

Trichobaris weevils distinguish amongst toxic host plants by sensing volatiles that do not affect larval performance

GISUK LEE,*†¹ YOUNGSUNG JOO,* CELIA DIEZEL,* EUN JU LEE,† IAN T. BALDWIN* and SANG-GYU KIM*¹

*Department of Molecular Ecology, Max Planck Institute for Chemical Ecology, Hans-Knöll-Straße 8, Jena D-07745, Germany,

†School of Biological Science, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 08826, Korea

Abstract

Herbivorous insects use plant metabolites to inform their host plant selection for oviposition. These host-selection behaviours are often consistent with the preference–performance hypothesis; females oviposit on hosts that maximize the performance of their offspring. However, the metabolites used for these oviposition choices and those responsible for differences in offspring performance remain unknown for ecologically relevant interactions. Here, we examined the host-selection behaviours of two sympatric weevils, the *Datura* (*Trichobaris compacta*) and tobacco (*T. mucorea*) weevils in field and glasshouse experiments with transgenic host plants specifically altered in different components of their secondary metabolism. Adult females of both species strongly preferred to feed on *D. wrightii* rather than on *N. attenuata* leaves, but *T. mucorea* preferred to oviposit on *N. attenuata*, while *T. compacta* oviposited only on *D. wrightii*. These oviposition behaviours increased offspring performance: *T. compacta* larvae only survived in *D. wrightii* stems and *T. mucorea* larvae survived better in *N. attenuata* than in *D. wrightii* stems. Choice assays with nicotine-free, JA-impaired, and sesquiterpene-over-produced isogenic *N. attenuata* plants revealed that although half of the *T. compacta* larvae survived in nicotine-free *N. attenuata* lines, nicotine did not influence the oviposition behaviours of both the nicotine-adapted and nicotine-sensitive species. JA-induced sesquiterpene volatiles are key compounds influencing *T. mucorea* females' oviposition choices, but these sesquiterpenes had no effect on larval performance. We conclude that adult females are able to choose the best host plant for their offspring and use chemicals different from those that influence larval performance to inform their oviposition decisions.

Keywords: *Datura wrightii*, host selection behaviour, *Nicotiana attenuata*, secondary metabolites, *Trichobaris compacta*, *Trichobaris mucorea*

Received 29 January 2016; revision received 24 April 2016; accepted 27 April 2016

Introduction

Herbivorous insects select their host plants for food or oviposition sites based on chemicals produced by plants (Thompson 1988; Price 1997). Several hypotheses have been suggested to explain why herbivorous insects choose certain plants over others. The preference–

performance hypothesis, also known as optimal oviposition theory (Jaenike 1978; Thompson 1988), predicts that female insects preferentially oviposit on certain host plants to maximize their larvae's performance (Jaenike 1978). When larvae are limited in their abilities to relocate, the female's choice of oviposition sites strongly influences their offspring's performance (Thompson & Pellmyr 1991; Zalucki *et al.* 2002; Bertheau *et al.* 2009; Clark *et al.* 2011; Mazaheri *et al.* 2011; Zhang *et al.* 2012). However, many other studies have revealed that adult choice and offspring performance are frequently poorly correlated (Jallow & Zalucki 2003; Brodbeck *et al.*

Correspondence: Ian T. Baldwin and Sang-Gyu Kim,
Fax: +82-42-878-8399; E-mails: baldwin@ice.mpg.de and
sgkim@ibs.re.kr

¹Current address: Center for Genome Engineering, Institute for Basic Science, Yuseong-gu, Daejeon 34047, Korea

2007; Gripenberg *et al.* 2007; Chatzigeorgiou *et al.* 2010; Shikano *et al.* 2010). Some adult females select host plants to obtain more nutrition for themselves, even when it decreases offspring performance (Scheirs & Bruyn 2002), or to minimize the influence of natural enemies or disease on their offspring, mortality factors frequently overlooked in laboratory assays (Barbosa & Krischik 1987; Valladares & Lawton 1991; Clark *et al.* 2011).

Plant secondary metabolites – the chemical cues mentioned above – affect host selection behaviour of insect herbivores and also act as attractants or deterrents influencing the location of oviposition sites (Rosenthal & Berenbaum 1992; Awmack & Leather 2002; Powell *et al.* 2006). For instance, high levels of glucosinolates in *Arabidopsis thaliana* negatively affect the oviposition behaviour of the phloem-feeding insect, *Bemisia tabaci*, and also the performance of both adults and nymphs in laboratory assays (Markovich *et al.* 2013), but some glucosinolates produced by *Brassicaceae* plants stimulate the oviposition behaviour of leaf herbivores, for example *Plutella xylostella* and *Delia radicum* (Marazzi & Städler 2004; Renwick *et al.* 2006; Sarfraz *et al.* 2006). In other examples, tobacco hornworms prefer to feed on indolide D-producing solanaceous plants (del Campo *et al.* 2001), while alder leaf beetles prefer to feed on *Salix* plants producing high levels of phenolic compounds (Ikonen *et al.* 2002); catechol in roasted coffee bean is an oviposition stimulant for cigarette beetles (Nagasawa *et al.* 2014), while volatile monoterpenes are deterrents for Christmas beetles (Matsuki *et al.* 2011).

Plants that are genetically engineered to silence the biosynthetic genes of certain metabolites have frequently been used to examine the function of metabolites in plant–insect interactions. Field experiments with isogenic *Nicotiana attenuata* plants altered in the levels of single metabolites, such as nicotine (Steppuhn *et al.* 2004), green leaf volatiles (Allmann & Baldwin 2010) or diterpene glycosides (Heiling *et al.* 2010), have clearly demonstrated that these metabolites play powerful roles in plant defence against herbivore attack. However, less is known about how altering the levels of single metabolites affects host selection behaviour and offspring performance. Species of the genus *Trichobaris*, known as *Solanaceae* weevils, attack tobacco, potato, eggplant, tomato or other cultivated solanaceous plants (O'Brien & Wibmer 1982). While *Trichobaris* adults feed mainly on leaves, their larvae feed inside the stems of plants (Barber 1935; Woodside 1949). In a previous study, we found more *Trichobaris mucorea* larvae infesting *N. attenuata* plants that were silenced in jasmonic acid (JA) production and therefore had less JA-elicited defence-related secondary metabolites compared to wild-type (WT) plants, indicating that these JA-induced

metabolites play an important role in resistance against *T. mucorea* attack. Because the larvae spend their entire lives inside the stems of a single plant until they emerge as adults (Diezel *et al.* 2011), the host selection of *T. mucorea* larvae depends entirely on the female's oviposition decisions.

Here, we identified another *Trichobaris* species, *Trichobaris compacta*, also known as the *Datura wrightii* weevil (Barber 1935), at our field station. Although both *T. compacta* and *T. mucorea* are distributed in similar habitats throughout Arizona, California and Utah in the southern United States and Mexico (www.sil.si.edu/bcaproject), their exact host range and host selection behaviour were not known. We examined the host range of *T. compacta* and *T. mucorea* in the field and investigated whether the feeding preferences of the adults of the two *Trichobaris* species were correlated with their oviposition preferences and if their oviposition preferences positively affected larval performance. We used transgenic lines of *N. attenuata*, which produced fewer defence metabolites or more terpenoid volatiles compared to control plants, to understand how plant metabolites affect the preference of adults and the performance of larvae in these two sympatric *Trichobaris* species.

