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Report

Changing Pollinators as a Means of Escaping Herbivores

Danny Kessler,[1] Celia Diezel,[1] and Ian T. Baldwin[1,*]
[1]Max Planck Institute for Chemical Ecology, Hans-Knöll-Straße 8, 07745 Jena, Germany

Summary

All animal-pollinated plants must solve the problem of attracting pollinators while remaining inconspicuous to herbivores, a dilemma exacerbated when voracious larval-stage herbivores mature into important pollinators for a plant [1]. Herbivory is known to alter pollination rates, by altering flower number [2], size [3, 4], nectar production [5], seasonal timing of flowering [6], or pollinator behavior [7]. *Nicotiana attenuata*, a night-flowering tobacco that germinates after fires in the Southwestern United States, normally produces flowers that open at night and release benzyl acetone (BA) to attract night-active hawkmoth pollinators (*Manduca quinquemaculata* and *M. sexta*), which are both herbivores and pollinators. When plants are attacked by hawkmoth larvae, the plants produce flowers with reduced BA emissions that open in the morning and are preferentially pollinated by day-active hummingbirds. This dramatic change in flower phenology, which is elicited by oral secretions (OSs) from feeding hawkmoth larvae and requires jasmonate (JA) signal transduction, causes the majority of outcrossed seeds to be produced by pollinations from day-active hummingbirds rather than night-active hawkmoths. Because oviposition and nectaring are frequently coupled behaviors in hawkmoths, we propose that this OS-elicited, JA-mediated change in flower phenology complements similarly elicited responses to herbivore attack (direct defenses, indirect defenses, and tolerance responses) that reduce the risk and fitness consequences of herbivory to plants.

Results and Discussion

During a *Manduca quinquemaculata* outbreak in a native population of *Nicotiana attenuata* in the 2007 field season in which nearly every plant was infested by a caterpillar, we noticed that the plants produced flowers that started to open their corollas at dawn. This was unusual in that *N. attenuata* flowers normally open their corollas at dusk, between 6 and 10 p.m. (night-opening flowers, NoFs), and keep their corollas open during the night and through the following morning. In contrast, the plants in this heavily *M. quinquemaculata*-infested population produced flowers that were starting to open their corollas in the morning, between 6 and 10 a.m. (morning-opening flowers, MoFs; Figure 1B; see also Figure S1 available online). In this population, 15% ± 20% (mean ± standard deviation; n = 37) of the plants' flowers were MoFs.

To determine whether caterpillar damage was responsible for the appearance of MoFs, we experimentally infested previously unattacked plants in another native population of *N. attenuata*, in which most plants had not been attacked by native caterpillars, with two *M. sexta* neonates. To minimize the amount of leaf area consumed, we exchanged the neonates every other day with two new neonates. Eight days after the start of the experimental infestation, an average of 35% of all flowers produced were now MoFs (Figure 1A), compared to only 11% on the uninfested plants. This shift in flower phenology was a discontinuous shift, in that flowers only opened their corollas and underwent anthesis at one of two times, either in the evening or in the morning (Figure 1B).

N. attenuata attracts and rewards *Manduca* spp. floral visitors by emitting a bouquet of floral volatiles and offering sugar-rich nectar, respectively [8]. The main constituent of the floral bouquet is benzyl acetone (BA), which is exclusively produced at night, with emissions beginning shortly after the opening of the corolla in NoFs [9]. BA emission is essential for pollination of NoFs by nocturnally active hawkmoths, such as *Manduca* spp. When plants are genetically transformed to silence BA emissions, capsule production of antherectomized flowers exposed only to nocturnal pollinators drops dramatically, demonstrating that without BA emissions, flowers are not visited by *Manduca* adults [10]. Remarkably, when we analyzed BA emissions from MoFs, we found that the large BA release at the beginning of the flowering period was completely missing and that BA emission in general was strongly reduced over the two-day lifetime of the flower (Figure 2). In addition, MoFs had lower nectar sugar concentrations (paired Student's t test, $t_9 = 7.80$, $p < 0.0001$; NoF: 28.7% ± 1.3%; MoF: 16.2% ± 0.6%) and presented in the morning only a third of the corolla diameter of NoFs ($t_9 = -20.70$, $p < 0.0001$; NoF: 15.6 ± 0.5 mm; MoF: 6.1 ± 1.1 mm; Figure S1 and Table S1). Nectar production ($t_9 = -0.50$, $p = 0.628$; 1.5 ± 0.2 μl), estimated from the standing nectar volume of flowers covered by mesh bags to exclude pollinators and the concentration of nicotine in the nectar ($t_{13} = -0.15$, $p = 0.885$; 31.3 ± 5.0 μM), did not differ between the two flower types. The three main differences between NoFs and MoFs, namely opening time, BA emissions, and nectar sugar concentrations, are likely to dramatically alter the community of pollinators responsible for cross-pollinating the flowers of *N. attenuata* [10].

