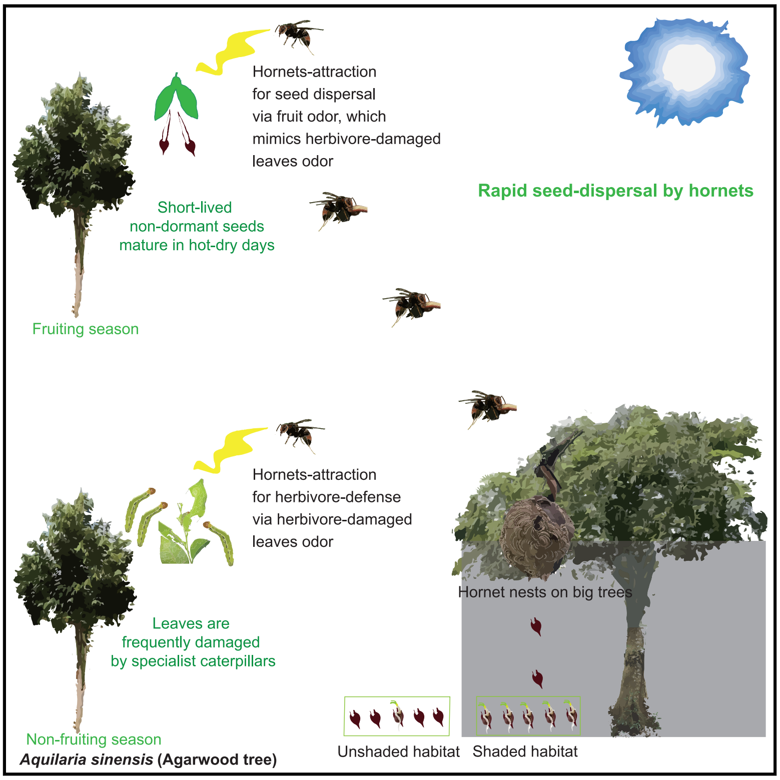
Report

Current Biology

Plant-defense mimicry facilitates rapid dispersal of short-lived seeds by hornets

Graphical abstract



Highlights

- Rapid seed dispersal is essential for the short-lived seeds of *Aquilaria sinensis*
- Hornets remove seeds within minutes of exposure and deposit most in damp shade
- Hornets are attracted by highly volatile fruit chemicals
- These volatiles mimic those that attract predators to herbivore-damaged leaves

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In brief

Qin et al. demonstrate that rapid seed dispersal by hornets is essential for short-lived seeds of an agarwood plant. Hornets are attracted rapidly by highly volatile fruit chemicals, which appear to have been achieved by re-purposing of volatiles utilized in plant defense. Rapid seed dispersal seems widespread, and volatiles are effective signals.

Qin et al., 2022, Current Biology *32*, 3429–3435
August 8, 2022 © 2022 Elsevier Inc.
https://doi.org/10.1016/j.cub.2022.06.034

Current Biology

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Report

Plant-defense mimicry facilitates rapid dispersal of short-lived seeds by hornets

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https://doi.org/10.1016/j.cub.2022.06.034

SUMMARY

Rates of seed dispersal have rarely been considered important. Here, we demonstrate through field observations and experiments that rapid dispersal is essential for the unusually short-lived seeds of *Aquilaria sinensis* (agarwood; Thymelaeaceae), which desiccate and die within hours of exposure by fruit dehiscence in the hot, dry forest canopy in tropical southwest China. We show that three species of *Vespa* hornets remove most seeds within minutes of exposure. The hornets consume only the fleshy elaiosomes and deposit most seeds in damp shade, where they can germinate, a mean of 166 m from the parent tree. Electrophysiological assays and field experiments demonstrate that the hornets are attracted by highly volatile short-carbon-chain (C5–C9) compounds, including aldehydes, ketones, alcohols, and acids, emitted from the dehiscent fruit capsule. These attractive fruit volatiles share 14 of 17 major electrophysiologically active compounds with those emitted from herbivore-damaged leaves, which attract predators, including hornets. Rapid seed dispersal thus appears to have been achieved in this species by the re-purposing of a rapid indirect defense mechanism. We predict that rapid seed dispersal by various mechanisms will be more widespread than currently documented and suggest that volatile attractants are more effective in facilitating this than visual signals, which are blocked by vegetation.

RESULTS AND DISCUSSION

The genus *Aquilaria* (Thymelaeaceae) includes 21 tree species in the rainforests of tropical and subtropical Asia (Figures S1A–S1D). The resinous agarwood they produce in response to damage or fungal infection is highly valued for use in medicine and perfume, and all species are threatened by overexploitation (https://www.iucnredlist.org/). In our study area in tropical southwest China, the two-valved capsular fruits of *A. sinensis* mature in the June–August hot season and dehisce longitudinally, releasing two diaspores (elaiosome and seed, ~236 mg per diaspore, n = 1,000) linked to the capsules by thread-like funicles. It has been suggested[1] that the color and shape of the hanging diaspores mimic swinging caterpillars hanging on silk (Figures 1A, 1B, and S2A; Video S1). Most fruit dehiscence occurred during the hot, dry afternoons of sunny days (Figures 2A and S2B), and seeds exposed to the canopy environment at this time became desiccated in 4 h and lost viability within 8 h (Figure 1C).

Rapid removal of short-lived seeds by hornets

During the same time period when the seeds are exposed, three species of *Vespa* hornets visited the canopy (Figures 2, S2B, and S2C; Table S1). They were attracted to the hanging diaspores, attacking them as if they were attacking prey.[2] The hornets removed the whole diaspore, including the seed, by cutting the funicle, in a medium time of 1 min (n = 482) (Figure 2B; Video S1). Most labeled diaspores were removed within 13 min (median, n = 469) after capsule dehiscence, 87% within 1 h, and 97% within 2 h (Figure 1B). Of the labeled seeds, 96.3% (488 of 507 seeds from 320 fruits) were removed by three *Vespa* species to a mean distance of 166 ± 12.4 m (mean ± SD, n = 169), with the remainder removed by ants, wind, or gravity (Figure 1C). *Vespa velutina* was the major disperser, accounting for 84.7% (508 of 600) of fruit visits and the longest observed dispersal distances of >400 m (Figure 1C).

