Odor-Mediated Push-Pull Pollination in Cycads

Irene Terry,[1]* Gimme H. Walter,[2] Chris Moore,[2] Robert Roemer,[3] Craig Hull[2]

Cycads are dioecious gymnosperms, with male and female individuals, of Permian origin. Cycads share obligate mutualisms with specialist insect pollinators, almost universally beetles (*1*). The known exception involves thrips of the genus *Cycadothrips* (*1*, *2*), which participate in obligate pollination mutualisms with endemic Australian *Macrozamia* cycads. *Cycadothrips* are in a basal thysanopteran family, proposed as arising by at least the Cretaceous (*3*). Thrips are primarily found on male cones, which provide food (pollen) for adults and larvae (*2*, *4*). During pollination, male and female *Macrozamia lucida* cones self-heat daily, up to 12°C above ambient temperatures between the hours of ~1100 and 1500, when nearly a millionfold increase in male volatile emissions occurs [females reach ~one-fifth that amount (*4*)]. In concert, adult *Cycadothrips chadwicki* leave male and female cones en masse (movie S1) (*4*), and larvae move to the exterior of their male cone habitat. Pollination is mediated by pollen-laden thrips that enter female cones.

We tested thrips' electrophysiological and behavioral responses to cone volatiles. A two-way choice between male sporophyll volatiles or air in a Y-tube olfactometer (*5*) demonstrated that thrips are attracted (or neutral) to sporophylls early in the day, repelled at midday, and attracted at later times (Fig. 1A). These phases parallel field results; cones retain (morning, low volatile emissions), repel (midday high emissions), and later attract (low emissions) thrips (*4*).

Electrophysiological tests (*5*) with volatiles from cones and their specific chemical components revealed that *Cycadothrips* respond to three components (Fig. 1, B and C): β-myrcene (>90% of total emissions during thermogenesis) and (*E*)-β-ocimene (2%), which change postthermogenesis (*4*, *5*), and allo-ocimene (~2%) (*5*). Y-tube tests at ecological concentrations of these chemicals (*5*) showed that ocimenes attract thrips (fig. S1), whereas β-myrcene attracts thrips at low concentrations but repels them at higher levels (Fig. 1D). When control and high-concentration β-myrcene vessels (*5*) were switched after thrips had entered the control arm, 77% moved directly into the new control arm. Most that remained died within 10 min, suggesting that by leaving cones thrips avoid toxic β-myrcene levels.

These cone volatile changes sufficiently explain the diel thrips behavior observed in situ (*4*), although temperature and light may modulate their effects. We characterize this as a "push-pull" pollination strategy. Flowers are generally portrayed as only "pulling" pollinators via visual or odor cues. Some orchids, though, chemically repel pollinators after pollination (*6*). Driving thrips from male cones increases pollen-laden thrips attendance at female cones, which presumably attract by deceit because their volatile components match those of males (*4*). Parallel attraction to male cones allows thrips to accrue pollen for the next day's cycle. This obligate pollination mutualism stands out for its push-pull behavior and because it involves an ancient gymnosperm lineage and a basal thrips clade (*2*). Floral scent may have originally evolved to deter herbivores (*7*), and this system may represent a conserved early intermediary in the evolution of seed plant pollination.

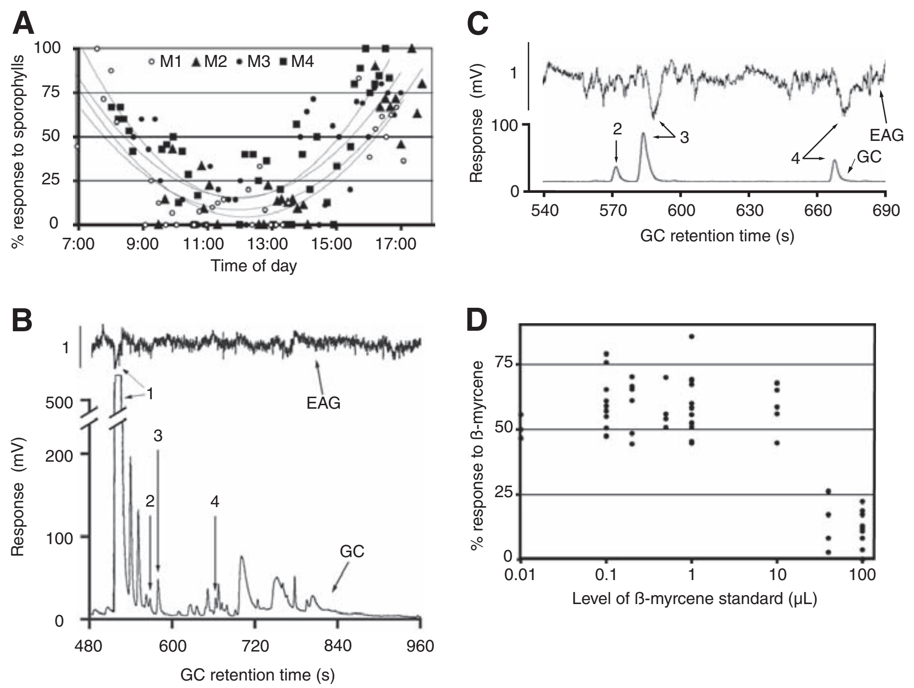

Fig. 1. *Cycadothrips* responses to *M. lucida* volatiles. (**A**) The % of insects going to sporophylls of four mid-pollination-stage male cones (M1 to M4) versus controls (Y-tube tests). Lines are quadratic fits to data from separate cones; fit parameters are not statistically different (*5*). (**B** and **C**) Male thrips physiologically respond [gas chromatography electroantennographic (EAG) detection, GC-EAD] (B) to β-myrcene (peak 1) from male cone volatiles and (C) to (*E*)-β-ocimene (peak 3) and allo-ocimene (peak 4) but not to (*Z*)-β-ocimene (peak 2) from ocimene standard. (**D**) The % of insects attracted to different concentrations of β-myrcene standard versus controls (Y-tube tests).

References and Notes

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8. Studies partially supported by National Geographic Society. Thanks to R. Raguso, A. Najar, F. Adler, and anonymous reviewers.

Supporting Online Material

www.science.org/cgi/content/full/318/5847/70/DC1
Materials and Methods
Fig. S1
Movie S1

15 May 2007; accepted 27 August 2007
10.1126/science.1145147

[1]Department of Biology, University of Utah, Salt Lake City, UT 84112, USA. [2]The School of Integrative Biology, University of Queensland, Brisbane, QLD 4072, Australia. [3]Department of Mechanical Engineering, University of Utah, Salt Lake City, UT 84112, USA.

*To whom correspondence should be addressed. E-mail: terry@biology.utah.edu