As *N. attenuata* is a fully self-compatible species that produces upwards of 30% of its seed production from opportunistic outcrossings [11, 10], we set about to determine the fitness consequences of this herbivory-induced phenological change in flower opening by antherectomizing [10] all flowers on a plant and measuring capsule production in plants that were experimentally exposed only to either nighttime (primarily hawkmoth) or daytime (primarily hummingbird) pollinators by covering plants with mesh-covered cones (Figure S2B). In a preliminary experiment conducted during the 2007 field season with uninfested plants producing mostly NoFs, antherectomized flowers exposed only to nighttime pollinators produced twice as many capsules (41.2% of 32 antherectomized flowers) compared to antherectomized flowers exposed only to daytime pollinators (21.9% of 32 antherectomized flowers). A similar experiment was conducted in the 2008 field season, but this time, half of the plants were experimentally infested with neonate *M. sexta* larvae. For the

*Correspondence: baldwin@ice.mpg.de

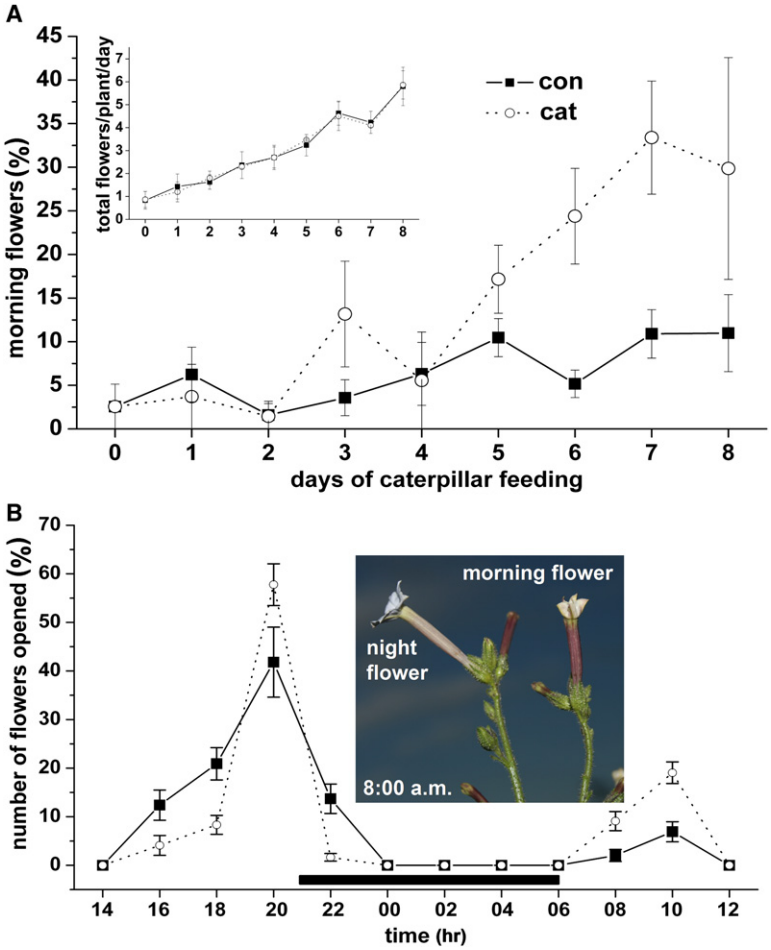

Figure 1. Phenological Change in the Timing of Flower Production when *Manduca sexta* Larvae Attack *Nicotiana attenuata* Plants

(A) *M. sexta* caterpillar feeding (cat) results in a proportional increase in number of flowers opening in the morning (morning flowers) in relation to total flower number, in comparison to undamaged plants (con). Inset: the total number of flowers (sum of night and morning flowers) produced by a plant was not influenced by herbivore attack. Means ± standard error of the mean (SEM) of three different experiments with native *N. attenuata* plants are shown.

