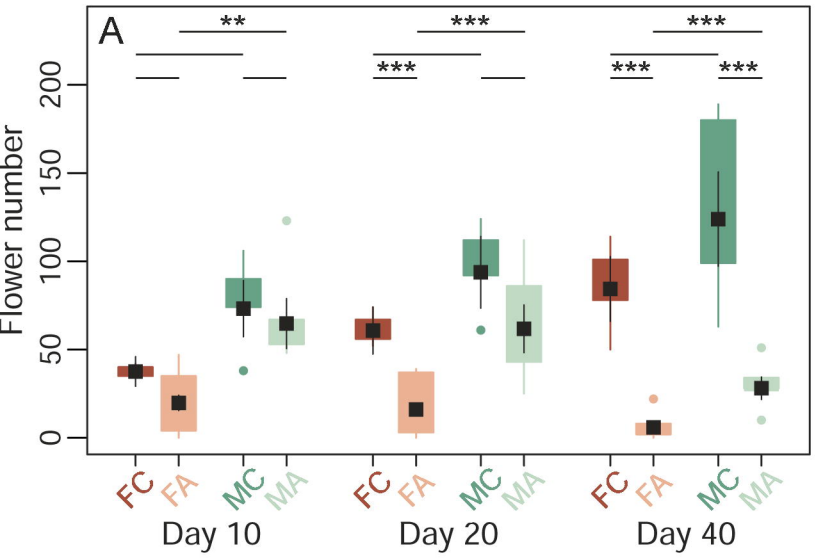
Figure 6: *Combined effects of Silene latifolia plant sex and Brachycaudus lychnidis aphid infestation treatment on (A) nectar volume, and (B-F) the metabolic composition of a standardized nectar volume. (A) Box-Whisker plots of raw data for female control (FC, dark red, n = 23), female infested (FA, light red, n = 13), male control (MC, dark turquoise, n = 25), and male infested (MA, light turquoise, n = 25) plants with the marginal means +/- standard error (black squares) for these groups estimated by the (G)LMMs. Significant effects detected via type III ANOVAs based on Wald χ^2 tests are denoted at the top of the plot, while the results of relevant post-hoc comparisons are denoted within the plots (significance levels: ***p<0.001, **p<0.01, *p<0.05). (B) The NMDS ordination for FC (n = 5), FA (n = 3), MC (n = 5), and MA (n = 5) plants shows plant individual scores (points), distances to group centroids (spiders), and their standard error (ellipses).(C-F) Volcano plots illustrate the results of model loops, i.e., the main and interactive effects of plant sex and aphid infestation treatment on the abundance of individual metabolic features. Modulated features are highlighted (FDR corrected [uncorrected] p-value <0.05 and log_2 fold change <-1 or >1 = coloured with black margin [coloured without margin]) with their effect direction (plant sex: F>M = red, M>F = turquoise, aphid infestation treatment: increased under aphid infestation (↑) = brown, decreased (↓) = beige). Interaction effect plots adopt the same modulation colour coding for (E) the sex effect within control and aphid infested plants and (F) the aphid infestation effect within females and males. Identical features within these group comparisons are connected by lines. Numbered metabolic features correspond to Table 3. A maximum of 15 identified features was highlighted for each modulation direction. For further details see* Supplementary Table S1B*.*