The elaiosome is removed and the seeds dropped either at the nests, which are usually on the main branches of big trees, or after landing briefly on a tree branch on the way to the nest; thus, most seeds are dropped under large tree crowns. *A. sinensis* seeds placed under such crowns had significantly higher seedling establishment over 38 days (61.3% ± 3.0%) than under the crown margin (45.6% ± 9.2%) or in open grassland (14.4% ± 2.4%) (Figure 2D; 120 seeds per group). In a parallel experiment with artificial shading, seedling establishment increased

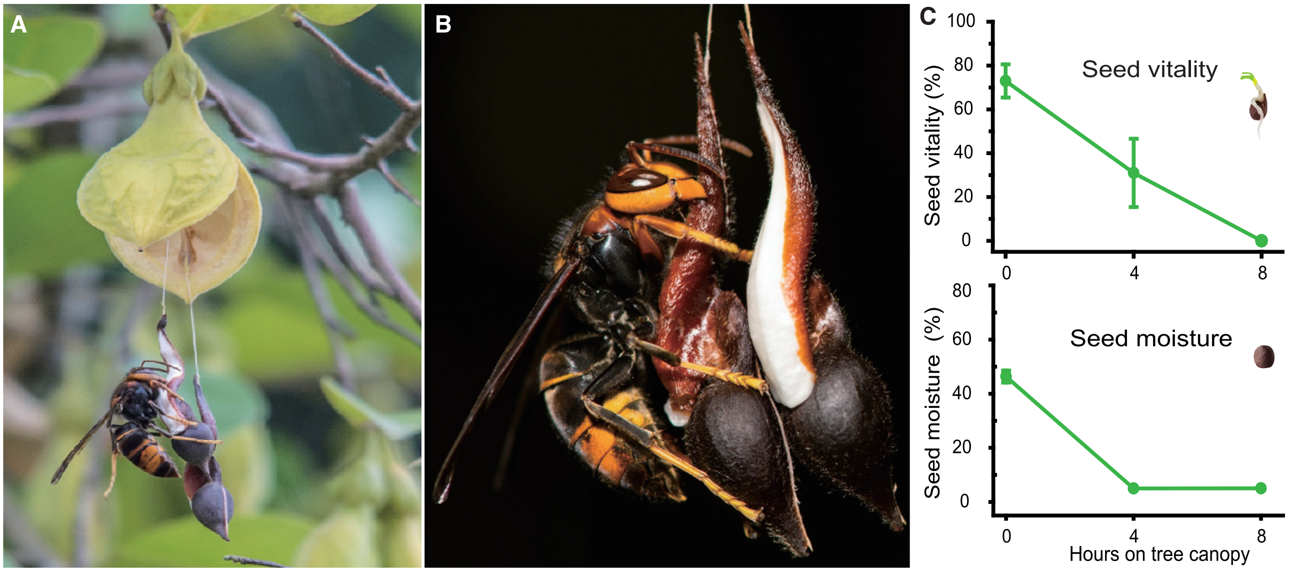

Figure 1. Hornet disperser and dry-sensitive seeds of *Aquilaria sinensis*

(A and B) Hornet (*Vespa velutina*) cutting the diaspore from a newly dehisced fruit of *Aquilaria sinensis* (see Video S1 for details).

(C) Seed vitality and seed moisture (mean ± SE) decline sharply with exposure time in the tree canopy after fruit dehiscence.

See also Figure S2B, Video S1, and Data S1.

significantly with increasing shade, from 0% without shade to 36.9%, 49.4%, and 53.8% with one, two, and four layers of 75%-shading mesh, respectively (Figure S3A; 120 seeds per group). Dispersal by hornets thus not only removes the seeds quickly from the rapidly lethal canopy environment, but also disperses them to habitats that are predictably favorable for seedling establishment.

Attraction of hornets by volatiles similar to those emitted by herbivore-damaged leaves

Dehiscent fruits hung in non-fruiting trees inside an *A. sinensis* plantation, and those hung in trees 3 km away from the plantation attracted hornets and remained attractive for at least 3 days, with hornets arriving within 1–10 min inside the plantation and 10–30 min outside it (Figures S3B and S3C). Fruit capsules emitted more diverse and abundant volatiles than diaspores (Figure S4A). Removing the odor from capsules significantly reduced the fruits' attraction to hornets, while removing the odor from diaspores did not (Figure 3A). Seventeen volatile compounds emitted from capsules elicited electrophysiological responses in hornet antennae. Most of the compounds were aldehydes, ketones, alcohols, and acids with short carbon chains (C5–C9) and small molecular weights (Figures 3B, S4B, and S4C; Table S2). We used the synthetic materials of five compounds (hexanal, (*Z*)-hex-3-en-1-ol, 1-octen-3-ol, 2-phenylacetaldehyde, and 2-phenylethan-1-ol) with the highest electrophysiological responses (Figure 3B) to make a mixture of standards. The attractiveness of this mixture to hornets was comparable to that of intact newly dehiscent fruits and was significantly higher than that of fruits with the odor removed (Figure 3C). The volatilities of the active compounds (estimated as vapor pressures[3]) from *A. sinensis* fruits were up to 1,280 Pa ($VP_{25^\circ C}$) and 30,000 times higher than those of the long carbon chain (C20–C30) aliphatic alkanes identified as attractants in another, unrelated hornet-dispersed plant, *Stemona tuberosa*[4] (Figure 3D). The high volatilities of the active compounds identified in our study are probably essential for the rapid attraction of hornets to the very short-lived seeds, while dispersal may be less urgent in *S. tuberosa*.

The leaves of *A. sinensis* are frequently damaged by a specialist lepidopteran herbivore *Heortia vitessoides* (Figure S2A).[5] The highly volatile attractants identified in this study overlap with compounds previously identified as attracting a predatory stinkbug (*Cantheconidea concinna*; Pentatomidae) as an indirect anti-herbivore defense in *Aquilaria*. We observed hornets predating *H. vitessoides* caterpillars during our study and therefore hypothesized that the rapid attraction of hornets for seed dispersal in *A. sinensis* evolved by repurposing the volatiles used to attract predatory insects for defense. To test this hypothesis, we introduced caterpillars of *H. vitessoides* to 2-year *A. sinensis* seedlings and analyzed the volatiles emitted by damaged leaves and the electrophysical responses of hornet antennae. The damaged leaf odor included 14 of the 17 (82%) electrophysiologically active compounds identified in the fruit odor (Table S2). Among these 14 compounds, eight (unknown 1, hexanal, (*Z*)-hex-3-en-1-ol, 1-octen-3-ol, octan-3-one, octan-3-ol, 2-phenylethan-1-ol, and indole) showed high electrophysiological activity in assays (Figures 3B and 3E), and the low activity of the other six may be due to their low concentrations in the damaged leaf odor. Furthermore, four of the eight compounds showing high electrophysiological activity were included in the five-compound mixture that was as attractive to hornets as dehiscent fruits (Figures 3B, 3C, and 3E; Table S2). Overall, these results are consistent with our hypothesis that the volatiles involved in the rapid attraction of predatory insects for plant