(B) Number of flowers opening at different times in attacked (cat) and unattacked (con) *N. attenuata* plants growing in native populations. Mean ± SEM percentage of flowers (n = 22 plants) opening at given times within 24 hr is shown. Horizontal black bar depicts the night phase. Inset: opening stage of corollas at 8 a.m.; night-opening flowers (left) remain open throughout the night, whereas morning-opening flowers (right) only begin to open their corollas at dawn (see also Figure S1 and Table S1).

uninfested plants producing NoFs, comparable results were found: antherectomized flowers exposed only to nighttime pollinators produced twice as many capsules as those exposed only to daytime pollinators (paired Student's t test, $t_8 = -1.44$, p = 0.188; Figure 3A). Interestingly, this ratio was reversed in the experimentally infested plants that produced MoFs: capsule production from antherectomized flowers exposed only to daytime pollinators was twice that of antherectomized flowers exposed to only nighttime pollinators ($t_9 = 3.99$, p = 0.004; Figure 3A). These experiments demonstrate that infested plants producing MoFs receive most of their outcrossed seed production from the activity of daytime pollinators and are therefore less attractive to nighttime pollinators. To understand how this occurs, we conducted detailed observations of the principal day-active pollinator, black-chinned hummingbirds (*Archilochus alexandri*), during the 2009 field season as they visited *N. attenuata* inflorescences, collecting nectar.

Collecting observational data on the visitations of hummingbirds to native plants is a challenge because the birds are difficult to track by video monitoring of focal plants in *N. attenuata* populations that typically consist of several thousand plants. During the 300 hr that we were in native *N. attenuata* populations in the 2009 field season, we were sufficiently close to nectaring hummingbirds 18 times to be able to record the order in which flowers were visited and how many MoFs and NoFs were on the plant. These detailed focal observations revealed that hummingbirds were

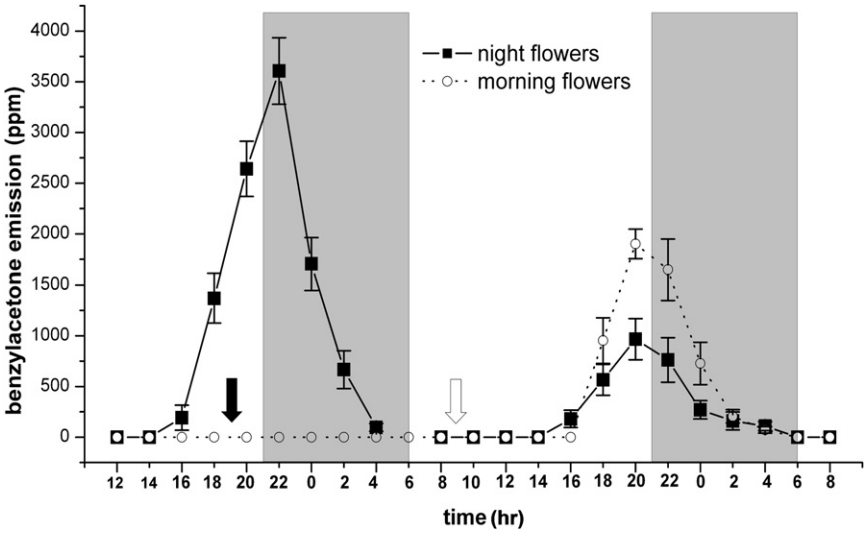

Figure 2. Emission of the Most Abundant and Attractive Floral Fragrance Constituent, Benzyl Acetone, from Morning- and Night-Opening Flowers of *N. attenuata* Plants

Diurnal patterns in benzyl acetone (BA) emission from individual flowers of night- and morning-opening flowers over 48 hr (mean ± SEM, n = 7). Gray bars depict the dark period. Whereas night-open flowers release a majority of their BA production at the time of anthesis and corolla opening (black arrow), BA emission is completely absent from morning-opening flowers when their corollas open and undergo anthesis (white arrow).

