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Antimicrobial nectar inhibits a florally transmitted pathogen of a wild *Cucurbita pepo* (Cucurbitaceae)[1]

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- *Premise of the study:* Floral nectars of many species contain antimicrobial chemicals, but their function in nectar is subject to debate. Previously, we have shown that *Erwinia tracheiphila*, the causative agent of bacterial wilt disease in cucurbits, can be transmitted via the floral nectaries.
- *Methods:* We used a disk diffusion assay (DDA) to determine the antimicrobial effects of nectar from a wild gourd on lawns of *Escherichia coli* and *Erwinia tracheiphila*. We also used *E. tracheiphila* to inoculate flowers of wild gourd plants, with and without nectar.
- *Key results:* The DDA showed that paper disks saturated with 10 μL of nectar inhibited the growth of *E. coli* on a larger area of the lawn than 40% glucose but a smaller area than 5% ampicillin for 12 h. On lawns of *E. tracheiphila*, nectar inhibited growth on a larger area than glucose for 24 h and there were no significant differences between ampicillin and nectar for12 h. A significantly larger proportion of the plants inoculated via flowers without nectar contracted wilt disease than plants with nectar.
- *Conclusions:* These findings indicate that nectar reduces transmission of *E. tracheiphila* via the nectaries and reveal the potential for florally transmitted pathogens to influence the evolution of floral traits.

Key words: antimicrobial nectar; bacterial wilt disease; cucumber beetles; *Cucurbita pepo* ssp. *texana*; disk diffusion assay; *Erwinia tracheiphila*; nectar.

The showy, often fragrant, flowers of animal-pollinated plants function to attract and reward pollinators. However, the same flowers can also serve as an easily exploited resource for a variety of natural enemies (Strauss and Whittall, 2006). Consequently, plants face a dilemma: their flowers must be apparent and readily accessible to the visitors that provide the pollination service while simultaneously discouraging the visitors that would exploit their resources without effecting pollination. For example, the presence of glucose, fructose, sucrose, and other simple sugars in floral nectar makes nectar an excellent medium for the growth of microbes (Herrera et al., 2008). Not surprisingly, microbes have been reported in the nectars of many species, and, for many species, the sugar concentration of nectar tends to decrease with time after anthesis whereas the ethanol concentration in nectar tends to increase—a finding that is consistent with microbial degradation of sugar (see Nicolson and Thornburg, 2007; Herrera et al., 2008). In addition to sugars, however, the nectars of many species contain secondary chemicals, such as phenolic compounds, and proteins that are known to have antimicrobial properties (Baker and Baker, 1983; Adler, 2000; Nicolson and Thornburg, 2007; Hancock et al., 2008). For example, a protein that generates very high levels of hydrogen peroxide (a powerful antimicrobial agent) was found in the nectar of nine of the 15 species examined (Carter and Thornburg, 2000), and some species are known to produce other proteins that have antifungal and antibacterial properties (Nicolson and Thornburg, 2007).

Because microbial degradation of the sugars in nectar would reduce the average nectar reward per flower and perhaps also decrease the efficiency of the pollinators as a result of the increased concentration of ethanol in nectar, antimicrobial compounds in nectar have been hypothesized to function in the attraction and reward of pollinators (Adler, 2000; but see Wiens et al., 2008). Recently, Carter and Thornburg (2004) noted that wind, pollinators, and other floral visitors can carry microbes, including plant-pathogenic microbes, into the reproductive tract of flowers. They hypothesize that antibiotic compounds in nectar function to protect the ovary from invading microorganisms. Although these hypotheses are neither exhaustive (e.g., the presence of antibiotic chemicals in the nectar could be a pleiotropic by-product of their production elsewhere in the plant; Adler, 2000) nor mutually exclusive, no studies have explicitly examined the effect of antimicrobial nectar on pollination or the incidence of a disease that is transmitted via the floral organs.