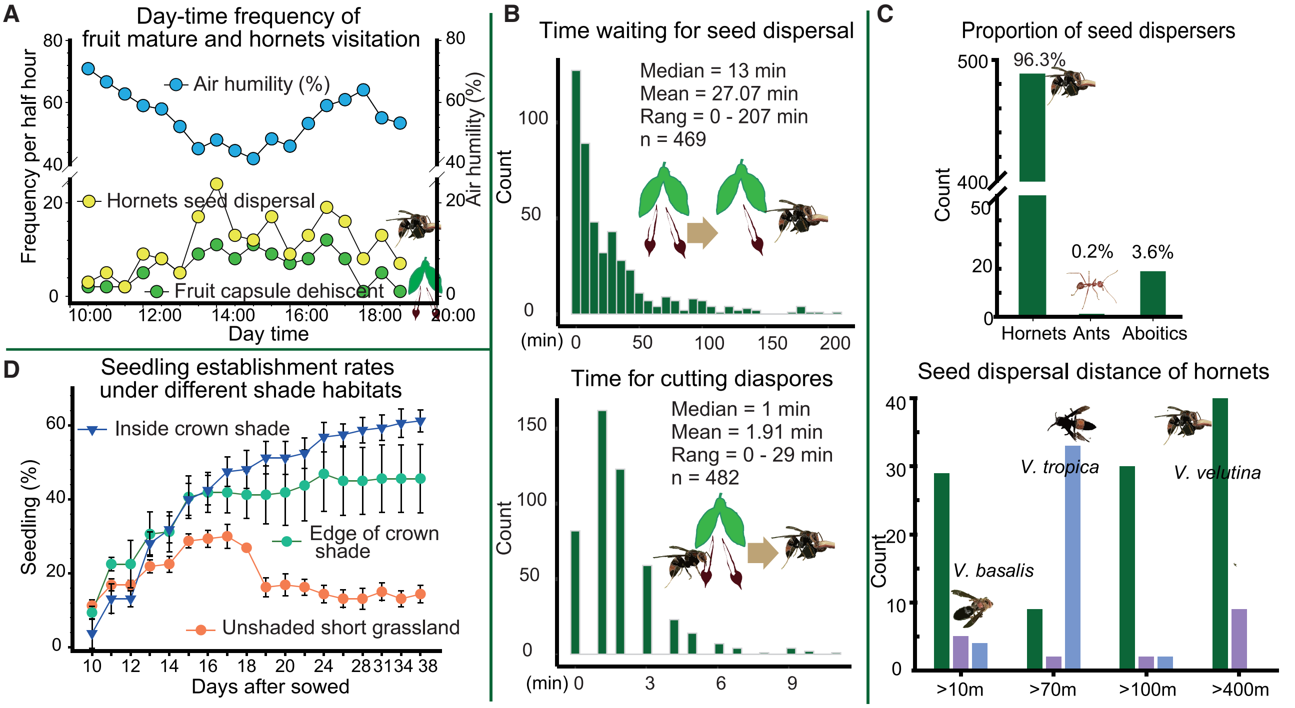

Figure 2. Rapid seed dispersal of *Aquilaria sinensis* by hornets

(A) Variation in air humidity and frequency of fruit dehiscence and seed dispersal by hornets during a day.

(B) The time between fruit dehiscence and seed dispersal, and the time taken for a hornet to remove a diaspore from a fruit.

(C) The proportions of different dispersal agents and the dispersal distances observed for the three major *Vespa* hornets.

(D) Seedling establishment (mean ± SE) under crown shade, at the edges of the crown shade, and in unshaded short grassland.

See also Figures S2C and S3A, Video S1, Table S1, and Data S1.

defense have been repurposed as attractants for rapid seed dispersal in *A. sinensis*.

Rapid seed dispersal by hornets in the genus *Aquilaria* and family Thymelaeaceae

All *Aquilaria* species tested have short-lived, drying-sensitive seeds,[6] and all species have structurally similar fruits and diaspores.[7] Seed dispersal by hornets has been previously reported in *A. sinensis*[2] and *A. malaccensis*,[8] and we have also observed this in two additional species, *A. yunnanensis* and *A. crassna*, within plantations during our field observations. All *Aquilaria* species also share the same specialist herbivore (*H. vitessoides*), which is the most serious pest in plantations[5] and is distributed in sympatry with *Vespa* hornets in Asia. Taken together, this evidence suggests that our observations for *A. sinensis* can probably be generalized to the other species in the genus. However, we would expect some variation in the details, and it would be very interesting to compare seed longevity, dispersal rapidity, hornet assemblages, and volatile profiles among *Aquilaria* species in both their natural ranges and under common garden conditions.

Based on the available fruit and seed information for the family Thymelaeaceae,[6] all species in the genus *Gyrinops* are also probably dispersed by hornets as they have similar fruit structures,[7] but the basal genus, *Gonystylus*, has larger, fleshy, vertebrate-dispersed fruits. These three tropical genera have desiccation-sensitive non-dormant seeds, but the other, more derived genera for which information is available produce dormant seeds, grow in seasonal climates, and are dispersed by vertebrates or wind (Figure 4). Unfortunately, the dispersal biology of most genera in the Thymelaeaceae is unknown, making it impossible to interpret the evolutionary history of dispersal by hornets in this family.

Rapid seed dispersal in other plants

Rates of dispersal have rarely been considered important and there is surprisingly little comparative information in the literature. Seed dispersal has usually been viewed as an essentially passive process in which plants produce fruits that depend on chance encounters with appropriate vectors (biotic or abiotic) for dispersal. At a site in tropical South China with a similar climate to our study area, fleshy vertebrate-dispersed fruits of 34 species had median persistence times on the plant of 5–71 days when accessible to frugivores and 6–205 days when frugivores were excluded.[10] Rapid dispersal is typical, however, in ant-dispersed species, and in a study in the same area as the one reported here,[11] most diaspores were removed in 1 min–4 h after capsule dehiscence. Similar removal rates have been reported from studies elsewhere.[12] Some cases of dispersal by ants have been shown to depend on volatile attractants,[13,14] as with the hornets in this study, but in other cases contact is apparently required.[15] Volatile attractants are expected to be more effective than visual cues for attraction from a distance in dense vegetation, but they may also attract seed predators. Relying on chance contacts with a high density of actively