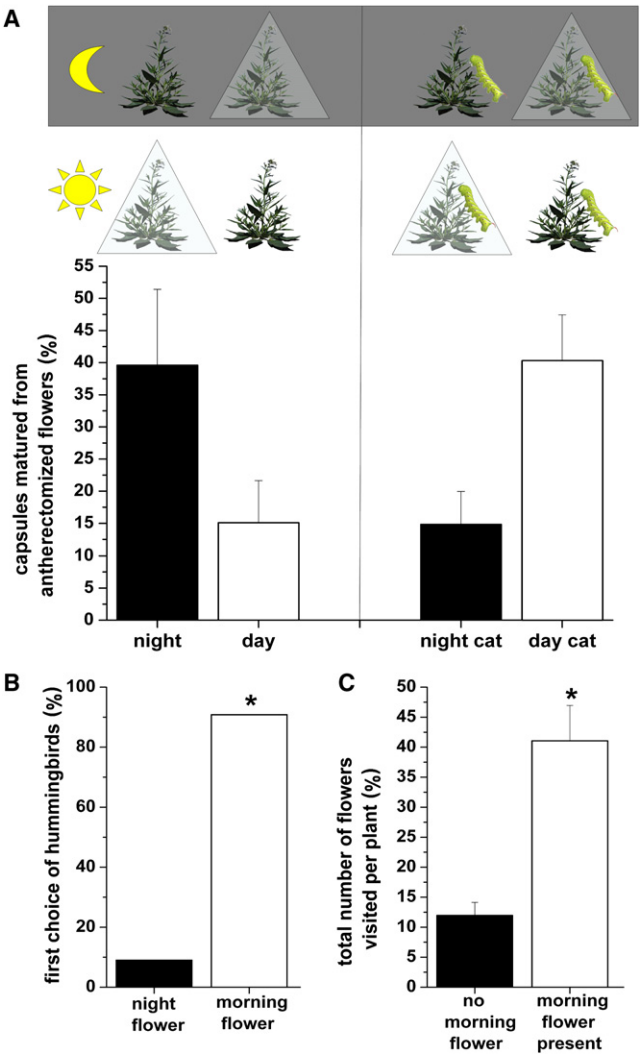

Figure 3. Fitness Consequences of the Herbivory-Elicited Change in Flower Phenology and the Influence of Morning- and Night-Opening Flowers on Hummingbird Visitation

(A) Mean ± SEM percentage of capsules matured from antherectomized flowers on each of ten plants previously experimentally infested with (cat) or without *M. sexta* larvae that were uncovered and therefore exposed to the native pollinator community from 8:00 p.m. to 6:00 a.m. (night) or from 6:00 a.m. to 8:00 p.m. (day). Plants were covered with mesh-covered wire cones (triangle; see Figure S2B) to prevent pollinator access to flowers at other times. All flowers of a plant were antherectomized on the eighth day after infestation (Figure S2A). All larvae were removed when plants were exposed to pollinators.

(B) The probability that morning-opening flowers were the first flower visited by hummingbird (*Archilochus alexandri*) pollinators was nine times higher than for night-opening flowers (n = 11; χ^2 = 7.4, df = 1, p = 0.007).

(C) Mean ± SEM number of flowers visited per hummingbird visitation to plants with and without a morning-opening flower (Student's t test, t_{16} = 3.82, p = 0.0015).

highly attracted to MoFs. In more than 90% of cases, hummingbirds visited MoFs first (Figure 3B), even though NoFs were more abundant on all plants. This strong preference for MoFs is likely due to the fact that these flowers provide a nectar reward that is untouched by nocturnal pollinators as a result of their opening times (Figure 2) and are unsullied by nectar-robbing carpenter bees (*Xylocopa* spp.), which do not recognize MoFs as a food source (n = 14, χ^2 = 8.3, df = 1, p = 0.004; Figure S3), possibly because of the different morphology of MoFs in the morning (Figure S1; Table S1). We propose that hummingbirds have learned to associate the particular floral shape of MoFs (Figure 1B) with a guaranteed nectar reward during their early-morning foraging excursions. This proposal is consistent with the established ability of hummingbirds to recognize flower morph-specific scheduling of nectar production [5]. In addition, our analysis revealed that if a hummingbird was attracted to a plant with at least one MoF, they visited three times more flowers within that plant's inflorescence compared to plants that did not have a MoF (Figure 3C). We propose that this strong preference of hummingbirds for MoFs is responsible for the increased outcrossing rates of *M. sexta*-infested plants exposed to daytime pollinators (Figure 3A).

We hypothesized that this herbivory-elicited change in flower phenology may reduce the herbivore load of the plant. The production of MoFs, which are primarily pollinated by hummingbirds, and the reduction of NoFs reduces the total BA emissions and nectar volume available for nighttime pollinators, because MoFs neither emit BA at this time nor are accessible for nectar collection by nocturnal pollinators. Reduction of BA emissions has been experimentally demonstrated to reduce hawkmoth visitations at night [10]. By producing MoFs, the plant becomes less apparent to hawkmoths and thereby reduces future herbivory by reducing the oviposition that is frequently associated with the pollination and nectaring behavior of the adult insects. Oviposition rates of *Manduca* adults on *N. attenuata* plants in native populations have been shown to increase with increasing flower number [12], and in laboratory studies of *Datura stramonium*, *Manduca* was shown to increase oviposition rates on plants with experimentally augmented nectar volumes [13]. We conducted a similar experiment in a native population of *N. attenuata* plants by adding 20 μl of a 12.5% sucrose solution per flower, thereby increasing the average flower nectar volume 10-fold, and recording oviposition rates the following day (n = 30 plant pairs). Nectar volume augmentation significantly increased the frequency of ovipositioning by *M. quinquemaculata*, the only *Manduca* species flying at the time of the experiment. Eggs were oviposited on 23.3% of the treated plants compared to 3.3% of the control plants (Fisher's exact probability test, p = 0.01; Figure S4), thereby confirming the close association between nectaring and ovipositioning.