A
B
C

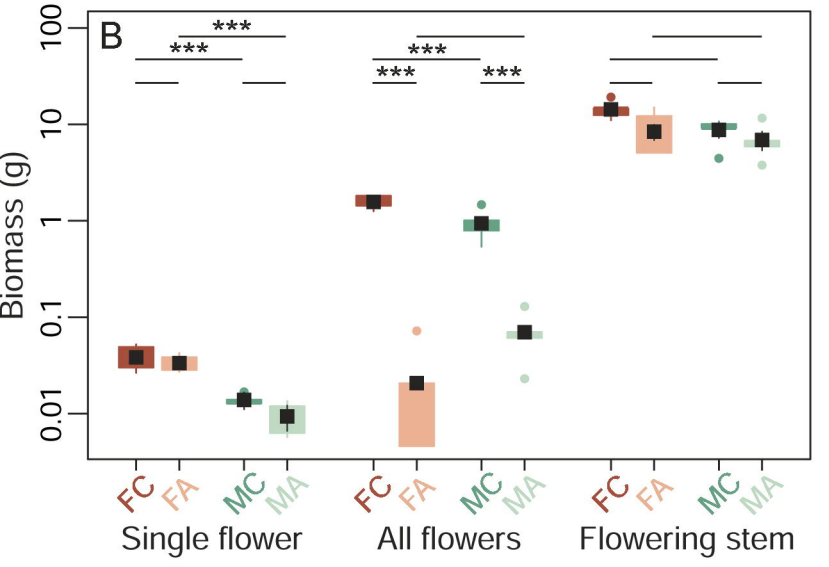

A) Setup
FC
FA
MC
MA
B) Plant stress indicators
i ABSORBANCE
ii APHID LOAD
0.000 g
G) Timeline
DAYS
0
B_i
10
D_{ii}
20
D_{ii}
$D_{i,iii}$
E
F
30
C
40
$B_{i,ii}$
D_{ii}
C) Pollinator choice
?
D) Visual flower traits
i COLOR
ii NUMBER
iii SIZE
1
2
3
E) Flower scent
F) Flower Nectar
µL
HO
OH
O
OH


A
B
Proportion
0.0
0.2
0.4
0.6
0.8
1.0
*
*
***
FC/FA
MC/MA
FC/FA
MC/MA
FC/FA
MC/MA
Visits
Feeding
Total time
FC/MC
FA/MA
FC/MC
FA/MA
FC/MC
FA/MA
Visits
Feeding
Total time

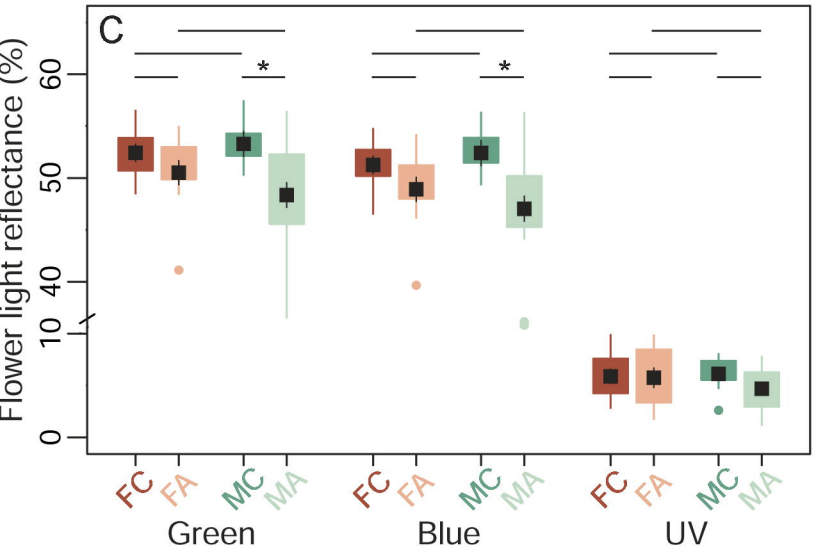
Sex × Atm × Time ($p<0.05$, $\chi_2=8.9$)