Erwinia tracheiphila (E. F. Smith) Holland (Enterobacteriaceae) is the causative agent of bacterial wilt disease, an important disease of cultivated cucurbits (cucumbers, melons, and squash) and wild gourds (*Cucurbita* spp.). *Erwinia tracheiphila* is vectored by cucumber beetles (*Diabrotica* spp. and *Acalymma* spp.). In the eastern United States, *E. tracheiphila* overwinters in the digestive tract of cucumber beetles (Fleischer et al., 1999; Garcia-Salazar et al., 2000), and transmission occurs when fecal pellets containing *E. tracheiphila* land on leaf wounds at the sites of feeding damage (Leach, 1964). After entering the plant, the bacteria proliferate in the xylem, where they secrete an exopolysaccharide matrix that cuts off the water supply, which results in wilting. Wilt symptoms typically develop

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7–15 d after infection. Once symptoms appear, death occurs in 1–3 wk (Yao et al., 1996).

After feeding on the leaves, cucumber beetles aggregate in the flowers in midmorning to feed and mate (Darlington, 2006). Our recent studies have shown that *E. tracheiphila* is also transmitted via the floral nectaries when cucumber-beetle fecal pellets contaminated by *E. tracheiphila* fall onto or near the floral nectaries (Sasu et al., 2010). The goals of the present study are to determine (1) whether the nectar of a wild gourd (*Cucurbita pepo* ssp. *texana* [L.] Andres) has antibiotic properties; (2) whether the antibiotic properties of the nectar change with the amount of leaf damage by cucumber beetles (because chemical defenses in the leaves of *Cucurbita pepo* are known to be induced by damage); (3) whether the strength of the antibiotic properties varies over time (because the flowers abscise 24–48 h after anthesis); and (4) whether the antibiotic properties of the nectar protect the nectaries from invasion by *E. tracheiphila*.

MATERIALS AND METHODS

Study species—*Cucurbita pepo* ssp. *texana* (wild gourd) is an annual monoecious vine with indeterminate growth and reproduction. It is native to northern Mexico, Texas, and the lower Mississippi River drainage area and is thought to be either the wild progenitor of the cultivated squashes (*C. pepo* ssp. *pepo*) or an early escape from cultivation (Decker and Wilson, 1987; Decker-Walters, 1990; Lira et al., 1995; Decker-Walters et al., 2002). After germination and seedling emergence, there is a period of vegetative growth (5–7 nodes) after which most nodes produce one large yellow flower (either staminate or pistillate) in the axils of each leaf. The flowers last for only one morning and are pollinated by bees, especially squash bees of the genera *Peponapis* and *Xenoglossa*. The fruits are round to oval in shape, with a volume of 175–450 mL, and typically contain 150–300 seeds that weigh 20–40 mg (Winsor et al., 2000; Avila-Sakar et al., 2001).

The leaves and other organs of this wild gourd produce bitter compounds called cucurbitacins (oxygenated tetracylic triterpenes) that deter most herbivores (Tallamy, 1985; Metcalf and Rhodes, 1990). However, cucumber beetles are adapted to feed on cucurbitacins in the leaves and are found throughout the native ranges of *Cucurbita* species (Robinson and Decker-Walters, 1997). Cucumber beetles feed on leaves and flowers and cause a characteristic pattern of holes (typically 1–1.5 cm in diameter) in the portions of the leaves serviced by the smallest veins. Leaf damage by cucumber beetles over the entire growing season has been shown to substantially reduce yield in cultivated squash (e.g., Tallamy and Krischik, 1989) and reduce reproductive output in the wild (free-living) gourd (Quesada et al., 1995; Stephenson et al., 2004; Du et al., 2008). Cucumber beetles are also the only known vector of the deadly bacterial pathogen *E. tracheiphila* Smith (Yao et al., 1996), which is the causative agent of bacterial wilt disease.