Materials and methods

Plant and insect growth conditions

Wild-type *Nicotiana attenuata* (Torr. ex Wats.) (WT) originated from a native population located 25 km north of the field station where the current work was performed (see Fig. S1, Supporting information) in southwestern Utah. The 14th and 31st, respectively, inbred generation of this accession was stably transformed to alter the expression of the following secondary metabolites and previously fully characterized in isogenic homozygous lines harbouring a single transgene insertion: irPMT (108-3 line) and irAOC (457-1 line) plants are reduced to 3–4% of WT nicotine levels (Steppuhn *et al.* 2004) and 0.01% of elicited WT jasmonic acid levels (Kallenbach *et al.* 2012), respectively, and ovTPS10 plants (10-3 line) that produce 50-fold more (E)- β -farnesene and 100-fold more (E)- α -bergamotene compared to elicited WT plants (Schuman *et al.* 2014). Germination procedures and glasshouse conditions have been described previously (Krügel *et al.* 2002). Young seedlings were planted in Teku plastic pots 10 days after germination; after 10 days, plants were transferred to 2-L pots. *Datura wrightii* (Regel) seeds were obtained from B & T World Seeds (Paguignan, France), and later, the seeds were harvested from inbred plants in the glasshouse. *D. wrightii* plants were

grown in 2-L pots under the same growth conditions as *N. attenuata* plants. All plants were grown in the glasshouse with 16-h light (26–28 °C) and 8-h dark (22–24 °C) cycles under Master Sun-T PIA Agro 400 or Master Sun-T PIA Plus 600 high-pressure sodium lights (Philips) with water supplied daily via an automatic watering system.

In 2013, *T. compacta* and *T. mucorea* adults were collected from their natural habitat, the Great Basin Desert in southwestern Utah (D1–D5 sites; Fig. S1, Supporting information). With these field-collected adults, a laboratory colony was established (Fig. S2, Supporting information). *T. mucorea* and *T. compacta* adults were fed *D. wrightii* leaves/floral buds and *Trichobaris* females laid eggs on the petioles of *N. attenuata* or floral buds of *D. wrightii*. Collected eggs were experimentally inoculated into the basal stems in *N. attenuata* or *D. wrightii* (Fig. S3, Supporting information). Three weeks later, the egg-inoculated stems were split to collect the larvae (in the WT plants, larvae were usually in the 2nd or 3rd instars). Each *Trichobaris* larva was transferred to a 15-mL tube filled with an artificial diet developed for stem weevil larvae (Malone & Wigley 1990). About 1 week later, we collected the newly emerged adults from the tubes. All steps were performed in a growth chamber with a 16-h light (26 °C) and 8-h dark (24 °C) cycle and 65% humidity (Snijders Scientific, Tillburg, The Netherlands).

Adult preference

Adult preference was evaluated with feeding and oviposition behaviour assays conducted in a white mesh cage (47.7 × 47.5 × 93.0 cm, BugDorm, BioQuip, Rancho Dominguez, CA). During these experiments, cages were placed in a glasshouse at 26–28 °C under 16-h supplemental lights from Philips SON-T Agro 400 (Philips) sodium lights. We used two size-matched plants both in the early elongation stage (about 13–15 cm) of growth (the stage at which natural ovipositions in the field are known to occur) in 2-L pots (diameter 19 cm × height 15 cm) per cage for a dual-choice assay. The orientation of plants and cages were randomly distributed in the glasshouse to avoid position and orientation effects. Newly emerged females were allowed to mate in single pairs for 2 days before being used for the experiments. In each cage, we placed a small plastic cup equidistant between the two plants and released three gravid females into the cup. After 3 days, we measured the leaf area consumed by females and counted the number of eggs oviposited to quantify the females' feeding and oviposition preferences ($8 \leq n \leq 14$; Fig. S4, Supporting information).

Larval performance

To examine the larval performance of two *Trichobaris* species, we used an experimental egg inoculation method which mimics the natural oviposition behaviour of both *Trichobaris* species (Fig. S3, Supporting information). *Trichobaris* eggs collected from *N. attenuata* or *D. wrightii* plants were singly inserted into the slightly abraded epidermal layers of the basal part of a plant stem. After 3 weeks, we split the stems of egg-inoculated plants, counted the larvae that had survived and measured larval mass ($n = 20$).

Statistical analysis

The total leaf area consumed of each pair of plants was analysed by a Student's *t*-test. The number of eggs deposited on each pair of plants was analysed by a replicated goodness-of-fit G-test with the null hypothesis of no preference (Sokal & Rohlf 1995). Larval mass was analysed by one-way ANOVA followed by Fisher's least significant difference (LSD) test, and survival rates were analysed by chi-square test. All statistical tests were conducted with Origin 8 SR1 (OriginLab Cop. Northampton, Massachusetts, USA) and the publically available R package (version 3.1.2., <http://www.r-project.org/>).

Results

Field observations and species identification of the two *Trichobaris* species

To examine the behaviour of *T. compacta* and *T. mucorea* in the field, we needed a rapid and reliable means of distinguishing these sibling species. From more than 200 *Trichobaris* adults collected from *Datura* and *Nicotiana* populations in 2013 and 2014 (Fig. S1, Supporting information), we were able to identify two *Trichobaris* species, *T. compacta* and *T. mucorea*, based on the shape of the male reproductive organs (Fig. S5, Supporting information; Barber 1935). After careful examination, we identified three additional morphological traits that distinguished the two species: an orange band on the head of *T. mucorea* (white inverted triangle) which was absent from *T. compacta*, a black strip on the lateral aspect of *T. mucorea* thorax (red inverted triangle), again absent from *T. compacta*, and three black spots (orange inverted triangle) on the dorsal part of *T. mucorea* which were larger than those in *T. compacta* (Fig. 1a, b). To quantify adult preferences for the two host plants, we counted in the 2014 field season the number of *Trichobaris* adults in five *D. wrightii* populations and four *N. attenuata* populations in the Great