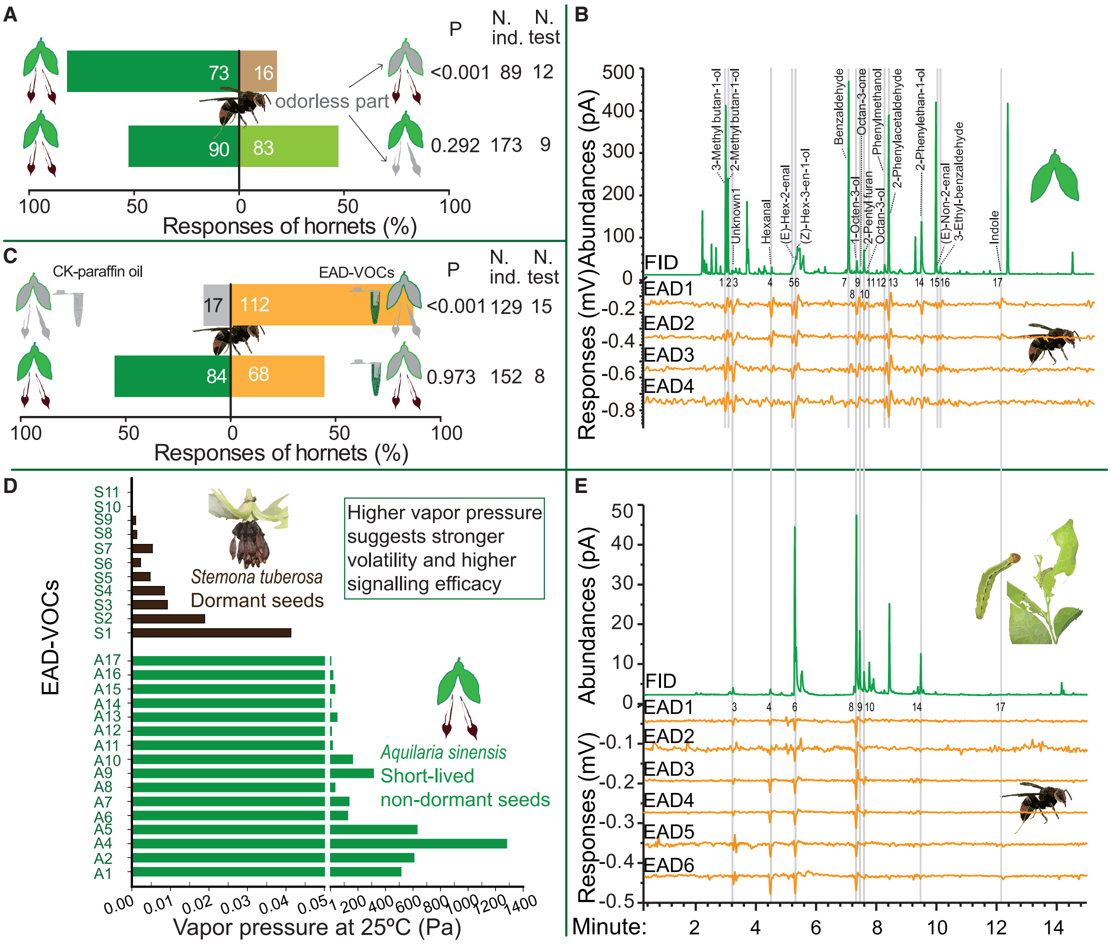

Figure 3. The odor from fruit capsules mimics that of herbivore-damaged leaves of *A. sinensis* and results in the rapid attraction of hornets

(A) Response of *V. velutina* to dual-choice bioassays between intact dehiscent fruits and fruits from which most odors had been removed by immersion in hexane for 24 h, then drying to 50°C for 5 h. Numbers in bars indicate hornets responding to either odor.

(B) Electrophysiological responses of *V. velutina* antennae to the fruit odor of *A. sinensis* with gas chromatography-electroantennographic detection (GC-EAD). The green line (FID) at the top represents volatile composition, and four orange lines (EAD1–4) indicate responses of the antennae of different hornet individuals to fruit volatiles. EAD-active compounds were identified as shown in the figure; see Table S2 for details.

(C) Responses of *V. velutina* to dual-choice bioassays between odorless fruits with paraffin oil solvent and a mixture of five EAD-active compounds (see Table S2 for details), or between intact fresh fruit and odorless fruits with EAD-active compounds. Numbers in bars indicate hornets responding to either odor.

(D) Vapor pressures of active volatile compounds of two hornet-dispersed plants, *Stemona tuberosa* and *A. sinensis*. Compounds of *Stemona tuberosa* from Chen et al.[4] See Data S1 for details.

(E) Electrophysiological responses of *V. velutina* antennae to the volatiles of leaves of *A. sinensis* damaged by the specialist lepidopteran herbivore, *H. vitessoides*, with GC-EAD. The green line at the top represents volatile composition (FID), and six orange lines (EAD1–6) indicate responses of the antennae of different hornet individuals to fruit volatiles. EAD-active compounds with the same number in (B) and (E) indicate the same compounds linked by long lines.

See also Figures S3B, S3C, and S4, Table S2, and Data S1.

foraging ants may be effective where seed crypsis is important, but this would clearly not work with wasps or other seed dispersal agents.

The contrast between the more than 11,000 angiosperms in 77 families known to be dispersed by ants and the few known cases of dispersal by hornets and other wasps (vespicochory) is hard to explain.[8,16–19] Most plants with diaspores dispersed by ants are herbaceous and low-growing, and the diaspores of most of these species are probably far more likely to be found by ants than wasps. For trees, however, ant dispersal covers too short a distance (1.99 m on average)[20] to move the seeds beyond the crown, and the targeted attraction of flying insects makes a lot more sense. It is striking that all known cases of vespicochory were found by chance, and that they are scattered across five plant orders and two continents. Wasps, including hornets, are more difficult to observe than birds, mammals, or ants, particularly in a tree canopy, so it is possible that more cases of wasp dispersal may be found for small-seeded tropical trees.

Rapid dispersal may be favored by high pre-dispersal predation risks[21–23] as well as the environmental extremes experienced by seeds in this study. Unfortunately, rates of seed mortality were not assessed for the plant species in any of the above-mentioned studies. Escape from environmental extremes, including fires, is likely to select for rapid dispersal in

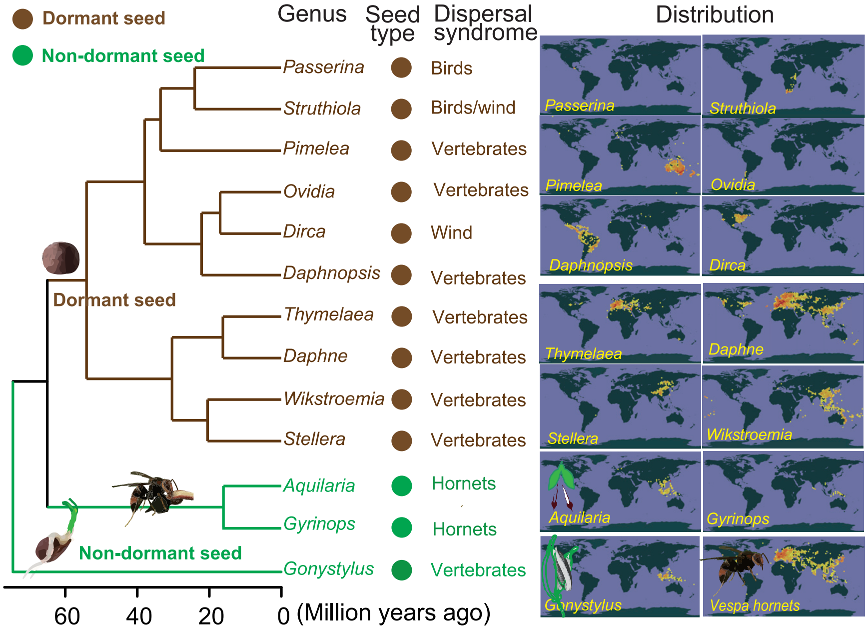

Figure 4. Phylogeny of the Thymelaeaceae for genera with information on seed dormancy type, dispersal syndrome, and distribution

Green branch and tip labels in phylogenetic tree[9] indicate plant genera with non-dormant (recalcitrant, often short-lived) seeds, while brown indicates dormant seeds. Seed dispersal syndromes were inferred from fruit traits and records,[7] and distributions are from GBIF (https://www.gbif.org/). See also Figure S1.

seeds exposed in the open dry habitats in which ant dispersal is most common,[16] while escape from predation is more likely in the understories of closed-canopy forests.