Disk diffusion assays—To determine whether the nectar of the wild gourd has antibiotic properties, we performed disk diffusion assays (DDA) (Gabhainn et al., 2004). In brief, we grew lawns of *Escherichia coli* and *Erwinia tracheiphila* on separate sterile culture plates and placed four filter-paper disks on each plate. The four disks were saturated with (1) 5% ampicillin solution, (2) 40% glucose, (3) nectar from field-grown plants with light beetle damage, and (4) nectar from field-grown plants with heavy beetle damage. We then measured the area of inhibition around each disk (see Fig. 1 for schematic).

Field nectar collection—During the 2008 field season, we collected nectar for the diffusion assays from male flowers on healthy (no visible symptoms of disease), unsprayed wild gourd plants grown at the Pennsylvania State University Agriculture Research Farms at Rock Springs, Pennsylvania. The day before the DDA was performed, we identified male flower buds that would open the next day and tied them shut with a twist tie so that the bees would not have early access to the nectar the following day. We also assessed foliar damage by cucumber beetles to the branch bearing the flower bud using a linear scale from 0 to 5, where 0 = no visible damage to foliage and 5 = significant visible damage to all leaves with at least one leaf having >50% of the leaf area removed (for details, see Stephenson et al., 2004). Damage levels of 0–2 were considered light beetle damage and damage levels of 3–5 were considered heavy beetle damage. Approximately 20 male flowers were collected in the early morning from plants with light damage, and 20 from plants with heavy damage, each day. The flowers were placed on ice and brought to the laboratory, where the nectar was collected using microcapillary tubes. The nectar from each damage level was pooled and stored on ice during the extraction process and then used immediately for the DDA.

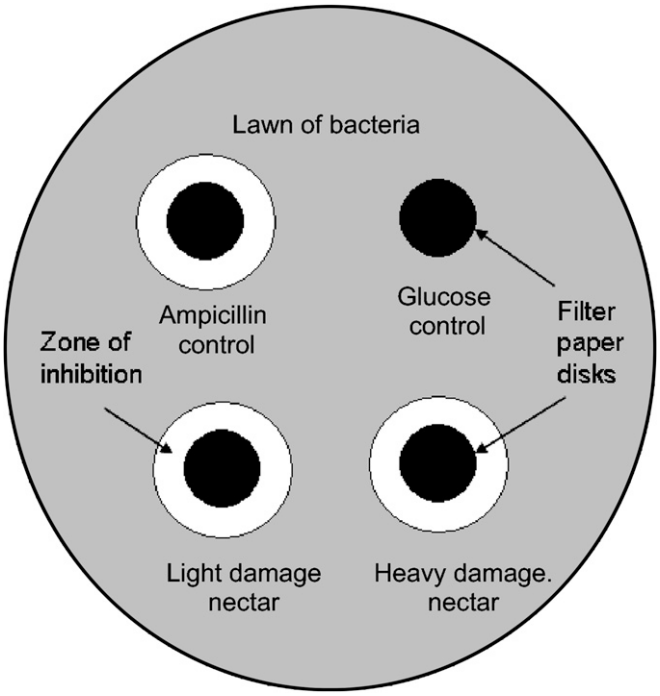

Fig. 1. Schematic of a disk-diffusion-assay plate. Each 100 × 15 mm sterile culture plate was inoculated with either *Escherichia coli* or *Erwinia tracheiphila*, and four 10-μL treatments were applied to the four 6-mm-diameter paper disks on each plate (5% ampicillin, 40% glucose, nectar from lightly damaged plants, and nectar from heavily damaged plants). The area of bacteria cleared by each paper disk was calculated at each of three times (8, 12, and 24 h).