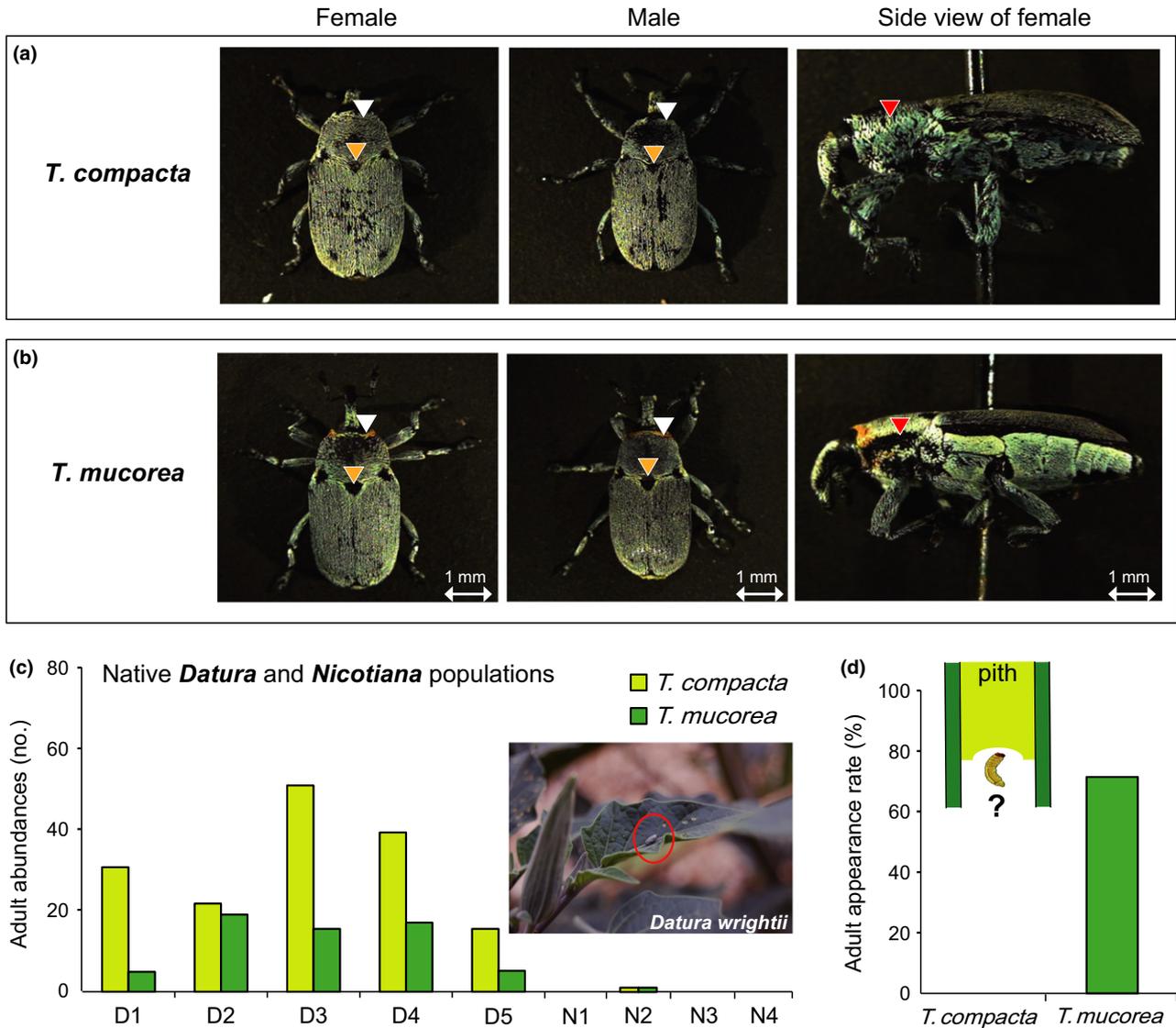


Fig. 1 Identification of two sympatric *Trichobaris* species from the Great Basin Desert. Morphological differences between two *Trichobaris* species from adults collected from the Great Basin Desert in southwestern Utah. (a) Dorsal view of *Trichobaris compacta* female, male and lateral view of female. (b) Dorsal view of *T. mucorea* female, male and lateral view of female. Three morphological traits highlighted by white, red and orange inverted triangles distinguish the adults of the two *Trichobaris* species: an orange band on the head part of *T. mucorea* (white inverted triangle) which is absent from *T. compacta*, a black strip on the lateral aspect of *T. mucorea* thorax (red inverted triangle), again absent from *T. compacta*, three black spots (orange inverted triangle) on the dorsal part of *T. mucorea* which is larger than those on *T. compacta*. Larval stages are not readily distinguished. (c) Relative abundance of *T. compacta* and *T. mucorea* adults found in *Datura wrightii* and *Nicotiana attenuata* populations (see Fig. S1, Supporting information). Both *Trichobaris* species were observed in the five populations of *D. wrightii* but rarely observed in the four *N. attenuata* populations. (d) Larvae collected which were 2nd or 3rd instar stage ($n = 14$) from *N. attenuata* plants ($n = 200$) growing in the N2 population (see Fig. S1, Supporting information). Ten larvae were successfully reared to adulthood and produced *T. mucorea* adults, and 4 larvae died at the larval stage.

Basin Desert (Fig. S1, Supporting information). Although *T. mucorea* is known as a tobacco stem borer of *N. attenuata* (Barber 1935; Diezel et al. 2011), we rarely observed *T. mucorea* adults in *Nicotiana* populations: most *T. mucorea* adults as well as *T. compacta* adults were found in the *Datura* populations (Fig. 1c).

However, all larvae collected from infested *N. attenuata* growing in the field (N2 population; see Fig. S1, Supporting information) that we were able to rear to adulthood (14 larvae collected) were identified as *T. mucorea* (10 larvae matured to adults and 4 larvae died during the larval stage; Fig. 1d).

Adult preference and larval performance of two Trichobaris species in D. wrightii and N. attenuata

To quantify adult host selection traits in two *Trichobaris* species, we conducted a dual-choice assay in the glass-house and measured feeding and oviposition preferences of *T. compacta* and *T. mucorea* for *D. wrightii* and *N. attenuata* plants. Both species consumed significantly more leaf area from *D. wrightii* than from *N. attenuata* plants (Fig. 2a, e; $P < 0.01$). However, oviposition preferences of the two *Trichobaris* species differed from each other: *T. compacta* females oviposited more on *D. wrightii* plants (Fig. 2b; $P < 0.001$) and *T. mucorea* females oviposited more on *N. attenuata* (Fig. 2f; $P < 0.001$). To examine larval performance, we

developed an experimental egg inoculation procedure (Fig. S4, Supporting information), and 3 weeks after inoculation, we measured survival rates and quantified larval mass. Because *T. compacta* larvae survived only in *D. wrightii* and not in *N. attenuata* plants (Fig. 2c; $P < 0.01$), we measured the larval mass of *T. compacta* fed only on *D. wrightii* (Fig. 2d; $P < 0.01$). *T. mucorea* larvae were able to survive in both *D. wrightii* and *N. attenuata* stems, but their survival rates in *N. attenuata* were much higher than in *D. wrightii* (Fig. 2g; $P < 0.01$) while the mass of the surviving larvae did not differ (Fig. 2h; $P = 0.836$). *T. compacta* females preferred to feed and oviposit exclusively on *D. wrightii* plants, and larval performance in this species was correlated