Attraction by volatiles is also widespread in plants whose seeds are dispersed by mammals, particularly fruit bats,[24,25] and we predict that cases of rapid dispersal will also be common among bat-dispersed plants. Many cultivated fruits with mammal-dispersed ancestors (e.g., peaches) are climacteric, with a sharp, ethylene-controlled peak in volatile emissions at maximum ripeness, followed by rapid senescence.[13] There have been very few studies of volatile production in wild fruits, but this short-lived period of maximum attractiveness both necessitates and facilitates rapid attraction of dispersal agents.

Conclusions

Our study demonstrates a need for extremely rapid seed dispersal in *Aquilaria sinensis*, where the seeds are exposed to the hot, dry, and rapidly lethal canopy environment of a tropical forest. This need is fulfilled by rapid removal by *Vespa* hornets, attracted by highly volatile, short-chain carbon compounds produced by the ripe fruit capsules. We show that these volatile attractants appear to mimic those produced by herbivore-damaged leaves, which attract predators—including hornets—in an equally rapid form of indirect defense. Indirect defense through herbivore-induced plant volatiles (HIPVs) has been called a "cry for help,"[26] and this characterization applies equally well when they are used to attract dispersal agents to short-lived seeds. In both cases, this appears to be an honest signal, since a reward is offered to the hornets and both partners benefit. A similar use of HIPVs has been reported in an orchid to attract social wasps for pollination,[27] but as far as we know, our study is the first to report the re-purposing of HIPVs as an attractant in seed dispersal. At least four other examples of seed dispersal by social wasps are known,[8,17–19] but there is no evidence yet that any of these involve either short-lived seeds or highly volatile attractants. Identifying cases in which rapid dispersal is essential may be important for plant conservation, since such plants will be more vulnerable to declines in populations of dispersal agents than those with "sit and wait" fruits in which the seeds remain viable until dispersed weeks or months later.

Finally, although it was not one of the aims of this study, we have identified a powerful synthetic attractant for the Asian yellow-legged hornet, *Vespa velutina*, which is an invasive threat to honeybees in multiple countries. The baits currently used for monitoring and controlling these invasions are not very effective or selective,[28] and we suggest that the compounds identified in this study could form the basis for improved baits.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - ○ Lead contact
  - ○ Materials availability
  - ○ Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - ○ Study description
  - ○ *Aquilaria* (agarwood)
  - ○ Hornets for seed-dispersal
  - ○ Study sites
- METHOD DETAILS
  - ○ Field observations of fruit maturation and seed dispersal
  - ○ Changes in seed viability and seed moisture content after fruit dehiscence
  - ○ Seedling growth experiments in habitats with different shade-levels
  - ○ Bioassays of the ability of fruit tissues to attract hornets and elicit dispersal behaviours
  - ○ Identification of the volatile compounds contributing to hornet attraction
  - ○ Behavioural bioassay of synthetic active compounds to hornets
  - ○ Seed evolution in the family Thymelaeaceae
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2022.06.034.

ACKNOWLEDGMENTS

We thank three anonymous reviewers, Chen Gao, and Chen ZhanQi for constructive comments; Zhang Jinyuan, Li Lisha, Wen Xiulian, Su Haoran, Ma Hongtao, and Kuang Linrui for assisting with fieldwork and chemical experiments; Zhu Renbin for taking photographs and videos; the Gardening and Horticulture Department of XTBG, CAS, Menglun primary school, and the Xishuangbanna Station for Tropical Rainforest Ecosystem Studies for allowing and assisting with sampling or observations; and the Institutional Center for Shared Technologies and Facilities of XTBG, CAS, for the GC-MS and GC-EAD. This work was supported by the Youth Innovation Promotion Association of CAS (grant number 2020392), the National Natural Science Foundation of China (grant numbers 31870359 and U1402264), and the Natural Science Foundation of Yunnan Province, China (grant numbers 202201AV070007).

AUTHOR CONTRIBUTIONS

G.W. and J.C. designed the study; R.-M.Q. and G.W. did the field observation, germination, and behavior experiments; R.-M.Q., P.W., and G.W. did the GC-MS and GC-EAD experiments; P.W. identified the chemicals and prepared standards; R.-M.Q. and G.W. did seed evolution analysis; and R.-M.Q., G.W., R.T.C., J.C., Y.Z., and P.W. wrote the original manuscript. G.W., R.T.C., R.-M.Q., and P.W. revised manuscript. All authors gave final approval for publication.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: May 9, 2022
Revised: June 4, 2022
Accepted: June 10, 2022
Published: June 30, 2022

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
3-Methyl butan-1-ol	J&K	CAS# 123-51-3
2-Methyl butan-1-ol	J&K	CAS# 137-32-6
(*Z*)-Hex-3-enal	TCI	CAS# 6789-80-6
Hexanal	TCI	CAS# 66-25-1
(*E*)-Hex-2-enal	TCI	CAS# 6728-26-3
(*Z*)-Hex-3-en-1-ol	Mayer	CAS# 928-96-1
(*E*)-hex-2-en-1-ol	TCI	CAS# 928-95-0
Hexan-1-ol	TCI	CAS# 111-27-3
1-Octen-3-ol	J&K	CAS# 3391-86-4
Octan-3-one	TCI	CAS# 106-68-3
Octan-3-ol	Mayer	CAS# 20296-29-1
2-Pentyl furan	J&K	CAS# 3777-69-3
(*Z*)-Hex-3-enyl acetate	TCI	CAS# 3681-71-8
Benzaldehyde	J&K	CAS# 100-52-7
Phenylmethanol	J&K	CAS# 100-51-6
2-Phenylacetaldehyde	J&K	CAS# 122-78-1
2-Phenylethan-1-ol	TCI	CAS# 60-12-8
2-Ethyl hexan-1-ol	Mayer	CAS# 104-76-7
Linalool	TCI	CAS# 78-70-6
(*E*)-Non-2-enal	J&K	CAS# 18829-56-6
3-Ethylbenzaldehyde	TCI	CAS# 34246-54-3
Indole	Mayer	CAS# 120-72-9
Software and algorithms		
MSD Chemstation e.02.01.1177	Agilent	Catalog# G1701EA
AMDIS software	NIST	http://amdis.net/
R	R development core team	https://www.r-project.org
Adobe Illustrator CS6 software	Adobe	https://www.adobe.com/products/illustrator
Other		
GBIF :Global Biodiversity Information Facility	N/A	https://www.gbif.org/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Gang Wang (wanggang@xtbg.org.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data generated in this study are available in the text and supplementary materials of this paper. Source data are provided with this paper.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study description

The analytical frameworks applied included: 1) Field observations and measurements of dispersal from *Aquilaria* plants by hornets; 2) Evaluation of benefits to plants from hornet-dispersal in the germination and seedling phases; 3) Determination of the mechanism of rapid attraction of hornets by identifying the active chemicals in fruit volatiles, with behavioural experiments, chemical analyses, and electrophysiological tests; 4) Identification of shared active chemicals between the odors of fruits and herbivore-damaged leaves, with chemical analyses and electrophysiological tests; 5) Exploration of the evolution of rapid seed-dispersal in the family Thymelaeaceae.