Plate preparation—The *E. tracheiphila* used in the DDA was originally isolated and cultured from our field plants with symptoms of wilt disease. To verify that the cultured bacteria were indeed *E. tracheiphila*, we inoculated seedlings grown in a greenhouse and recorded wilt symptoms (see Ferrari et al., 2007). The *E. coli* used in the DDA was obtained from Invitrogen (Carlsbad, California, USA; One Shot Top 10 chemically competent *E. coli*). Both bacteria were stored separately in 15% glycerol solution at –80°C. Sterile culture plates (100 × 15 mm) were prepared using nutrient agar supplemented with agar and peptone (NAP). To prepare bacteria for the DDA, we thawed the glycerol stock to room temperature and applied 100 μL inoculum of either *E. tracheiphila* or *E. coli* directly onto the NAP plates using a sterile L-rod (see DeMackiewicz et al., 1998). We allowed the surface of the plates to dry and then placed four sterile individual filter-paper disks with a diameter of 6 mm equidistant from each other on the surface of the agar medium. To each disk we added 10 μL of one of four treatment solutions: 5% ampicillin solution (50 mg mL^{-1}), 40% glucose solution, nectar from lightly damaged plants, and nectar from heavily damaged plants (Fig. 1). The DDA was performed on each of 4 d (16 and 25 July, 8 and 13 August). Each day, 15 plates were prepared per bacterium for a total of 30 plates d^{-1} (60 plates *E. coli* and 60 plates *E. tracheiphila* in total). The plates were incubated at 26°C in the dark, and the diameter of the inhibition zone was measured at 8, 12, and 24 h. The area of the inhibition zone was later calculated ($A = \pi r^2$ – area of filter-paper disk).

Statistical analysis—To determine the effects of treatment (the four solutions added to the filter paper), time (8, 12, or 24 h), date (the four dates on which the DDA was performed), and the interaction between date and

treatment on the area cleared around each filter-paper disk on each plate, we performed a repeated-measures analysis of variance (ANOVA; Proc Mixed; SAS Institute, 2002) for each of the two types of bacterial lawns. Date was treated as a fixed effect because preliminary studies indicated that nectar volume per flower varied with soil moisture (recent rainfall) and we felt that the antimicrobial properties of the nectar might vary with nectar volume or dilution. The repeated variable was time (random), and we used autoregressive covariance structure. To measure the variance among inhibition zones across time, the repeated variable was calculated on each treatment within each plate on each date that the assay was performed. We also performed multiple pairwise comparisons among the four treatments at each point in time (separately for 8, 12, and 24 h) while controlling for Type I error rates, to identify differences among the least-square means of the four treatments at each point in time (Tukey-Kramer; SAS Institute, 2002) for each of the two types of bacteria.

***Nectary inoculations of* E. tracheiphila**—To determine whether floral nectar alters the probability of infection by *E. tracheiphila* via the nectaries, we grew 120 wild gourd plants in February 2009 in 1-gallon pots in Pro-Mix BX potting soil with fungicide (Premier Horticulture, Riviere-du-Loup, Quebec, Canada) in a greenhouse. At ~10 wk post-emergence, we began to inoculate the nectaries of male flowers. On the day that a flower was inoculated, we either (1) removed the nectar using a 50-μL capillary tube and placed 100 μL of *E. tracheiphila* inoculum onto the nectary with a blunt 18-gauge needle and 1-mL Tuberculin syringe or (2) placed 100 μL of *E. tracheiphila* inoculum onto the nectary with a blunt 18-gauge needle and 1-mL tuberculin syringe without removing the nectar. The inoculum was prepared from *E. tracheiphila* isolated the previous summer from field-grown plants. The isolates were grown in nutrient broth supplemented with extra peptone and placed in a 15% glycerol solution and frozen at – 80°C (see Ferrari et al., 2007). Samples of the frozen isolates were thawed and streaked onto NAP plates and incubated at 26°C for 5–7 d. The resulting colonies were dislodged with a sterile L-rod and transferred into deionized water (diH2O). The average concentration of *E. tracheiphila* cells in each inoculum was 5.47×10^8 cells mL^{-1}, determined using a spectrophotometer at OD 600. Each day that we performed inoculations, we also inoculated two control plants, one with diH20 and another directly through the stem vasculature with the *E. tracheiphila* used that day. Five flowers were inoculated on each plant (all with nectar or all without nectar) unless a plant developed symptoms of wilt disease, in which case we performed no additional floral inoculations. We recorded the first day of wilt symptoms and followed disease progression until the plant died. To be certain that the wilting was caused by *E. tracheiphila*, we reisolated the bacteria from the wilting plants and confirmed colony morphology.