If producing MoFs reduces herbivore loads and hummingbirds can provide excellent pollination services, why don't *N. attenuata* plants always produce MoFs? The answer to this question remains unknown, but it is possible that for a plant that frequently occurs in large, synchronized, almost monoculture populations after fires, hummingbird pollination may not be as reliable as the pollination services provided by hawkmoths. Hawkmoths can be attracted via volatiles over great distances, whereas the pollination services of hummingbirds may be more restricted by other requirements such as the location of nest sites in trees. Furthermore, hummingbird pollination may result in more within-plant pollen transfer (geitonogamy) than is mediated by hawkmoth pollination. More certain is our understanding of the mechanisms by which *N. attenuata* plants perceive attack by *Manduca* larvae and the signaling cascade that mediates the switch from NoF to MoF production.

When *N. attenuata* plants are attacked by *M. sexta* larvae, fatty acid-amino acid conjugates (FACs) from larval oral secretions and regurgitants (OSs) are introduced into wounds

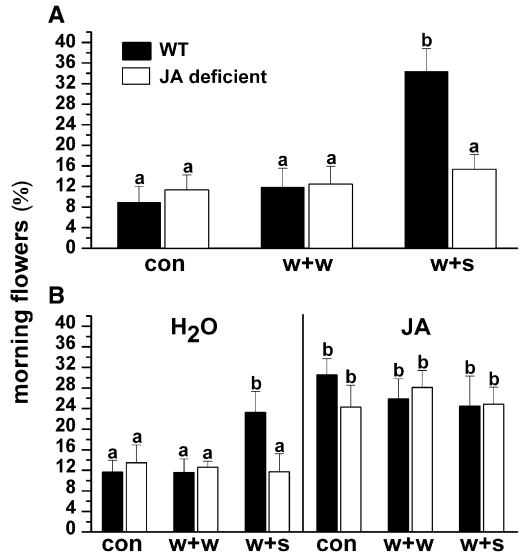

Figure 4. Production of Morning-Opening Flowers Is Elicited by *M. sexta* Larval Oral Secretions and Requires Jasmonate Signaling

Production of morning-opening flowers is elicited by treatment of mechanical wounds with *M. sexta* larval oral secretions in wild-type (WT) *N. attenuata* plants and requires jasmonate (JA) signaling, as revealed by the lack of elicitation in JA-deficient plants and its restoration by exogenous JA treatment.

(A) Mean ± SEM percentage of morning-opening flowers in WT and isogenic JA-deficient plants (genetically transformed to silence the expression of lipoxygenase 3; *Na*LOX3) that either remained untreated (con) or were mechanical damaged and had their wounds immediately treated with water (w+w) or oral secretions of *M. sexta* larvae (w+s) every second day for eight days. Morning- and night-opening flowers were counted after eight days of continuous elicitation (n = 14 plants per treatment).

(B) Mean ± SEM percentage of morning-opening flowers in con, w+w-elicited, and w+s-elicited plants of WT and LOX3-silenced lines sprayed with either water (H_2O) or jasmonic acid (JA) every other day for eight days (n = 8–10 plants per treatment).

"a" and "b" designate significantly different means as determined by a Fisher's protected least significant difference post hoc test ($p < 0.05$) from an analysis of variance.

during feeding [14, 15]. These elicitors activate a complex MAP kinase cascade [16] that in turn activates WRKY transcription factors [17] that eventually elicit a burst of jasmonic acid (JA), a plant hormone that acts as a major transducer of signals and is essential for the production of a complicated suite of plant defenses [18, 19]. The defenses include the production of toxins [20–22] and antidigestive direct defenses [23] as well as indirect defenses that attract predators of herbivores by emitting volatile attractants [14, 17, 24]. The FACs in larval OSs also activate tolerance mechanisms that involve the bunkering of recently fixed carbon to roots, but these do not require JA signaling [25]. To determine whether herbivory-elicited production of MoFs is also elicited by OSs and requires JA signaling, we wounded wild-type (WT) plants and treated the wounds with OSs of *M. sexta.* After a week of OS elicitations, 34% of the flowers produced were MoFs, compared to 11% of the flowers produced in plants that were only mechanically damaged (Figure 4A). When plants genetically transformed to be JA deficient by the silencing of lipoxygenase 3 (*Na*LOX3), which produces the fatty acid hydroperoxides essential for JA biosynthesis, were similarly elicited, neither mechanical damage nor damage plus treatment with OSs from *M. sexta* larvae increased the production of MoFs. However when these LOX3-silenced plants were sprayed with JA, the OS-elicited increase in MoFs observed in WT plants could be fully restored (Figure 4B). LOX3 plants sprayed with JA also significantly increased their production of MoFs (25%–28%) compared to plants sprayed with H_2O (12%–13%), independently of their previous elicitation treatment. From these experiments, we conclude that JA signaling is required for the herbivory-induced change in flower opening, which in turn is elicited when larval OSs are introduced into wounds as larvae feed.