Aquilaria (agarwood)

Aquilaria species (Thymelaeaceae) are forest trees distributed in South and Southeast Asia (Figure S1) and are highly valued for the resinous wood (agarwood) produced in response to damage or fungal infection. Because of the threat from trade, all *Aquilaria* species have been listed on Appendix II of CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) since 2005 (https://cites.org). On the IUCN Red List of Threatened Species, four species are listed as Critically Endangered, one as Endangered, eight as Vulnerable, and the others as Data Deficient (https://www.iucnredlist.org/). We studied *Aquilaria sinensis*, the principal source of Chinese agarwood. *A. sinensis* is a Vulnerable evergreen tree native to lowland forest in southern China and widely cultivated. Its fruits, which mature during summer (mid-June to the end of July), are bilocular leathery dehiscent capsules, with each locule harbouring a single dark brown seed attached to a conspicuous white-brown elaiosome on the seed base (Figure 1A). Mature fruits split longitudinally while they are still on the tree and the diaspore (seed + elaiosome) remains hanging from a thread-like structure (Figure 1A) for a few days after fruit dehiscence if not dispersed. When capsules dehisce, the diaspores are exposed to air, awaiting potential dispersers (Figure 1; Video S1). Previous studies have shown that the seeds of *A. sinensis* and other *Aquilaria* species are desiccation-sensitive and die soon if not germinated rapidly after maturity.[6]

Hornets for seed-dispersal

Three hornet species (Hymenoptera: Vespidae) were recorded as the primary dispersers of the diaspores of *A. sinensis* during our study; *Vespa velutina*, *V. tropica*, and *V. basalis*, in the same genus as previously reported for *Aquilaria*.[2,8] According to the specimen records from the Global Biodiversity Information Facility (GBIF, https://www.gbif.org/) (Figure S1), the ranges of all these species widely overlap with those of the genus *Aquilaria* in South and Southeast Asia. *V. velutina* (Asian hornet) was the commonest disperser in our study and was used for the behavioural and electrophysiological studies.

Study sites

Our study sites are located in Menglun township, Yunnan, Southwest China (21°56´N, 146 101°15´E, and elevation 580 m), near the northern margin of the Asian tropics. Field observations of fruit dehiscence and seed dispersal by hornets, behavioural assays, and collection of fruit volatiles were done mainly in two *Aquilaria sinensis* plantations within the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (XTBG), and at a primary school nearby. The Garden retains a 255-ha patch of relatively undisturbed tropical rain forest, does not use pesticides, and minimizes the use of chemical fertilizers, so the native fauna is still diverse. The climate is dominated by the southwest monsoon, with a well-defined alternation of wet and dry seasons. Mean annual precipitation is about 1500 mm with 80% occurring during the May-October wet season. The mean annual air temperature is 22.7°C, with monthly means ranging from 14.8°C in January to 25.5°C in June.[29] This study was mainly conducted during the June–August fruiting seasons of 2017–2020.

METHOD DETAILS

Field observations of fruit maturation and seed dispersal

We first evaluated the contribution of hornets to the seed dispersal of *A. sinensis* and estimated the potential maximum dispersal distance. We tracked the fate of 600 freshly opened diaspores from 420 fruits on 10 branches of four trees growing in the playground of the primary school in 2017. The field observations were conducted in June–July, from 10:00 to 20:00 hr, the time of maximum fruit dehiscence and seed dispersal. Only hornets (three *Vespa* species) and weaver ants (*Oecophylla smaragdina*) were observed visiting seeds. Some seeds were removed by wind, gravity, or unobserved agents and were recorded as unknown. The three dispersal types (hornets, ants, and unknown) were recorded by five observers during a continuous 5-day period in June 2017. We also evaluated the potential maximum dispersal distance by visually tracking 200 diaspores carried away by hornets at this relatively open site. The potential maximum dispersal distance for each event was estimated as the distance from the tree to the point where we lost track of the hornet, and was measured with a laser rangefinder (SW-1000A, Sndway, China, max-rang: 1000m).

To explore the timing of fruit dehiscence and hornet seed-dispersal, and their potential relationships with environmental factors, we systematically surveyed the frequency of hornet visitation, fruit dehiscence, and hornet dispersal from 9:00 to 19:00 hr, for a continuous 5-day period in July 2020. We also measured the time (minutes) needed for a hornet to remove a seed after exposure the air following capsule dehiscence, and the time needed for hornets to remove a diaspore from a capsule after landing on the fruit. Around 550 undehisced fruits from two trees were closely observed, and the dispersal of 507 diaspores from 320 dehisced fruits and 2736 visits were recorded. We recorded air temperature, humidity with a hygrothermograph (Testo 610, SE & Co. KGaA, Germany), and light intensity with an illuminometer (Testo 540, SE & Co. KGaA, Germany) every ten minutes around the outside of the tree canopy during the whole observation phase. We counted the frequency of fruit dehiscence and hornet seed-dispersal per 30 minutes, and the mean values of air temperature, light intensity, and air humidity for each 30 minutes from 9:00 to 19:00.

Changes in seed viability and seed moisture content after fruit dehiscence

Fruit dehiscence is concentrated in the warmer, drier hours of summer days, exposing the desiccation-sensitive seeds to potentially lethal conditions. This could make rapid location and dispersal of the seeds by hornets critical for survival. To investigate the potential negative effects of seed exposure to the canopy environment on sunny days, we bagged the freshly dehiscent fruits in the tree canopy to exclude hornets and exposed them from 10:00 to 18:00 on normal bright sunny days, in August 2019. We then measured the seed moisture content and viability of the seeds and compared these with seeds from newly dehisced capsules.

A total of 230 fruits (460 seeds) near dehiscence were sampled from the same tree in the morning. When the fruits dehisced, we weighed lots of 10 fresh seeds each and put them in 46 individual mesh bags (hold size: 0.4 mm). Twelve bags (120 seeds) were chosen randomly and sown in a seeding tray with wet sandy soil (4 seeds per cell) as the control (zero hour group), and 24 bags (240 seeds) were hung on the branches near the outside of tree canopy at c. 2 m at 10:00 am on a normal sunny day. Of these 24 bags, 12 were weighed four hours later (14:00) and sown in the same conditions as the control (4-hour group), and another 12 were weighed eight hours later (18:00) and sown in the same way (8 hours group). The last 10 bags (100 seeds) were dried at 70°C in an oven until they reached constant weight for three successive days. The mean dry weight of the last 10 bags was treated as the dry weight of each bag of sown seeds (*Wd*). The weight of each seed bag after exposure was treated as the fresh weight (*Wf*). The seed moisture (SM) was calculated as SM = (*Wf* - *Wd*)/ *Wf*. We then compared the seed viability of these different treatment groups by measuring the germination percentage. Seeds of each group (i.e., exposure in the tree canopy for zero, four, and eight hours) were grown in optimal germination conditions (normal outdoor temperature, with 75%-80% normal light filtered by a shade net) and number of germinated seeds were recorded every day. For each seed group, 120 seeds were sown and four seeds in one cell were treated as a replicate for calculating the germination percentage. The observations stopped on the 35th day when no more germination occurred on three successive days.