RESULTS

The repeated-measures ANOVA revealed that the treatment applied to the filter-paper disks, the date on which the disk diffusion assay was performed, and the interaction between treatment and date all had significant effects on the growth of *E. coli* (Table 1A). The significant treatment × date interaction was attributable primarily to the strong effects of the ampicillin treatment and the relatively weak effects of the two types of nectar on the second date (although both types of nectar had larger zones of inhibition than the glucose treatment). The analysis also showed that the area of each inhibition zone varied over time and that time had a significant effect on the growth of *E. coli* on each treatment within each plate on each date the assay was performed (Table 1A). At 8, 12, and 24 h, the ampicillin treatment had cleared a significantly larger area of the *E. coli* lawn than the glucose treatment or either of the two types of nectar (from plants lightly and heavily damaged by cucumber beetles; Fig. 2). At 8 and 12 h, the nectar from lightly damaged plants had cleared a significantly larger area of the *E. coli* lawn than the glucose treatment, but by 24 h there were no significant differences in the area cleared by nectar and by glucose (Fig. 2). At no time did the two types of nectar differ in the area they cleared on the lawns of *E. coli*.

TABLE 1. Results of a repeated-measures analysis of variance for the effects of treatment (5% ampicillin, 40% glucose, nectar from lightly damaged plants, and nectar from heavily damaged plants), date, and the interaction of date and treatment on area of inhibition (cm^2) on sterile culture plate lawns of (A) *Escherichia coli* and (B) *Erwinia tracheiphila*. In this model, time was a repeated measures variable; it was treated as a random variable, and it was specified for each treatment within each plate on each date the experiment was performed. Autoregressive covariance structure was specified (Proc Mixed; SAS Institute, 2002).

(A) *E. coli*

Effect	Numerator df	Denominator df	*F*	*P*
Treatment	3	224	94.00	<0.0001
Date	3	224	3.62	0.0140
Date × treatment	9	224	3.06	0.0018
Covariate	**Estimate**	**SE**	***Z***	***P***
Time on each plate (date × treatment)	0.44	0.04	11.60	<0.0001

(B) *E. tracheiphila*

Effect	Numerator df	Denominator df	*F*	*P*
Treatment	3	224	20.30	<0.0001
Date	3	224	0.97	0.4057
Date × treatment	9	224	2.97	0.0027
Covariate	**Estimate**	**SE**	***Z***	***P***
Time on each plate (date × treatment)	0.41	0.04	9.98	<0.0001

The repeated-measures ANOVA also revealed that treatment and the treatment × date interaction had significant effects but that date did not have a significant effect on the growth of *E. tracheiphila* (Table 1B). The treatment × date interaction was attributable primarily to the strong effects of both types of nectar on the first date of the experiment (which exceeded the ampicillin treatment) and the complete lack of inhibition by the glucose treatment on the fourth date of the experiment. The analysis also showed that the area of each inhibition zone varied over time and that time had a significant effect on the growth of *E. tracheiphila* on each treatment within each plate on each date the assay was performed (Table 1B). At 8 and 12 h, the ampicillin and both types of nectar had cleared a significantly larger area of the *E. tracheiphila* lawn than the glucose treatment (Fig. 2). There were, however, no significant differences in the area of the lawn cleared by the ampicillin or either of the two types of nectar. At 24 h, the ampicillin had cleared a larger area of the *E. tracheiphila* lawn than the glucose or either type of nectar. At 24 h, the nectar from the lightly damaged plants had cleared a significantly larger area of the *E. tracheiphila* lawn than the glucose treatment, whereas the nectar from the heavily damaged plants had cleared a nearly significantly larger area than the glucose treatment ($P = 0.06$) (Fig. 2). At no time point did the two types of nectar differ significantly in their effect on the growth of *E. tracheiphila*.