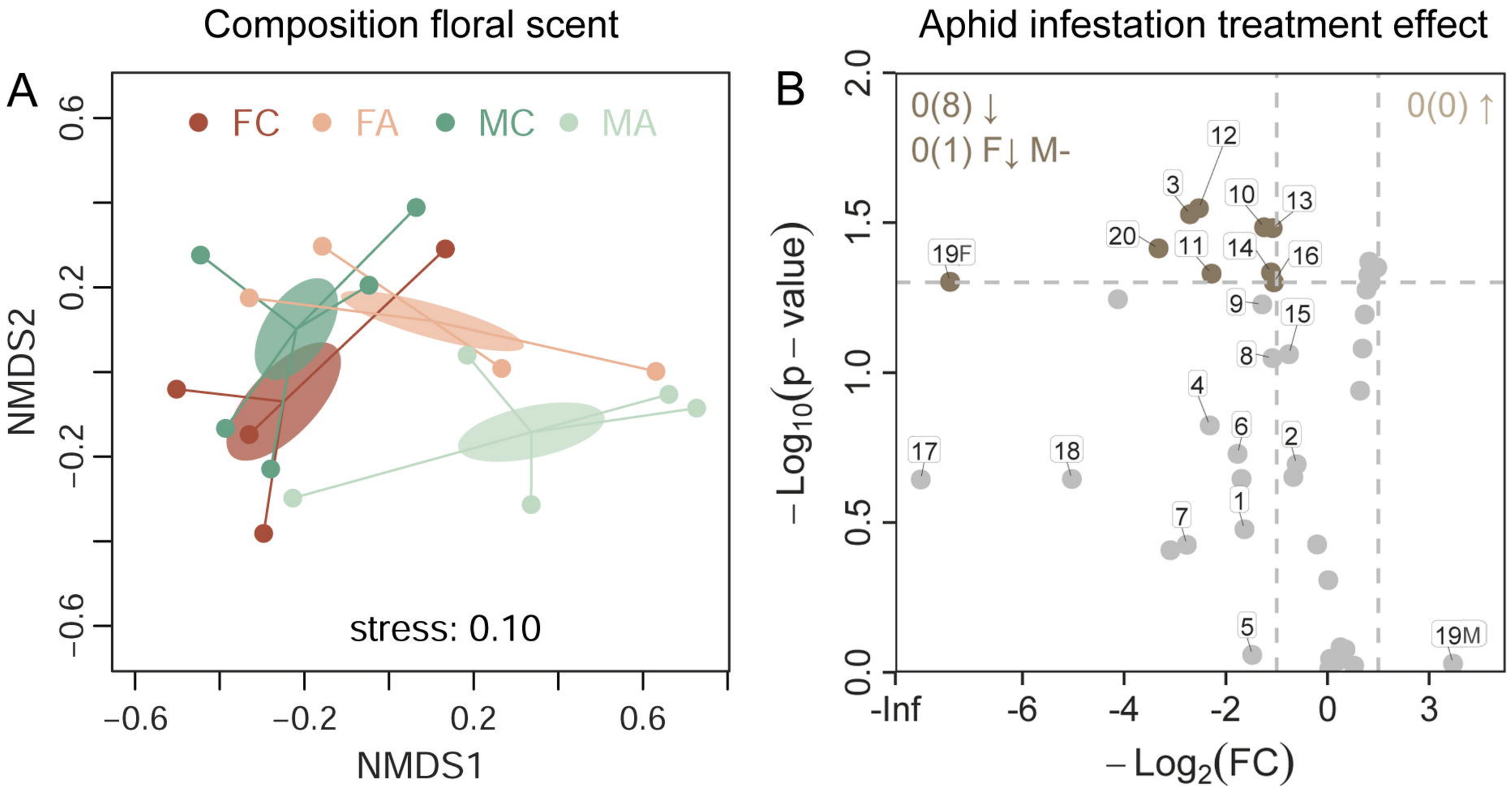


Sex × Atm × Component ($p<0.001$, $\chi_2=17.6$)