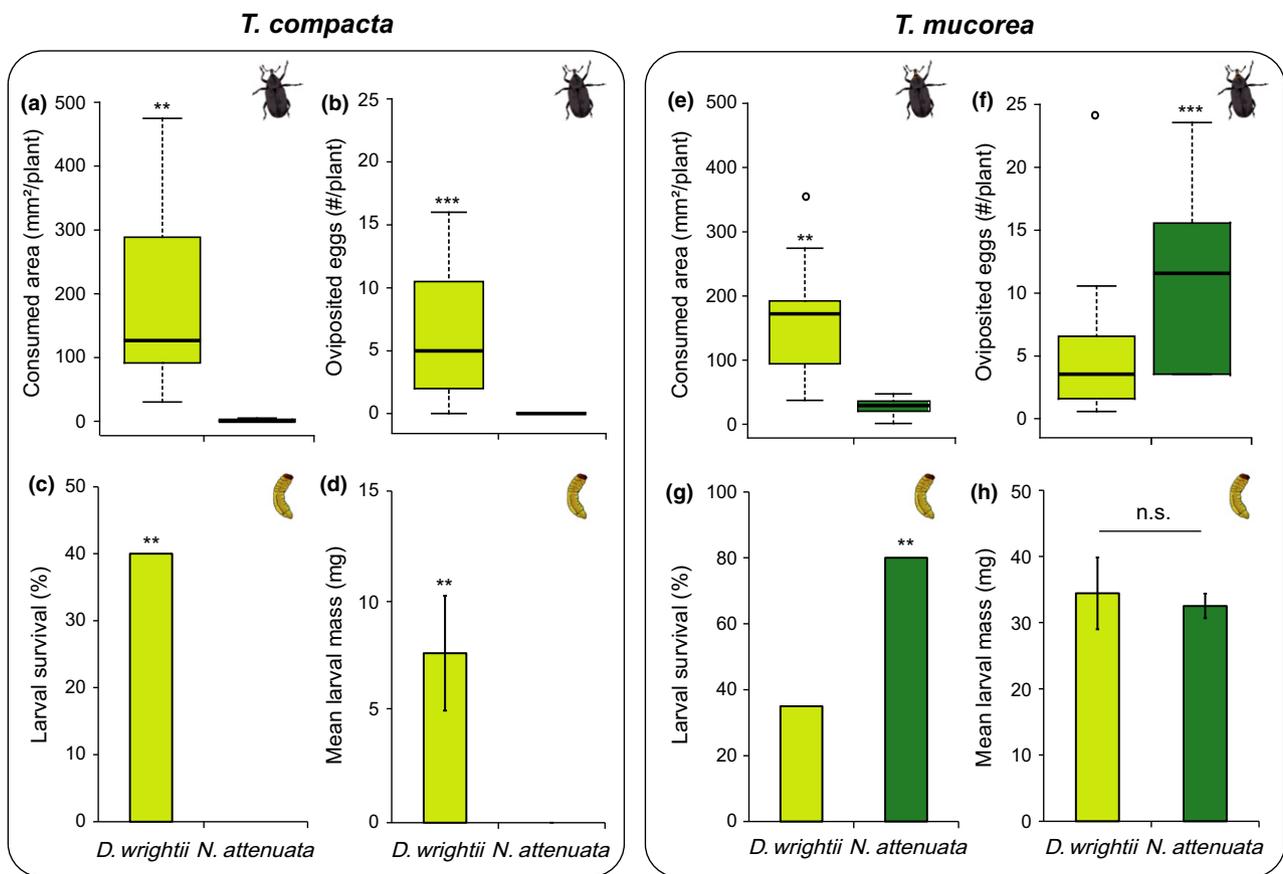


Fig. 2 *T. compacta* and *T. mucorea* adult preference and larval performance in *D. wrightii* versus *N. attenuata* host plants. (a) Leaf area consumed by *T. compacta* adult females in choice tests: *T. compacta* adults fed more on *D. wrightii* than on *N. attenuata* plants (G-test; **, $P < 0.01$; $n = 8$). (b) Numbers of eggs oviposited by *T. compacta*: females oviposited more on *D. wrightii* than on *N. attenuata* plants (G-test; ***, $P < 0.001$; $n = 8$). (c) Percentages of surviving larvae of *T. compacta* 3 weeks after egg inoculation: *T. compacta* larvae survived only in *D. wrightii* plants (χ^2 -test; **, $P < 0.01$; $n = 20$). (d) Mean (\pm SE) larval mass of *T. compacta* that fed on *D. wrightii* plants (one-way ANOVA; **, $P < 0.01$, $n = 20$). (e) Leaf area consumed by *T. mucorea* females: *T. mucorea* adults fed more on *D. wrightii* than on *N. attenuata* plants (G-test; **, $P < 0.01$; $n = 8$). (f) Numbers of eggs oviposited by *T. mucorea*: females oviposited more into *N. attenuata* stems than into *D. wrightii* plants (G-test; ***, $P < 0.001$; $n = 8$). (g) Percentages of surviving larvae of *T. mucorea* 3 weeks after egg inoculation: survival rates of *T. mucorea* larvae were higher when the larvae fed on *N. attenuata* than on *D. wrightii* plants (χ^2 -test; **, $P < 0.01$; $n = 20$). (h) Mean (\pm SE) larval mass of *T. mucorea* that fed on *D. wrightii* and *N. attenuata* plants: there was no significant difference in the larval mass (one-way ANOVA; n.s., not significant; $n = 20$).

with adult preference. *T. mucorea* adult females preferred to feed on *D. wrightii* leaves, but to oviposit into *N. attenuata* stems, and larval performance in this species was correlated with oviposition preference in the adult female.

Adult preference and larval performance of two *Trichobaris* species in nicotine-free *N. attenuata* plants

Nicotine, the well-known-induced chemical defence in *N. attenuata*, protects this plant from attack from a wide variety of insect and mammalian herbivores (Steppuhn *et al.* 2004). Therefore, we hypothesized that nicotine would strongly influence the host selection behaviour of the two *Trichobaris* species. To rigorously investigate the role of nicotine in adult oviposition and feeding preferences and larval performance, we conducted choice assays with each of the two *Trichobaris* species with EV plants (empty-vector transformed wild-type *N. attenuata*) and irPMT plants (silenced in *putrescine N-methyltransferase*, which is a key enzyme required for nicotine biosynthesis; see Fig. 3a). In this assay, neither *T. compacta* nor *T. mucorea* females consumed more irPMT than EV plants (Fig. 3b, f; $P = 0.203$ for *T. mucorea*, $P = 0.808$ for *T. compacta*). *T. compacta* females, as we observed in nature (Fig. 1c), rarely laid eggs on EV plants or on irPMT plants (Fig. 3c; $P = 0.095$), and *T. mucorea* females laid similar numbers of eggs on both EV and irPMT plants (Fig. 3g; $P = 0.291$). These results demonstrate that nicotine is neither an attractant nor a deterrent for females for feeding or oviposition of either species.

To measure the effect of nicotine on the larval performance of the two *Trichobaris* species, we inoculated one egg each into stems of EV or irPMT plants and measured larval mass 3 weeks later. That *T. compacta* larvae did not survive in EV plants (Fig. 3d) highlights the adaptive value of the oviposition preference of *T. compacta* females. More than 50% of the *T. compacta* larvae inoculated into irPMT plants survived, demonstrating that nicotine is responsible for the inability of *T. compacta* larvae to survive in WT *N. attenuata* stems (Fig. 3d, e; $P < 0.001$). *T. mucorea* larvae in irPMT plants had the same survival rates (Fig. 3h; $P = 0.705$) and gained similar amounts of body mass compared to the larvae in EV plants (Fig. 3i; $P = 0.574$). From these results, we conclude that nicotine is toxic to *T. compacta* larvae but not *T. mucorea* larvae.

Adult preference and larval performance of *T. mucorea* in JA-deficient *N. attenuata* plants

Jasmonic acid (JA) and its derivatives play a key role in plant–herbivore interaction by activating direct and indirect defences and functioning as proximate feeding cues

for some insects (Kessler *et al.* 2004; Wu & Baldwin 2010; Kallenbach *et al.* 2012). To examine whether JA-induced signalling has an effect on either adult preference or larval performance of *T. mucorea*, we conducted a dual-choice assay with irAOC (*allene oxidase cyclase*-silenced) transgenic lines impaired in their ability to synthesize JA (Fig. 4a). *T. mucorea* adult females preferred to feed on irAOC rather than on EV plants (Fig. 4b; $P < 0.001$), which may reflect the role of JA in plant defence against herbivore attack. Interestingly, females oviposited more eggs on EV than on irAOC plants (Fig. 4c; $P < 0.05$). Such behaviour suggests that JA signalling induces unknown attractants or suppresses deterrents, influencing the oviposition preference of *T. mucorea* females. While there was no significant difference in survival rates between larvae that fed on EV and those that fed on irAOC plants (Fig. 4d; $P = 0.450$), larvae that fed on irAOC plants grew larger than those that fed on EV plants (Fig. 4e; $P < 0.001$).