In the greenhouse inoculation experiment, we found that the probability that a plant will contract wilt disease following inoculation of the nectary with *E. tracheiphila* is dependent upon the presence of nectar at the time of inoculation ($\chi^2 = 15.4$; df = 1; $P < 0.001$). Twenty-four of the 50 plants whose male flowers were inoculated with *E. tracheiphila* after nectar removal contracted wilt disease. By contrast, only six of the 50 plants whose male flowers were inoculated without nectar removal contracted wilt disease. All 10 of the plants that received stem inoculations

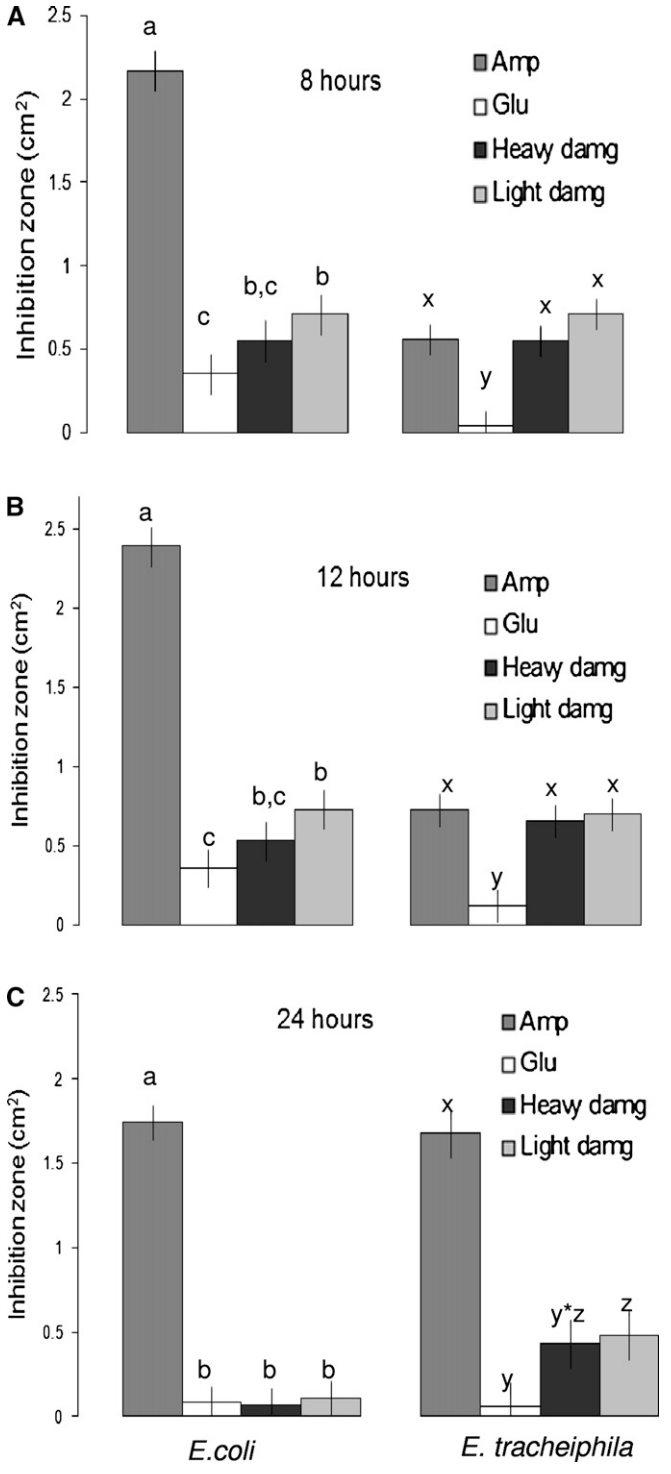

Fig. 2. Disk-diffusion-assay inhibition zones (cm^2) on lawns of *Escherichia coli* and *Erwinia tracheiphila*. Graphs A, B, and C show the least-square means ± SE of the inhibition zones at 8, 12, and 24 h for each of the four treatments. Least-square means and standard errors were obtained from a general linear model analysis of variance including the following effects: date (four dates), treatment (5% ampicillin, 40% glucose, nectar from lightly damaged plants, nectar from severely damaged plants), plate nested within date, and the treatment × date interaction. Lowercase letters (a, b, and c for *E. coli* and x, y, and z for *E. tracheiphila*) show significant differences resulting from multiple pairwise comparisons performed at each time point (8, 12, and 24 h, respectively) (Tukey-Kramer; SAS Institute, 2002). y* represents a nearly significant ($P > 0.06$) difference between the glucose and heavy-damage treatments at 24 h.

(positive control for *E. tracheiphila* virulence) contracted wilt disease, whereas none of the plants whose flowers were inoculated with diH_2O contracted wilt disease (negative control).

DISCUSSION

The floral nectar of many species contains secondary chemical compounds and/or proteins that are known to have antimicrobial properties (Martini et al., 1990; Adler, 2000: Thornburg et al., 2003; Nicolson and Thornburg, 2007; Sala Junior et al., 2008). The nectars of other species are known to have antimicrobial properties, but the specific chemicals responsible for these properties have not been investigated (Adler, 2000; Nicolson and Thornburg, 2007). Here, we have shown that the nectar produced by wild gourd plants has a short-term (12 h) effect on the growth of *E. coli* in culture and a pronounced effect on the growth of *E. tracheiphila* in culture for 24 h. In fact, wild gourd nectar is as effective at inhibiting *E. tracheiphila* growth as 5% ampicillin for 12 h. Moreover, our