Seedling growth experiments in habitats with different shade-levels

Field observations showed that hornets often nest on the main branches of big trees and also may land in such big trees temporally to feed on the elaiosomes of *A. sinensis* seeds they are carrying. The seeds are therefore often dropped in the shaded and relatively moist environment under the canopy of these trees, rather in open sites. We predicted that this directed dispersal could facilitate seedling establishment of *A. sinensis*. Seedling growth experiments were conducted under both natural and artificial shade gradients during August to September 2020. Under natural conditions, we chose three shade-levels spanning the range available in our study sites, 1) under the crowns of big trees with hornet nests, 2) under the margins of tree crowns, and 3) in unshaded short grassland. In each habitat, 160 seeds were sown, 5 seeds per cell, in seedling trays with 32 cells filled with damp sandy soil. We treated the 40 seeds in 8 cells in one row as a replicate, giving 4 replicates for observing the number of germinated seeds or seedlings every day. The seedling establishment percentages (proportion of seeds becoming seedlings) were compared among treatments. We measured the maximum light intensity of each habitat with an illuminometer (Testo 540, SE & Co. KGaA, Germany) at 14:00 h on several sunny days. These values were ∼80,000 lux in short grassland (full light), ∼10,000 lux under the margin of the crown (12.5% of full light), and ∼3000 lux under the crown (3.75% of full light). Similar experiments were conducted with four artificial shade-levels created in short grassland by covering seedling trays (at 10 cm height) with nothing or one-, two-, or four-layers of 75%-shading mesh, with 120 seeds sown under each treatment. Both experiments were surveyed for 38 days until no more germination occurred on 3 successive days. At the end of the experiment, most living seedlings were 2-5 cm tall. The experiments were done in the wet season and the trays were watered on days when it did not rain, so water was never limiting.

Bioassays of the ability of fruit tissues to attract hornets and elicit dispersal behaviours

Dehisced fruits are the main target for hornet visits, so we first tested whether they could attract hornets and elicit seed dispersal behaviours, and how long this ability was maintained after dehiscence. We tested this within the *A. sinensis* plantation at XTBG and also at around 3 km away from the nearest *Aquilaria* trees. We used freshly dehisced fruits (1st-day fruits) and those that had dehisced 2-4 days earlier (2nd-4th-day fruits) and been kept within mesh bags to exclude hornets. Within the plantation, ten fruits of each group were hung on the branches of non-fruiting *A. sinensis* trees with 10-cm spacing in the order 1st-, 2nd-, 3rd-, and 4th-day, or the inverse, along the branch. The frequencies of hornet visits and the numbers of dispersed diaspores of each group were recorded within 1-2 hours until all diaspores of one group were dispersed (in practice, always the 1st-day fruit group) during10:00–18:00 h of a sunny day during August in 2019. The test was repeated 7 times inside the plantation. Similar experiments were conducted using a non-fruiting banyan tree (*Ficus concinna*) at least 3 km away from the nearest *A. sinensis* trees. Three fruits groups (1st-, 2nd-, 3rd-day fruit) were used, with 18-26 seeds per group in each trial, and 12 trials were conducted in sunny weather in August 2019. The number of dispersed diaspores in each trial was recorded for 2–6 h until all diaspores of the 1st-day group were dispersed.

We further tested the role and source(s) of odor in hornet attraction by comparing the attraction of odorless fruit tissues, in which the odor had been removed by immersion in HPLC hexane for 24 h then drying at 50°C for 5 hours, and normal fresh tissues in the plantation during June-August 2018. The comparisons were: (1) Fresh fruits vs. odorless capsules + fresh diaspores; and (2) Fresh fruits vs. odorless diaspores + fresh capsules. To test their attraction to hornets, five fruits of each group were placed in plastic Petri dishes on a 2.5-m platform, following the protocol used in the behaviour experiments. The test was repeated 12 times for the first comparison and 9 times for the second, with each test lasting 30–120 min, until all diaspores of one group were removed by hornets. Hornet visitation to different fruit groups was recorded and hornet preference was compared with the binomial test.

Identification of the volatile compounds contributing to hornet attraction

We collected the odors of whole fresh fruits and of different fruit parts with the solid phase micro-extraction (SPME) equipped with a 65 μm PDMS/DVB film coated blue fibre (Supelco, CA). SPME extracts the headspace volatiles of the glass cup with samples for one hour at indoor ambient temperature. Odor samples of the capsule (N=9), elaiosomes (N=6), and seeds (N=8) were collected in 2017. All trees used for behaviour tests and field observations were sampled. We then analysed the volatile compounds with an HP7890A-5975C gas chromatograph and mass spectrometer (GC-MS). An HP-5MS capillary column (30 m × 250 μm × 0.25 μm, Agilent, US) was used with 37 cm/s helium as the carrier gas. The oven ramp was set as 50°C for 2 min, followed by 8°C/min and then 280°C for 10 min. For the quadrupole mass spectrometry, a 70 eV EI ion source was used at 230°C. The mass range scanned was m/z 28.5 - m/z 300 at a rate of 2×4 scans/s. The detection threshold was set to 10. Data were analyzed using Agilent Chemstation software (Agilent Technologies) and AMDIS software (http://amdis.net/). Compounds were identified by comparing mass spectra and with records from the NIST 17 mass spectral database and authentic standard compounds analysed by the same GC-MS method. Synthetic compounds, including 3-methyl butan-1-ol (CAS 123-51-3), 2-methyl butan-1-ol (CAS 137-32-6), (*Z*)-hex-3-enal (CAS 6789-80-6), hexanal (CAS 66-25-1), (*E*)-hex-2-enal (CAS 6728-26-3), (*Z*)-hex-3-en-1-ol (CAS 928-96-1), (*E*)-hex-2-en-1-ol (CAS 928-95-0), hexan-1-ol (CAS 111-27-3), 1-octen-3-ol (CAS 3391-86-4), octan-3-one (CAS 106-68-3), octan-3-ol (CAS 20296-29-1), 2-pentyl furan (CAS 3777-69-3), (*Z*)-hex-3-enyl acetate (CAS 3681-71-8), benzaldehyde (CAS 100-52-7), phenylmethanol (CAS 100-51-6), 2-phenylacetaldehyde (CAS 122-78-1), 2-phenylethan-1-ol (CAS 60-12-8), 2-ethyl hexan-1-ol (CAS 104-76-7), Linalool (CAS 78-70-6), (*E*)-non-2-enal (CAS 122-78-1), 3-ethyl benzaldehyde (CAS 34246-54-3), and Indole (CAS 120-72-9), were obtained from commercial sources (TCI, Shanghai; J&K, Beijing; Mayer, Shanghai).