Volatile cues guide *T. mucorea* females to the host plant for oviposition

In a previous field study, we found increased infestation of *T. mucorea* in the *TERPENE SYNTHASE 10* (*TPS10*)-overexpressing plants (ovTPS10), which produce more (*E*)- α -bergamotene (TAB) and (*E*)- β -farnesene (TBF) sesquiterpene volatiles than WT *N. attenuata* plants, and in WT plants that were surrounded by ovTPS10 in small populations (Fig. 5a; Schuman *et al.* 2015). Three explanations could account for these observations: (i) ovTPS10 plants produce fewer defence metabolites against *T. mucorea* female attack; (ii) TAB and TBF sesquiterpenes are chemical cues that attract *T. mucorea* females; (iii) ovTPS10 plants produce fewer defence metabolites against *T. mucorea* larval attack. To test these hypotheses, we conducted dual-choice assays, measuring EV and ovTPS10 leaf area consumed by *T. mucorea* females and the eggs oviposited by *T. mucorea* females into EV and ovTPS10 plants. *T. mucorea* females consumed similar amounts of leaf area from both EV and ovTPS10 plants (Fig. 5b; $P = 0.073$), but oviposited more eggs into the stems of ovTPS10 plants than into EV plants (Fig. 5c; $P < 0.001$). Larval performance of *T. mucorea* did not differ significantly between EV and ovTPS10 plants (Fig. 5d, e; $P = 0.467$ for larval survival, $P = 0.868$ for larval mass). These results clearly demonstrate that TAB and TBF are oviposition cues for *T. mucorea* females.

Discussion

In the choice assays, *T. mucorea* females preferred to oviposit on *N. attenuata* rather than on *D. wrightii* to

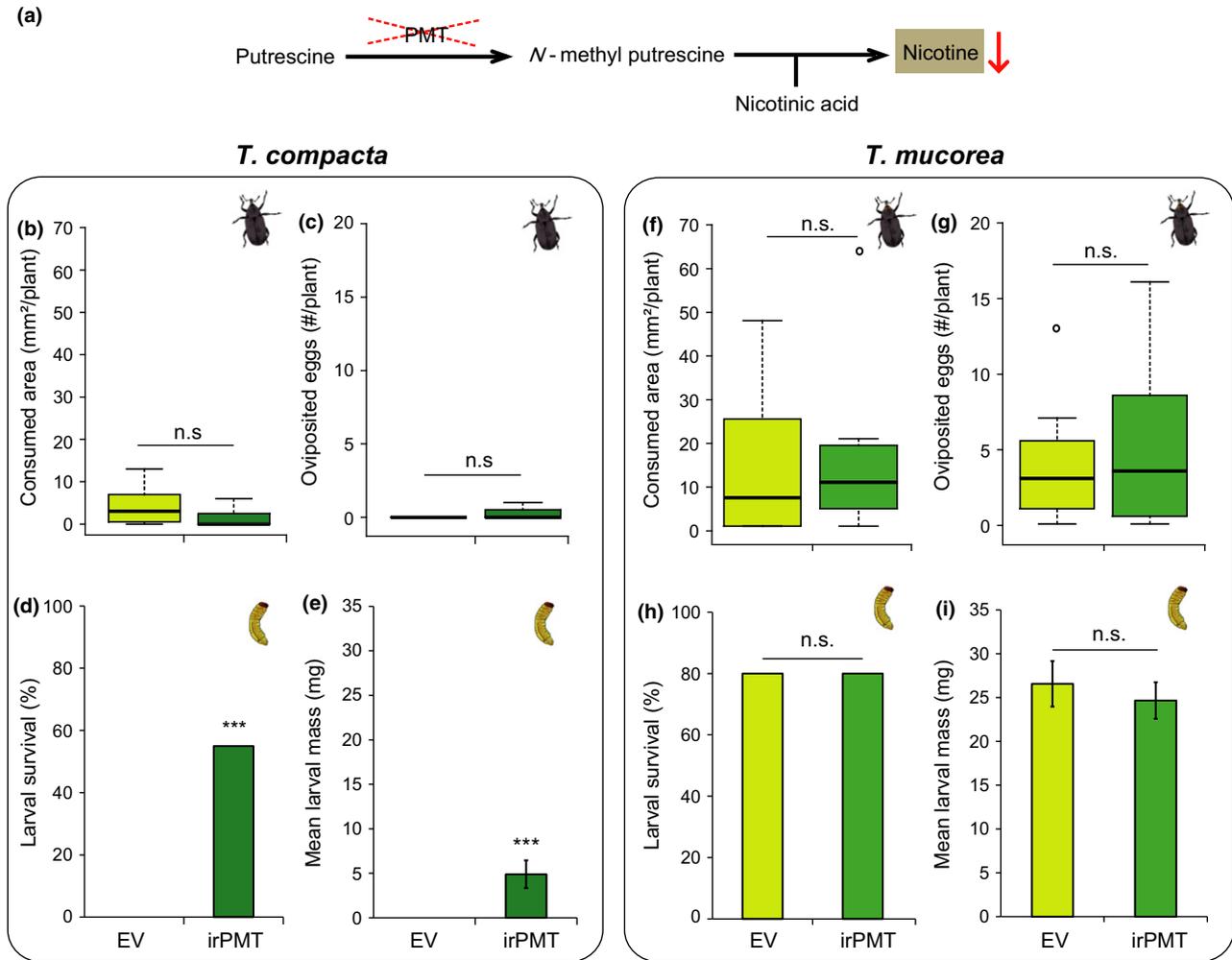


Fig. 3 Feeding and oviposition preference of *T. compacta* and *T. mucorea* females and larval performance in nicotine-producing versus nicotine-free plants. (a) Simplified biosynthetic pathway of nicotine. To generate a nicotine-free plant, one of the key nicotine biosynthesis enzymes, *putrescine N-methyltransferase*, was silenced in *N. attenuata* plants (irPMT; Steppuhn *et al.* 2004). (b) Leaf area of EV and irPMT plants consumed by *T. compacta* females. (c) Numbers of eggs on EV and irPMT plants oviposited by *T. compacta* females: there were no significant differences in feeding and oviposition preferences (G-test; n.s., not significant; $n = 8$). (d) Percentages of surviving larvae of *T. compacta* in EV and irPMT plants 3 weeks after egg inoculation: *T. compacta* larvae survived only in irPMT plants (χ^2 -test; $P < 0.001$; $n = 20$). (e) Mean (\pm SE) larval mass of *T. compacta* that fed on irPMT plants (one-way ANOVA; ***, $P < 0.001$; $n = 20$). (f) Leaf area consumed of EV and irPMT plants by *T. mucorea* females. (g) Numbers of eggs on EV and irPMT plants oviposited by *T. mucorea* females: no differences in feeding and oviposition preference (G-test; n.s., not significant; $n = 8$). (h) Percentages of surviving larvae of *T. mucorea* in EV and irPMT plants 3 weeks after egg inoculation: no significant difference in survival rates (χ^2 -test; $P = 0.143$; $n = 20$). (i) Mean (\pm SE) larval mass of *T. mucorea* fed on EV and irPMT plants: no significant difference in larval mass (one-way ANOVA; $P = 0.574$; n.s., not significant; $n = 20$).

maximize offspring performance, although these females fed mainly on *D. wrightii* (Fig. 2e); this result explains why *T. mucorea* adults were observed mainly on *D. wrightii* plants, but larvae collected from *N. attenuata* plants that developed into adults were identified as *T. mucorea* (Fig. 1d). These results show that females of these two *Trichobaris* species choose host plants that optimize offspring performance. However, in several species, adult's preference differs from larval performance: the root feeding vine weevil, *Otiiorhynchus*

sulcatus, shows no strong correlation between larval feeding preference and female oviposition preference (Clark *et al.* 2011), and adults of the glass miner, *Chromatomyia nigra*, tend to choose oviposition sites that maximize their own performance rather than that of their offspring (Scheirs *et al.* 2000; Janz *et al.* 2005).