In conclusion, the herbivory-elicited shift in floral phenology adds another dimension to the repertoire of defense responses that are known to be activated by the elicitors in insect larval OSs in *N. attenuata* and suggests that changes in flower phenology are another of the complex responses that reduce the fitness consequences of herbivore attack and help plants to solve the dilemma of attracting herbivores while advertising for pollinators.

Experimental Procedures

Herbivory-Elicited Change in Flower-Opening Phenology

In a native population of *N. attenuata* in southwestern Utah, groups of four size-matched plants were selected, of which two plants were experimentally infested with two first-instar *Manduca sexta* larvae while the other two remained caterpillar-free. Larvae were removed every second day and replaced by two new neonates to minimize the loss of leaf area by consumption while maintaining continuous elicitation. All naturally oviposited eggs laid by wild *Manduca* spp. were removed each day.

Night-opening flowers (NoFs) and morning-opening flowers (MoFs) were counted and removed each morning between 6:00 and 8:00 a.m.—the time when MoFs were still distinguishable from NoFs. The experiment was conducted in three blocks, each with five replicate plants per treatment, and two of the sets were used to measure fitness consequences of this phenology change in flower opening time.

Fitness Consequences

After seven days of continuous caterpillar feeding, when the production of MoFs reached its maximum, all flowers that were about to open within the next 24 hr were antherectomized prior to anthesis and labeled. One undamaged plant (day) and one infested plant (cat day) of the four plants within one group were completely covered by a mesh-covered wire cone [10] (Figure S2B) until dawn on the next day. The other two plants, again one undamaged plant (night) and one infested plant (cat night), remained uncovered over the following night to allow for exposure to nighttime pollinators. Just prior to dawn on the following morning, mesh-covered cones were exchanged between the covered and uncovered plants to allow daytime pollinators to visit the flowers of "day" and "day cat" plants. At dusk on the following evening, "day" and "day cat" plants were covered. All plants remained covered until the corollas of the antherectomized flowers had senesced. Capsules produced from antherectomized and successfully pollinated flowers were counted and collected 14 days after antherectomization.

The effects shown in Figure 3A for night pollination could be enhanced by the fact that MoFs are not open at night, cannot be outcrossed by nocturnal pollinators, and therefore will not set capsules. To evaluate the strength of this potential effect, we counted MoFs on all experimental plants in the morning after the experiment (see also Figure S2A). If the number of MoFs was subtracted from the original number of antherectomized flowers (which included NoFs and MoFs) and the percentage of capsules produced was recalculated, we found no differences from the original data (Figure 3A). In other words, uninfested plants still produced twice as many capsules from their NoFs (41% ± 12% standard error of the mean) via the activities of nocturnal pollinators as compared to infested plants from their NoFs (20% ± 7%).

In a preliminary experiment in 2007, 19 pairs of undamaged *N. attenuata* plants were chosen in a native population, and two NoFs per plant were antherectomized in the morning before they opened [10]. All other flowers were removed. One plant was covered with a mesh-covered cone between 6:00 p.m. and 6:00 a.m. to exclude nocturnal pollinators, whereas the other plant remained uncovered during the night. On the next morning, the cones

were exchanged, and the plant accessible to the activities of nocturnal pollinators was covered at 6:00 am, whereas the night-covered plant remained uncovered from 6:00 a.m. to 6:00 p.m., allowing access to day-active pollinators. At 6:00 p.m., all experimental plants were covered until the senescence of all experimental flowers.

Nectar Volume and Nectar Sugar Concentrations

Nectar from single flowers was collected as described in [8] between 7:00 and 9:00 a.m. [10] from NoFs and MoFs on the same plant to compare standing nectar volume and nectar sugar concentration in a native *N. attenuata* population at the time of hummingbird visitations in the morning. To compare NoFs and MoFs at the same developmental stage, at anthesis just as the corolla opens, we collected nectar from NoFs between 6:00 and 7:00 p.m. and from MoFs between 7:00 and 8.00 a.m., from the same plants grown in the glasshouse. The standing nectar volume was significantly larger for MoFs (paired Student's t test, $t_{19} = 8.83$, $p < 0.0001$; MoF: 2.7 ± 0.2 μl; NoF: 1.2 ± 0.2 μl), but the nectar sugar concentration was the same ($t_{19} = 0.15$, $p = 0.91$; 13.0% ± 0.2%).