To identify volatile compounds emitted from the fresh fruits with potential for attracting hornets, we measured the electrophysiological responses of *V. velutina* to volatiles with a custom-made gas chromatography-electroantennogram detector (GC-EAD) (based on LMP7721 operational amplifier (TI, US) and HP34465A digital multimeter (Keysight, US)) recording[30] in June, 2018. The *V. velutina* hornets were collected from the *A. sinensis* plantation during the fruiting season. For each GC-EAD test, the top head part, with two antennae and a little head tissue at the base of the antennae, was used. The antenna tip was cut open with iris scissors under a microscope. The head opening was mounted to the reference electrode and the tips of two antennae were mounted to the recording electrode. All electrodes were filled with bee Ringer's solution [NaCl (130 mM), KCl (6 mM), MgCl2 (4 mM), CaCl2 (5 mM)]. For the separation of SPME samples, a Trace GC (Thermo, US) was used. The splitless injector was heated to 250°C. The volatiles were separated on an HP-5 (30 m × 320 μm × 0.32 μm; Agilent, USA) column with 1.0 ml/min helium as carrier gas. The GC oven ramp was set as 50°C for 2 min and then 10 °C/min to 280°C for 5 min. Half of the GC effluents were recorded with a flame ionization detector (FID) and the other half was transferred to the EAD part via a transfer line at 250°C, then cooled and diluted with a clean, wet, and static-free airflow at 37 cm/s before delivered to the prepared antenna. The GC-EAD tests were repeated six times. Identification of active compounds was also confirmed with synthetic standard compounds with the same protocols of GC-EAD by checking hornets' responses to artificial blends and some individual synthetics.

The above chemical and electrophysiological studies suggested that the fruit odor may attract hornets by mimicking herbivore-induced plant volatiles (HIPVs), as this odor shares the same or similar compounds to those reported from the herbivore-damaged leaves of *Aquilaria* species.[31] Also, hornets were observed to prey on caterpillars of *Heortia vitessoides*, a major specialist pest of *A. sinensis* plantations. To test this hypothesis, we collected odors of both *H. vitessoides*-damaged and normal leaves with SPME, identified the odor compounds with GC-MS, and detected the electrophysiological responses of *V. velutina* hornets to leaf odors with GC-EAD. To collect the odor of *H. vitessoides*-damaged leaves, six fourth-instar caterpillars of *H. vitessoides* were introduced to one branch (10-15 leaf) each of six two-year old *A. sinensis* seedlings around 1 m tall, and the leaf odor was collected after 1 hr, 3 hr, and 6 hr. Seven leaf odor samples were also collected from the nondamaged leaves in control seedlings of same species. All testing protocols followed those used in the fruit odor analysis. In addition, we compared the potential signalling efficiency of the active chemicals identified in this study to those identified in an unrelated hornet-dispersed herb, *Stemona tuberosa* (Family Stemonaceae).[4] Vapour pressures affect the release rate of a compound to the atmosphere and so are expected to reflect the signalling efficiency.[3] We calculated the vapour pressures under ambient conditions ($VP_{25^\circ C}$) of these compounds using the MPBPVP module in EPI Suite 4.11 software (EPA, New York, USA). The values based on the Anotoine method or modified grain methods[32] were calculated and an optimal value fitting the molecular structure of chemicals was supplied.

Behavioural bioassay of synthetic active compounds to hornets

To test the attraction of the electrophysiologically active compounds (EAD-VOCs) to hornets, we did a field behaviour experiment during June-August 2018. Synthetic samples of five major compounds (hexanal, (*Z*)-hex-3-en-1-ol, 1-octen-3-ol, 2-phenylacetaldehyde, 2-phenylethan-1-ol) with high electrophysiological responses were mixed to make the EAD-VOCs solution. Two comparisons were made: (1) odorless fruits with synthetic EAD-VOCs solution vs. odorless fruit with paraffin oil solution (negative control), and (2) odorless fruits with synthetic EAD-VOCs solution vs. normal fresh fruits (positive control). The EAD-VOCs solution contains the five compounds with equal concentration at 1 mg/ml with hexane (HPLC) as solvent and 100 μl of EAD-VOCs or paraffin oil solution within a 0.5 ml centrifuge tube was supplied with odorless fruit during each trial. Five fruit sets were used in each group of one trial. The behaviour experiments closely followed the protocol used in the previous studies: on a 2.5-m high platform, hornet visitation to different fruit groups was recorded, and the preference was determined with the binominal test.

Seed evolution in the family Thymelaeaceae

To investigate the evolution of rapid seed dispersal by hornets in *Aquilaria*, we surveyed genus-level information on seed dormancy, seed dispersal syndrome, and distribution in the family Thymelaeaceae. The most recent genus-level phylogeny[9] includes 34 of the 54 genera. Seed dormancy information (non-dormant or dormant seed) of all studied species in this family was collected from Baskin and Baskin.[6] We found seed information for 51 species of 13 genera, representing all the main clades of this family, so a sub-phylogeny for these 13 genera was extracted from above big phylogeny in R with package ape (http://ape-package.ird.fr/). The distribution of each genus was downloaded from GBIF (https://www.gbif.org/) to assess if it overlapped with that of hornets and if it was predominantly tropical or temperate, since short-lived non-dormant seeds are more common in the tropics.[33] The seed dispersal syndrome of each genus was inferred from records in the Flora Malesiana[7] and the fruit traits described. All species in *Aquilaria* and *Gyrinops* have capsular fruits with seeds and elaiosome that dangle from the apex of the fruit valves on a filiform funicle, so we inferred that they both should be hornet-dispersed. All other genera were inferred to be dispersed by birds, mammals, or wind, based on their colored drupaceous or dry fruits.[7] A genus-level phylogeny of family Thymelaeaceae with seed dormancy type, dispersal syndrome and distribution map was plotted with R package ape and Adobe Illustrator CS6 software (https://www.adobe.com/products/illustrator).

QUANTIFICATION AND STATISTICAL ANALYSIS

Data description, statistical analysis, and plotting were mainly conducted in R (R Core Team, https://www.R-project.org). To analyze the effect of environment factors on the frequency of fruit dehiscence and seed-dispersal, we inferred the regression relationship under a linear model, based on the dataset of the mean values of air temperature, light intensity, and air humidity within each half-hour, and the numbers of fruit dehiscence and hornet visits per half-hour. To test the direct dispersal function of hornets, we did single factor ANOVA analysis for comparing the germination and seedling establishment under different shade-levels in nature and with artificial shade treatments. The ANOVA analysis was also used to compare hornet visitation or seed dispersal frequency to fruits with different day-ages inside and outside the plantation, to confirm the function of fruits in hornet attraction. To confirm the fruit part responsible for hornet attraction and the function of fruit odor within the process, the paired t test was used to compare hornet numbers visiting differently treated fruits in dual-choice bioassays, such as between fresh intact fruits and partly odor removed fruits, between EAD-active compounds modified fruits and odorless fruits or intact fresh fruits separately.