Nicotine, the most abundant alkaloid in *N. attenuata*, is known to be an efficient defence compound against herbivores (Baldwin 1999; Steppuhn *et al.* 2004). Survival rates of *T. compacta* larvae in *N. attenuata* were

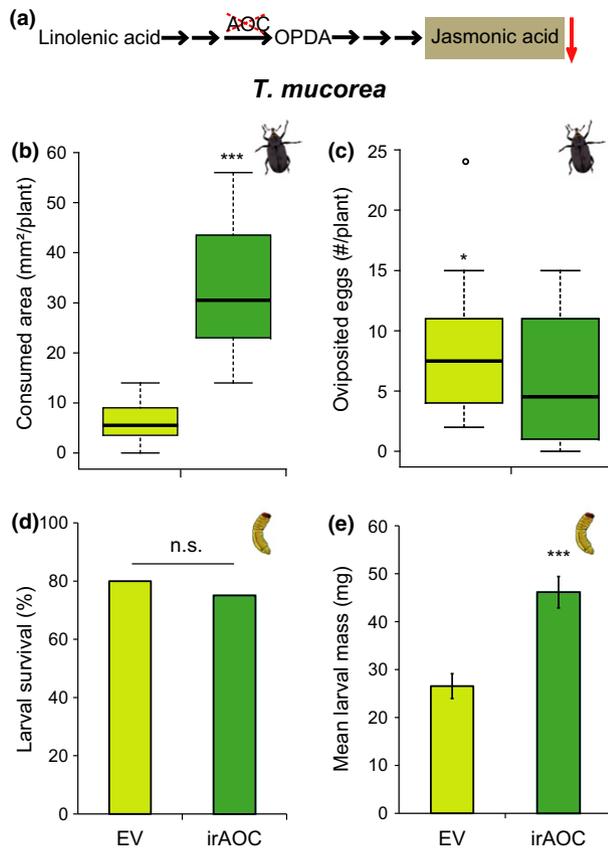


Fig. 4 *T. mucorea* female preference and larval performance in EV versus irAOC plants. (a) Simplified biosynthetic pathway of JA. irAOC, silenced the jasmonic acid (JA) biosynthesis gene, *allene oxide cyclase* in *N. attenuata* (Kallenbach *et al.* 2012). OPDA; 12-oxo-phytyldienoic acid. (b) Leaf area of EV and irAOC plants consumed by *T. mucorea* females: *T. mucorea* adult fed more on irAOC than on EV plants (G-test; ***, $P < 0.001$; $n = 14$). (c) Numbers of eggs on EV and irAOC plants oviposited by *T. mucorea* females: *T. mucorea* oviposited more into EV plants than into irAOC plants (G-test; *, $P < 0.05$; $n = 14$). (d) Percentages of surviving larvae of *T. mucorea* in EV and irAOC plants 3 weeks after egg inoculation: no significant difference in survival rates (χ^2 -test; $P = 0.450$; $n = 16$). (e) Mean (\pm SE) larval mass of *T. mucorea* fed on EV and irAOC plants: *T. mucorea* larva fed on irAOC plants had significantly higher mass than those fed on EV plants (one-way ANOVA; $n = 16$).

dramatically increased in nicotine-free *N. attenuata* plants (irPMT), indicating that *T. compacta* larvae cannot tolerate or detoxify nicotine. Although nicotine is toxic for *T. compacta* larvae, *T. compacta* females did not choose the nicotine-free plants for oviposition sites, indicating that nicotine is not a deterrent signal to *T. compacta* adult females for feeding and oviposition. In addition, nicotine had no effect on the oviposition preference and larval performance of *T. mucorea*, consistent with the larvae's ability to tolerate/detoxify nicotine.

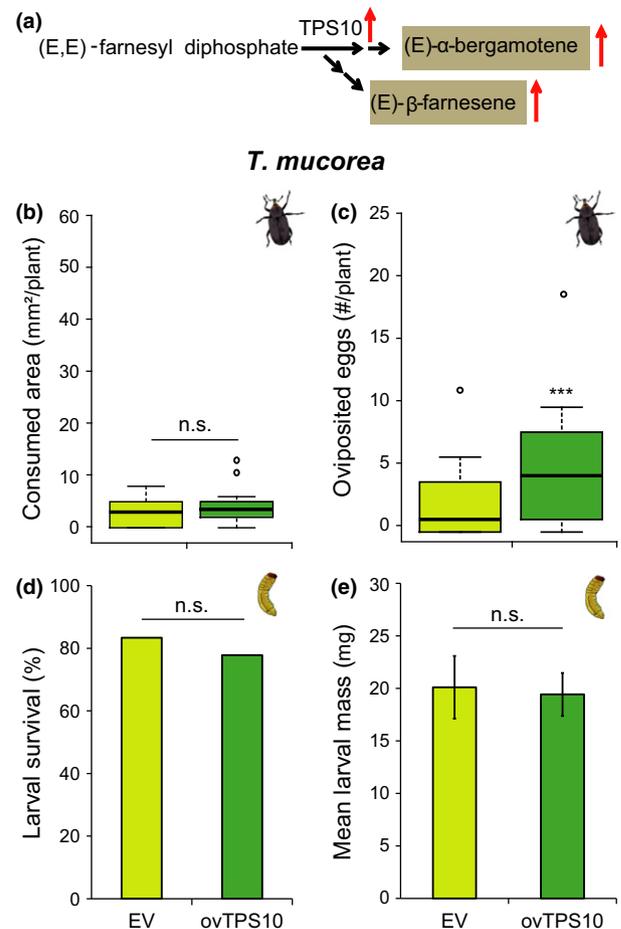


Fig. 5 *T. mucorea* adult female preference and larval performance in EV versus ovTPS10 plants. (a) Simplified biosynthetic pathway of sesquiterpene (*E*)- α -bergamotene and (*E*)- β -farnesene. ovTPS10, overexpressed of *Zea mays* TERPENE SYNTHASE 10 gene (Schuman *et al.* 2014). (b) Leaf area of EV and ovTPS10 plants consumed by *T. mucorea* females: no feeding preference of *T. mucorea* (G-test; n.s., not significant; $n = 14$). (c) Numbers of eggs on EV and ovTPS10 plants oviposited by *T. mucorea* females: females oviposited more into ovTPS10 than into EV plants (G-test; ***, $P < 0.001$; $n = 14$). (d) Percentages of surviving larvae of *T. mucorea* in EV and ovTPS10 plants 3 weeks after egg inoculation: no significant difference in survival rates (χ^2 -test; $P = 0.467$; $n = 10$). (e) Mean (\pm SE) larval mass of *T. mucorea* that fed on EV and ovTPS10 plants: no mass difference (one-way ANOVA; $P = 0.868$; $n = 10$).

We focused here on the interaction between *N. attenuata* and *T. mucorea*, hoping to identify an oviposition stimulant of *Trichobaris* species with various transgenic plants. We first tested the hypothesis that jasmonic acid (JA)-induced metabolites affect females' oviposition preference. In a previous field study, Diezel *et al.* (2011) found that JA-deficient lines of *N. attenuata* were highly infested by *T. mucorea*, and *T. mucorea* larvae preferred to feed on JA-deficient piths compared to WT piths in a

split-pith two-choice assay. However, this pith choice test cannot fully explain the large numbers of *T. mucorea* in JA-deficient plants, because the larvae do not move from the plants in which they are oviposited. Unexpectedly, we found *T. mucorea* larvae laid more eggs on EV than on JA-deficient plants (Fig. 4c), suggesting that the high infestation rates in the field-grown JA-deficient plants were not due to the oviposition behaviour of *T. mucorea* females and likely due to reductions of unknown toxic compounds in JA-deficient plants grown in the field. This result also suggested that *T. mucorea* females respond to JA signalling-mediated stimulants when choosing oviposition sites, although their offspring perform much better on irAOC plants than on EV plants (Fig. 4e). Insects frequently rely on plant volatiles to locate their host plants (Knolhoff & Heckel 2014). A previous study of Schuman *et al.* (2015) showed that numbers of *T. mucorea* larvae in heterogeneous habitats (mixed WT and ovTPS10 *N. attenuata* plant populations) are higher than those in homogeneous habitats (only WT plant populations), suggesting that the sesquiterpenes, TAB and TBF, might function as attractants for insects seeking oviposition sites. Moreover, these sesquiterpenes play a central role in the JA-mediated volatiles that are systemically released by herbivore attacked plants that function as indirect defences (Schuman *et al.* 2014, 2015). This inference was clearly consistent with the results of this study: *T. mucorea* females clearly preferred to oviposit into ovTPS10 compared to EV plants, and these sesquiterpenes did not affect larval performance.