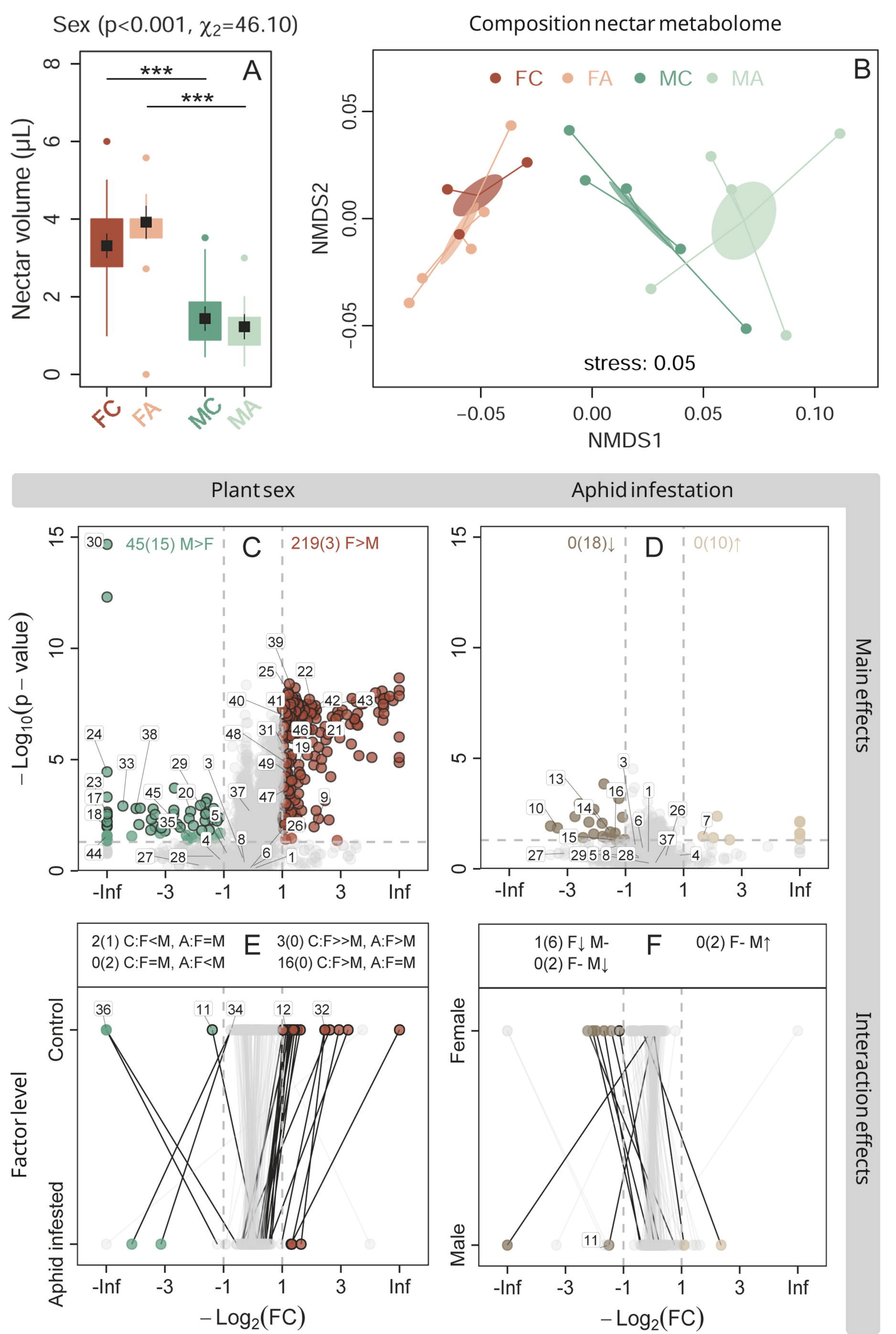


Sex × Atm ($p<0.05$, $\chi_2=5.0$), Atm × Color ($p<0.05$, $\chi_2=8.3$)





Composition floral scent
A
FC
FA
MC
MA
stress: 0.10
NMDS1
NMDS2
−0.6
−0.2
0.2
0.6
Aphid infestation treatment effect
B
0(8) ↓
0(1) F↓ M-
0(0) ↑
12
3
10
13
20
11
14
16
19F
9
15
8
4
6
2
17
18
1
7
5
19M
−Log10(p − value)
−Log2(FC)
-Inf
-6
-4
-2
0
3
0.0
0.5
1.0
1.5
2.0


Sex (p<0.001, χ2=46.10)
A
***
***
Nectar volume (µL)
FC
FA
MC
MA
Composition nectar metabolome
B
FC
FA
MC
MA
NMDS2
NMDS1
stress: 0.05
Plant sex
Aphid infestation
Main effects
C
45(15) M>F
219(3) F>M
D
0(18)↓
0(10)↑
−Log10(p−value)
Interaction effects
E
2(1) C:F<M, A:F=M
0(2) C:F=M, A:F<M
3(0) C:F>>M, A:F>M
16(0) C:F>M, A:F=M
F
1(6) F↓ M-
0(2) F- M↓
0(2) F- M↑
Factor level
Control
Aphid infested
Female
Male
−Log2(FC)