Nectar Nicotine Concentrations

Nectar was collected in the morning (6:00 to 8:00 a.m.) from newly opened flowers. Nectar of five flowers per plant was pooled, and 10 μl of nectar was transferred in a 1.5 ml Eppendorf tube containing 50 μl MeOH, 0.04% (v/v) acetic acid, and 1 ng/μl nicotine-D_3 as an internal standard. Particulate matter was removed by centrifugation (10 min, 12,000 × g). Ten microliters of this solution was analyzed on an Agilent 1100 high-pressure liquid chromatography (HPLC) system connected with a Bruker-MicroToF mass spectrometer operated in ESI-positive mode with a capillary exit voltage of 130V. Separations were achieved with a gradient solvent program (solvent A: 10 mM ammonium bicarbonate [pH 10]; solvent B: acetonitrile) on a Phenomenex Gemini NX, 3 μm, 2 × 50 mm C-18 reverse phase column with the following HPLC program: 10% B isocratic for 2 min, linear gradient to 80% B for 5 min, isocratic at 80% B for 3 min. The column was reconditioned for 7 min between injections.

Oviposition Experiment

Experiments were conducted on two successive nights in June 2004. Fifteen pairs of same-size *N. attenuata* plants were selected from a 150 m^2 area of a native *N. attenuata* population in southwestern Utah. The number of NoFs was reduced to five flowers per plant. The nectar volume of these flowers was experimentally increased by adding 20 μl of a 12.5% sucrose solution at dusk (7:00 to 8:00 p.m.); control flowers were left untreated. All eggs were removed from plants at the time of nectar supplementation. Newly oviposited *M. quinquemaculata* eggs were counted the morning after nectar supplementation.

Requirement of JA Signaling

WT *N. attenuata* plants selfed for 30 generations (seeds derived from a collection from a native population at the DI Ranch in Santa Clara, UT, USA) and line A300, in which *N. attenuata* lipoxygenase 3 (*Na*LOX3) is expressed in an antisense orientation in the same WT genotype (as characterized in [19]), were used in the experiment. Germination was carried out according to the procedures described in [26]. Every other day, WT and LOX3-silenced plants were treated by wounding a fully expanded leaf at the early flowering stage. Leaves were wounded with a pattern wheel, and 20 μl water (w+w) or 20 μl *M. sexta* oral secretions diluted 1:1 (v/v) with water (w+s) was applied immediately after wounding. Control plants (con) remained untreated. After eight days of continuous elicitation, con, w+w-treated, and w+s-treated plants were sprayed every other day with either 30% ethanol—the solvent control solution—or a 1 mM JA solution. Unwounded control plants were also sprayed with either water or the JA solution. w+s and w+w elicitation of leaves continued throughout the experimental period, which lasted another eight days. Seven days after the first treatment, all open flowers were removed in the morning between 9:00 and 10:00 a.m., and the numbers of MoFs and NoFs were counted the following morning between 7:00 a.m. and 9:00 a.m.

Supplemental Information

Supplemental Information includes four figures and one table and can be found with this article online at doi:10.1016/j.cub.2009.11.071.