Female solanaceous weevils of the species *T. compacta* and *T. mucorea* are able to choose suitable plants for their offspring: the positive correlation between female preference for oviposition and larval performance is consistent with the expectations of the optimal oviposition theory (Karungi *et al.* 2010; Zhang *et al.* 2012; Akol *et al.* 2013; Beuzelin *et al.* 2013). However, *T. compacta* and *T. mucorea* females did not choose nicotine-free and JA-deficient *N. attenuata* plants, respectively, for their offspring, although their larvae performed better in these transgenic lines. In addition, *T. mucorea* females respond to the emission of the sesquiterpene volatiles, TAB and TBF, when choosing oviposition sites, and these choices did not correlate with the larval performance. These data demonstrate that the plant metabolites that affect female oviposition choice differ from the metabolites that affect offspring performance. Further experiments will be necessary to reveal the mechanisms of host selection in *Trichobaris*, specifically, to clarify which oviposition cues used by *T. compacta* females' are emitted by *D. wrightii* and how JA-related defence compounds defend *N. attenuata* plants from attack by *T. mucorea* larvae.

Acknowledgements

We thank E. Rothe for help with *Trichobaris* adult preference experiments and colony maintenance; D. Kessler for assistance with *Trichobaris* abundance experiments at our field station; S.Y. Lee for graphic supporting; Emily Wheeler for editorial assistance; Brigham Young University for the use of the Lytle Ranch Preserve. All authors declare that they have no conflict of interests. This work is supported by European Research Council advanced grant ClockworkGreen (No. 293926) to ITB, the Global research Lab programme (2012055546) from the National Research Foundation of Korea, Institute for Basic Science (IBS-R021-D1) and the Max Planck Society.

References

- Akol AM, Masembe C, Isabirye BE, Kukiriza CK, Rwomushana I (2013) Oviposition preference and offspring performance in phytophagous fruit flies (Diptera: Tephritidae): the african invader, *Bactrocera invadens*. *International Research Journal of Horticulture*, **1**, 1–14.
- Allmann S, Baldwin IT (2010) Insects betray themselves in nature to predators by rapid isomerization of green leaf volatiles. *Science*, **329**, 1075–1078.
- Awmack CS, Leather SR (2002) Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, **47**, 817–844.
- Baldwin IT (1999) Inducible nicotine production in native *Nicotiana* as an example of adaptive phenotypic plasticity. *Journal of Chemical Ecology*, **25**, 3–30.
- Barber HS (1935) *The Tobacco and Solanum Weevils of the Genus Trichobaris*. US Dept. of Agriculture, Miscellaneous Publication, Washington, District of Columbia.
- Barbosa P, Krischik VA (1987) Influence of alkaloids on feeding preference of eastern deciduous forest trees by the gypsy moth *Lymantria dispar*. *American Naturalist*, **130**, 53–69.
- Bertheau C, Salle A, Roux-Morabito G *et al.* (2009) Preference-performance relationship and influence of plant relatedness on host use by *Pityogenes chalcographus* L. *Agricultural and Forest Entomology*, **11**, 389–396.
- Beuzelin JM, Wilson LT, Showler AT *et al.* (2013) Oviposition and larval development of a stem borer, *Eoreuma loftini*, on rice and non-crop grass hosts. *Entomologia Experimentalis et Applicata*, **146**, 332–346.
- Brodbeck BV, Andersen PC, Oden S, Mizell RF (2007) Preference-performance linkage of the xylem feeding leafhopper, *Homalodisca vitripennis* (Hemiptera: Cicadellidae). *Environmental Entomology*, **36**, 1512–1522.
- del Campo ML, Miles CI, Schroeder FC, Mueller C, Booker R, Renwick JA (2001) Host recognition by the tobacco hornworm is mediated by a host plant compound. *Nature*, **411**, 186–189.
- Chatzigeorgiou AC, Papadopoulos NT, Prophetou-Athanasiadou DA (2010) Effect of cotton cultivars on the oviposition preference of pink bollworm (Lepidoptera: Gelechiidae). *Journal of Pest Science*, **83**, 289–296.
- Clark KE, Hartley SE, Johnson SN (2011) Does mother know best? The preference-performance hypothesis and parent-offspring conflict in aboveground-belowground herbivore life cycles. *Ecological Entomology*, **36**, 117–124.