greenhouse inoculation study showed that when the nectar is removed from flowers, the plants are 4× more likely to contract wilt disease than plants whose nectar was not removed prior to inoculation. Although we exercised great care when removing the nectar from the flowers in the greenhouse, we cannot rule out the possibility that the process of nectar removal may, perhaps by damaging floral tissue, have facilitated infection by *E. tracheiphila*. It appears, however, that the nectar was able to inhibit *E. tracheiphila* infection even though the volume (100 μL) of the inoculum and the concentration of the *E. tracheiphila* in the inoculum (5.47×10^8 cells mL^{-1}) were far greater than what is likely to be encountered in nature.

These findings suggest that the anti–*E. tracheiphila* properties of the nectar play a role in the transmission of wilt disease via the floral nectaries of *Cucurbita* species. We focused on the antimicrobial properties of nectar from male flowers and transmission in the greenhouse via the inoculation of male flowers because (1) male flowers attract more beetles per flower than female flowers under field conditions (Sasu et al., 2009); (2) male flowers are oriented vertically and the fecal pellets of the beetles accumulate at the base of the flower near the nectary, whereas female flowers are oriented horizontally; and (3) wild gourd plants make approximately 7× more male flowers than female flowers and, consequently, there are greater opportunities for exposure to *E. tracheiphila* via male flowers (Sasu et al., 2010). Previous greenhouse inoculation studies, however, have shown that *E. tracheiphila* can also be transmitted via female flowers (Sasu et al., 2010), and a pilot study that we conducted suggested that the nectar of female flowers also has antimicrobial properties (data not shown).

Both male and females flowers of *Cucurbita* species open at sunrise and close in the late morning. Nectar secretion begins a few hours before anthesis and is produced continuously until the flowers close (Nepi et al., 1996). In our fields, total nectar secretion by the late morning ranges from 10 to 100 μL in unvisited (bagged) flowers and seems to vary with soil moisture. However, the flowers are visited by pollinators repeatedly

throughout the morning: first by squash bees, which forage for both nectar and pollen shortly after anthesis, and then by squash bees, bumble bees, and honey bees, which forage for the newly secreted nectar throughout the remainder of the morning (Winsor et al., 2000).