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References

1. Bronstein, J.L., Huxman, T., Horvath, B., Farabee, M., and Davidowitz, G. (2009). Reproductive biology of *Datura wrightii*: The benefits of a herbivorous pollinator. Ann. Bot. (Lond.) *103*, 1435–1443.
2. Cresswell, J.E., Hagen, C., and Woolnough, J.M. (2001). Attributes of individual flowers of *Brassica napus* L. are affected by defoliation but not by intraspecific competition. Ann. Bot. *88*, 111–117.
3. Steets, J.A., and Ashman, T.-L. (2004). Herbivory alters the expression of a mixed-mating system. Am. J. Bot. *91*, 1046–1051.
4. Ivey, C.T., and Carr, D.E. (2005). Effects of herbivory and inbreeding on the pollinators and mating system of *Mimulus guttatus* (Phrymaceae). Am. J. Bot. *92*, 1641–1649.
5. Ornelas, J.F., Gonzalez, C., Jimenez, L., Lara, C., and Martinez, A.J. (2004). Reproductive ecology of distylous *Palicourea padifolia* (Rubiaceae) in a tropical montane cloud forest. II. Attracting and rewarding mutualistic and antagonistic visitors. Am. J. Bot. *91*, 1061–1069.
6. Agrawal, A.A., Sharon, Y.S., and Stout, M.J. (1999). Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. Evolution *53*, 1093–1104.
7. Adler, L.S., Karban, R., and Strauss, S.Y. (2001). Direct and indirect effects of alkaloids on plant fitness via herbivory and pollination. Ecology *82*, 2032–2044.
8. Kessler, D., and Baldwin, I.T. (2007). Making sense of nectar scents: The effects of nectar secondary metabolites on floral visitors of *Nicotiana attenuata*. Plant J. *49*, 840–854.
9. Euler, M., and Baldwin, I.T. (1996). The chemistry of defense and apparency in the corollas of *Nicotiana attenuata*. Oecologia *107*, 102–112.
10. Kessler, D., Gase, K., and Baldwin, I.T. (2008). Field experiments with transformed plants reveal the sense of floral scents. Science *321*, 1200–1202.
11. Sime, K.R., and Baldwin, I.T. (2003). Opportunistic out-crossing in *Nicotiana attenuata* (Solanaceae), a predominantly self-fertilizing native tobacco. BMC Ecol. *3*, 6.
12. Karban, R. (1997). Neighbourhood affects a plant's risk of herbivory and subsequent success. Ecol. Entomol. *22*, 433–439.
13. Adler, L.S., and Bronstein, J.L. (2004). Attracting antagonists: Does floral nectar increase leaf herbivory? Ecology *85*, 1519–1526.
14. Halitschke, R., Schittko, U., Pohnert, G., Boland, W., and Baldwin, I.T. (2001). Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. III. Fatty acid-amino acid conjugates in herbivore oral secretions are necessary and sufficient for herbivore-specific plant responses. Plant Physiol. *125*, 711–717.
15. Diezel, C., von Dahl, C.C., Gaquerel, E., and Baldwin, I.T. (2009). Different lepidopteran elicitors account for cross-talk in herbivory-induced phytohormone signaling. Plant Physiol. *150*, 1576–1586.
16. Wu, J., Hettenhausen, C., Meldau, S., and Baldwin, I.T. (2007). Herbivory rapidly activates MAPK signaling in attacked and unattacked leaf regions but not between leaves of *Nicotiana attenuata*. Plant Cell *19*, 1096–1122.
17. Skibbe, M., Qu, N., Galis, I., and Baldwin, I.T. (2008). Induced plant defenses in the natural environment: *Nicotiana attenuata* WRKY3 and WRKY6 coordinate responses to herbivory. Plant Cell *20*, 1984–2000.
18. Paschold, A., Halitschke, R., and Baldwin, I.T. (2007). Co(i)-ordinating defenses: NaCOI1 mediates herbivore- induced resistance in *Nicotiana attenuata* and reveals the role of herbivore movement in avoiding defenses. Plant J. *51*, 79–91.
19. Halitschke, R., and Baldwin, I.T. (2003). Antisense LOX expression increases herbivore performance by decreasing defense responses and inhibiting growth-related transcriptional reorganization in *Nicotiana attenuata*. Plant J. *36*, 794–807.

20. Baldwin, I.T. (1999). Inducible nicotine production in native *Nicotiana* as an example of adaptive phenotypic plasticity. J. Chem. Ecol. *25*, 3–30.
21. Jassbi, A.R., Zamanizadehnajari, S., Kessler, D., and Baldwin, I.T. (2006). A new acyclic diterpene glycoside from *Nicotiana attenuata* with a mild deterrent effect on feeding *Manduca sexta* larvae. Z. Naturforsch. [B] *61*, 1138–1142.
22. Steppuhn, A., Gase, K., Krock, B., Halitschke, R., and Baldwin, I.T. (2004). Nicotine's defensive function in nature. PLoS Biol. *2*, E217.
23. Zavala, J.A., Patankar, A.G., Gase, K., Hui, D., and Baldwin, I.T. (2004). Manipulation of endogenous trypsin proteinase inhibitor production in *Nicotiana attenuata* demonstrates their function as antiherbivore defenses. Plant Physiol. *134*, 1181–1190.
24. Kessler, A., and Baldwin, I.T. (2002). Plant responses to insect herbivory: The emerging molecular analysis. Annu. Rev. Plant Biol. *53*, 299–328.
25. Schwachtje, J., Minchin, P.E.H., Jahnke, S., van Dongen, J.T., Schittko, U., and Baldwin, I.T. (2006). SNF1-related kinases allow plants to tolerate herbivory by allocating carbon to roots. Proc. Natl. Acad. Sci. USA *103*, 12935–12940.
26. Krügel, T., Lim, M., Gase, K., Halitschke, R., and Baldwin, I.T. (2002). *Agrobacterium*-mediated transformation of *Nicotiana attenuata*, a model ecological expression system. Chemoecology *12*, 177–183.