- Diezel C, Kessler D, Baldwin IT (2011) Pithy protection: *Nicotiana attenuata*'s jasmonic acid-mediated defenses are required to resist stem-boring weevil larvae. *Plant Physiology*, **155**, 1936–1946.
- Gripenberg S, Morriën E, Cudmore A, Salminen J, Roslin T (2007) Resource selection by female moths in a heterogeneous environment: what is a poor girl to do? *Journal of Animal Ecology*, **76**, 854–865.
- Heiling S, Schuman MC, Schoettner M *et al.* (2010) Jasmonate and ppHsystemin regulate key malonylation steps in the biosynthesis of 17-hydroxygeranylinalool diterpene glycosides, an abundant and effective direct defense against herbivores in *Nicotiana attenuata*. *The Plant Cell*, **22**, 273–292.
- Ikonen A, Tahvanainen J, Roininen H (2002) Phenolic secondary compounds as determinants of the host plant preferences of the leaf beetle *Agelastica alni*. *Chemoecology*, **12**, 125–131.
- Jaenike J (1978) On optimal oviposition behavior in phytophagous insects. *Theoretical Population Biology*, **14**, 350–356.
- Jallow MFA, Zalucki MP (2003) Relationship between oviposition preference and offspring performance in Australian *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Australian Journal of Entomology*, **42**, 343–348.
- Janz N, Bergström A, Sjögren A (2005) The role of nectar sources for oviposition decisions of the common blue butterfly *Polyommatus icarus*. *Oikos*, **109**, 535–538.
- Kallenbach M, Bonaventure G, Gilardoni PA, Wissgott A, Baldwin IT (2012) *Empoasca* leafhoppers attack wild tobacco plants in a jasmonate-dependent manner and identify jasmonate mutants in natural populations. *Proceedings of the National Academy of Sciences*, **109**, E1548–E1557.
- Karungi J, Lubanga UK, Kyamanywa S, Ekbohm B (2010) Oviposition preference and offspring performance of *Crocidolomia pavonana* (Lepidoptera: Pyralidae) on different host plants. *Journal of Applied Entomology*, **134**, 704–713.
- Kessler A, Halitschke R, Baldwin IT (2004) Silencing the jasmonate cascade: induced plant defenses and insect populations. *Science*, **305**, 665–668.
- Knolhoff LM, Heckel DG (2014) Behavioral assays for studies of host plant choice and adaptation in herbivorous insects. *Annual Review of Entomology*, **59**, 263–278.
- Krügel T, Lim M, Gase K, Halitschke R, Baldwin IT (2002) *Agrobacterium*-mediated transformation of *Nicotiana attenuata*, a model ecological expression system. *Chemoecology*, **12**, 177–183.
- Malone LA, Wigley PJ (1990) A practical method for rearing Argentine stem weevil, *Listronotus bonariensis* (Coleoptera: Curculionidae) in the laboratory. *New Zealand Entomologist*, **13**, 87–89.
- Marazzi C, Städler E (2004) *Arabidopsis thaliana* leaf-surface extracts are detected by the cabbage root fly (*Delia radicum*) and stimulate oviposition. *Physiological Entomology*, **29**, 192–198.
- Markovich O, Kafle D, Elbaz M *et al.* (2013) *Arabidopsis thaliana* plants with different levels of aliphatic- and indolyl-glucosinolates affect host selection and performance of *Bemisia tabaci*. *Journal of Chemical Ecology*, **39**, 1361–1372.
- Matsuki M, Foley WJ, Floyd RB (2011) Role of volatile and non-volatile plant secondary metabolites in host tree selection by Christmas beetles. *Journal of Chemical Ecology*, **37**, 286–300.
- Mazaheri A, Khajehali J, Hatami B (2011) Oviposition preference and larval performance of *Aeolesthes sarta* (Coleoptera: Cerambycidae) in six hardwood tree species. *Journal of Pest Science*, **84**, 355–361.
- Nagasawa A, Kamada Y, Kosaka Y, Arakida N, Hori M (2014) Catechol – an oviposition stimulant for cigarette beetle in roasted coffee beans. *Journal of Chemical Ecology*, **40**, 452–457.
- O'Brien CW, Wibmer GJ (1982) Annotated checklist of the weevils (Curculionidae sensu lato) of North America, Central America, and the West Indies (Coleoptera: Curculionoidea). *Memoirs of the American Entomological Institute (Gainesville)*, **34**, 1–382 i–ix.
- Powell G, Toshi CR, Hardie J (2006) Host plant selection by aphids: behavioral, evolutionary, and applied perspectives. *Annual Review of Entomology*, **51**, 309–330.
- Price PW (1997) *Insect Ecology*. John Wiley & Son, New York.
- Renwick Jaa, Haribal M, Gouinguéné S, Städler E (2006) Isothiocyanates stimulating oviposition by the diamondback moth, *Plutella xylostella*. *Journal of Chemical Ecology*, **32**, 755–766.
- Rosenthal GA, Berenbaum MR (1992) *Herbivores: Their Interactions with Secondary Plant Metabolites: Ecological and Evolutionary Processes*. Academic Press, San Diego, California.
- Sarfraz M, Dossdall LM, Keddie BA (2006) Diamondback moth–host plant interactions: implications for pest management. *Crop Protection*, **25**, 625–639.
- Scheirs J, Bruyn L De (2002) Integrating optimal foraging and optimal oviposition theory in plant–insect research. *Oikos*, **96**, 187–191.
- Scheirs J, Bruyn LD, Verhagen R (2000) Optimization of adult performance determines host choice in a grass miner. *Proceedings of the Royal Society B: Biological Sciences*, **267**, 2065–2069.
- Schuman MC, Palmer-Young EC, Schmidt A, Gershenzon J, Baldwin IT (2014) Ectopic terpene synthase expression enhances sesquiterpene emission in *Nicotiana attenuata* without altering defense or development of transgenic plants or neighbors. *Plant Physiology*, **166**, 779–797.
- Schuman MC, Allmann S, Baldwin IT (2015) Plant defense phenotypes determine the consequences of volatile emission for individuals and neighbors. *eLife*, **4**, 1–43.
- Shikano I, Akhtar Y, Isman MB (2010) Relationship between adult and larval host plant selection and larval performance in the generalist moth, *Trichoplusia ni*. *Arthropod-Plant Interactions*, **4**, 197–205.
- Sokal RR, Rohlf FJ (1995) *Biometry: The Principles and Practice of Statistics in Biological Research*. State University of New York at Stony Brook, New York.
- Steppuhn A, Gase K, Krock B, Halitschke R, Baldwin IT (2004) Nicotine's defensive function in nature. *PLoS Biology*, **2**, 1074–1080.
- Thompson JN (1988) Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomologia Experimentalis et Applicata*, **47**, 3–14.
- Thompson JN, Pellmyr O (1991) Evolution of oviposition behavior and host. *Annual Review of Entomology*, **36**, 65–89.
- Valladares G, Lawton JH (1991) Host-plant selection in the holly leaf-miner: does mother know best? *The Journal of Animal Ecology*, **60**, 227–240.
- Woodside AM (1949) The tobacco stalk borer in Western Mexico. *Journal of Economic Entomology*, **42**, 63–67.
- Wu J, Baldwin IT (2010) New insights into plant responses to the attack from insect herbivores. *Annual Review of Genetics*, **44**, 1–24.

Zalucki MP, Clarke Anthony R, Malcolm SB (2002) Ecology and behavior of first instar larval Lepidoptera. *Annual Review of Entomology*, **47**, 361–393.

Zhang PJ, Y Bin Lu, Zalucki MP, Liu SS (2012) Relationship between adult oviposition preference and larval performance of the diamondback moth, *Plutella xylostella*. *Journal of Pest Science*, **85**, 247–252.

G.L., Y.J. and C.D. performed experiments. I.T.B. and S.G.K. conceived and designed experiments.

Data accessibility

All DNA sequences are available in GenBank database: *Nicotiana attenuata* AOC, EF467332.1; *NaPMT*, AF280402; *Zea mays* TPS10, NM_001112380. Sampling locations are provided in Fig. S1 (Supporting information). All performance data are available at the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.j7t92>.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1. Description of site surveyed in the Great Basin Desert of southwestern Utah, USA. *D. wrightii* populations (D1, 37°14′14.09N, 114°02′82.41W, *n* = 81; D2, 37°14′66.91N, 114°01′97.08

n = 110; D3, 37°14′36.24N, 114°02′19.9W, *n* = 45; D4, 37°15′03.61N, 114°01′86.07W, *n* = 76; D5, 36°95′56.45N, 113°92′24.54W, *n* = 77). *N. attenuata* populations (N1, 37°14′12.52N, 114°02′76.20W, *n* = 200; N2, 37°14′63.29N, 114°01′97.56W, *n* = 200; N3, 37°13′27.80N, 113°23′49.50W, *n* = 20; N4, 37°07′45.60N, 113°28′52.80W, *n* = 97).

Fig. S2. Setup for the culturing of *Trichobaris* species in the laboratory (a) *T. mucorea* and *T. compacta* adults were collected from their native habitat. Eggs of each *Trichobaris* species were collected from *N. attenuata* petioles and *D. wrightii* buds. (b) One egg was inoculated into the basal part of each *N. attenuata* stem during the early elongation stage of plant growth. Five to 7 days after inoculation, the eggs hatched, and the neonates burrowed into the pith and started feeding. (c) After 3 weeks, egg-inoculated stems were cut carefully, and larvae were extracted and weighed before being transferred to individual 15 mL falcon tubes with artificial diet and reared to adults. (d) Newly emerged adults were transferred to the colony containers with *D. wrightii* and *N. attenuata* plants for mating and oviposition.

Fig. S3. Method for *Trichobaris* egg inoculation into the stems of early elongation-stage *N. attenuata* plants.

Fig. S4. Experimental device of adult preference experiments.

Fig. S5. Rigid male reproductive organ (median lobe) of two *Trichobaris* species, lateral view of the median lobe (a, c). In *T. compacta*, the median lobe was much more strongly deflexed and narrow than in *T. mucorea*. Dorsal view of the median lobe (b, d). In *T. mucorea* males, the sclerotized (orificial) plates were widely separated, small and subconical compared with the more closed form found in *T. compacta* males.