In the mid- to late morning, cucumber beetles aggregate in the flowers to mate and feed. At the same field site during the same time of the growing season in which the flowers were collected for the DDA, we have found, using real-time polymerase chain reaction with *E. tracheiphila*–specific primers, that >50% of the flowers have cucumber-beetle frass on or in the nectary (Sasu et al., 2010). Moreover, by 1100 hours, >90% of the flowers contain cucumber-beetle frass contaminated with *E. tracheiphila* (Sasu et al., 2010). In late morning the flower closes, and within 24–48 h a complete abscission layer is formed between the flower and the pedicel and the flower falls to the ground. For a plant to become infected, it is necessary for the *E. tracheiphila* to traverse the nectary and move into the xylem of the pedicel before the flower abscises. Infection by *E. tracheiphila* may be facilitated by repeated visitation by nectar-foraging bees throughout the morning. In addition, *Cucurbita* flowers are known to resorb the nectar left in the flowers before abscission (Nepi et al., 1996), which may also facilitate bacterial transport through the nectary tissue.

At our field site, a typical wild gourd plant produces 90–150 male flowers during July and August (Stephenson et al., 2004; Du et al., 2008; Sasu et al., 2009). Given the very high exposure rates to *E. tracheiphila* of the flowers that we have observed in our fields (>90% with *E. tracheiphila*–contaminated frass; Sasu et al., 2010), it is surprising that only 5–40% of the plants contracted wilt disease each year over a 7-yr period with 720 plants yr^{-1} (four fields with 180 plants; Ferrari et al., 2007; Du et al., 2008; Sasu et al., 2009). We suspect that the low incidence of wilt disease in our fields is attributable to the anti–*E. tracheiphila* properties of the nectar sufficiently slowing the growth of the bacteria until floral abscission occurs. These findings support the hypothesis proposed by Carter and Thornburg (2004) that the antibiotic properties of nectar function to prevent pathogens from gaining access to the plants via the nectaries.

The production of defensive chemicals (cucurbitacins) in the leaves and other organs of *C. pepo* (squash and wild gourds) are known to have both a constitutive component and a component that is inducible by cucumber-beetle herbivory (Tallamy, 1985; Metcalf and Rhodes, 1990). Adler et al. (2006) found that nectar alkaloids increased with herbivory in *Nicotiana tobacum*. However, some floral nectars contain antimicrobial proteins, hydrogen peroxide, and other compounds that may not be upregulated as a response to herbivory (Martini et al., 1990; Thornburg et al., 2003; Nicolson and Thornburg, 2007; Hancock et al., 2008; Sala Junior et al., 2008). We found that the antibiotic properties of the wild-gourd nectar did not differ significantly between plants with light and heavy damage by cucumber beetles. This finding suggests that either (1) the nectar components responsible for the antibiotic properties are not inducible by cucumber-beetle herbivory or (2) both light and heavy damage by cucumber beetles are sufficient to induce the antibiotic compounds.

Historically, studies of the evolution of floral traits (such as their color, odor, size, shape, longevity, and quantity and composition of nectar) have focused on their roles in attracting and rewarding pollinators, disseminating pollen, and producing fruit and seed. However, there is a growing realization that floral traits can also evolve in response to selective pressures imposed by natural enemies (e.g., Stephenson, 1981, 1982; Galen, 1983; Strauss et al., 1996; Baldwin et al., 1997; Adler, 2000; Ashman, 2002; Irwin et al., 2004). Although most of these studies focused on herbivores, recent studies have argued that the evolution of floral longevity can be influenced by exposure rates to florally transmitted pathogens (Shykoff et al., 1996; Kaltz and Shykoff, 2001; Valdivia et al., 2006). The data presented here, in showing that the antimicrobial nectar of a wild gourd reduces transmission of *E. tracheiphila* via the floral nectaries, suggest the possibility that florally transmitted pathogens can also influence the evolution of nectar composition.

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