

# Capitate Glandular Trichomes Fail to Provide Significant Resistance to Banded Sunflower Moth (Lepidoptera: Tortricidae)

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## Abstract

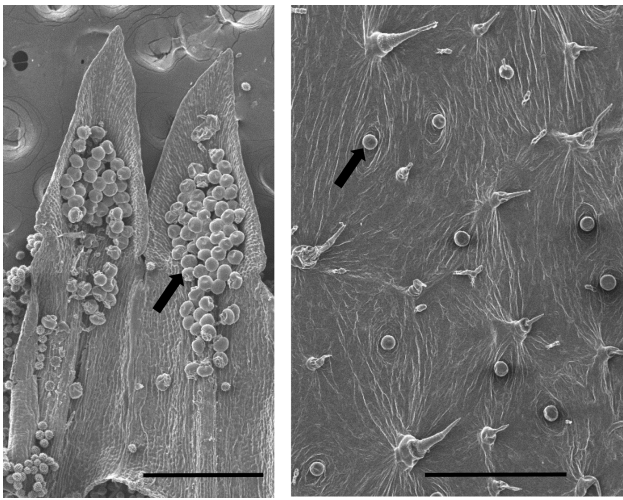
Extracts from capitate glandular trichomes (CGT) of wild and cultivated sunflowers, *Helianthus* spp., have repellent or toxic effects on sunflower specialists and generalist herbivores less closely associated with sunflower. Though CGT have been primarily examined for their potential to provide partial resistance to the sunflower moth, *Homoeosoma electellum* Hulst (Lepidoptera: Pyralidae), a floret- and seed-feeding pest, the banded sunflower moth (*Cochylis hospes* Walsingham [Lepidoptera: Tortricidae]) is a similar species more common in the primary sunflower-producing states of North Dakota and South Dakota. Replicated field trials using partially inbred lines with low or high CGT densities were used to evaluate possible reductions to seed damage by *C. hospes* larvae in 2016–2017. Results failed to support the idea that CGT are a useful defense against larvae of *C. hospes*; the putative plant defense of high trichome density corresponded to slightly more, rather than less, insect damage. A test of a secondary explanation, that strength of sunflower hulls could help determine patterns of seed damage among tested lines, produced similarly negative results. Though timing of bloom differed between groups of most- and least-damaged lines, prior research and pheromone-trapping data suggest differences in plant maturity also cannot adequately explain the observed results. While the specific mechanisms remain unclear, significant differences in susceptibility to *C. hospes* exist for cultivated sunflower and limit losses from this primary insect pest.

**Key words:** host plant resistance, antibiosis, *Helianthus annuus*, *Homoeosoma electellum*, sesquiterpene lactone

Plant hairs, or trichomes, are a common feature across various plant taxa and structures. As pointed out by Levin (1973), early research on plant trichomes focused on their utility in plant systematics or role in water balance. However, even prior to Levin (1973), the value of trichomes in plant defense was known for several crop and noncrop plants, and subsequent original research and reviews (Peter et al. 1995, Glas et al. 2012) established a rich literature exploring the role of trichomes in plant defense. Many varieties of trichomes have been described, with tomato (Simmons and Gurr 2005) and potato (Glas et al. 2012) providing well-known examples of plants with diverse trichomes, but it is simplest to categorize plant hairs as glandular or nonglandular. Glandular hairs contain mixtures of plant metabolites which often have roles in plant defense; contents of glandular trichomes in tomato and its wild relatives (*Lycopersicon* spp.) can limit herbivore movement by release of sticky exudate (Simmons et al. 2004), and also delay development or cause mortality of susceptible larvae (Frelichowski and Juvik 2001). Nonglandular trichomes also contribute to plant defense though multiple mechanisms. Insect pests may exhibit nonpreference for varieties with greater densities

of nonglandular trichomes (McAuslane 1996), though in other cases pests exhibit increased oviposition in response to plant hairs (Lambert et al. 1992). Nonglandular trichomes also can entrap or impale insects, leading to substantial mortality (Pillemer and Tingey 1976). Because of the potential benefits to plant defense, modifying trichome number or activity (i.e., composition of metabolites in glandular trichomes) has been a recurring goal for plant breeding (Wang et al. 2004, Simmons and Gurr 2005, Glas et al. 2012).

Wild sunflowers, *Helianthus* spp., have abundant nonglandular and glandular trichomes studied for their value to systematics (Spring and Schilling 1989) and potential use for host plant resistance. Most research on sunflower trichomes has been on capitate glandular trichomes (CGT) and their potential to provide partial resistance to the sunflower moth, *Homoeosoma electellum* Hulst (Lepidoptera: Pyralidae), a floret- and seed-feeding pest of wild and cultivated sunflowers. Though CGT are located on various sunflower structures, they are most concentrated at the tips of fused anther tubes on disc florets (Spring 1989; Fig. 1). Because *H. electellum* larvae feed in succession on pollen, other floret tissues, and developing achenes



**Fig. 1.** Scanning electron micrographs showing capitulate glandular trichomes (indicated by arrows) on sunflower florets (right, 43 $\times$ ) and leaves (left, 50 $\times$ ) for sunflower inbred line HA 300. Scale bars equal 500  $\mu$ m, both images. Sunflower pollen grains are also visible as smaller scattered spheres on the floret and nonglandular trichomes present on the leaf.

(seeds) (Rogers 1978), early instars' feeding habits may bring them into contact with glandular trichomes. When incorporated into an artificial diet, a sesquiterpene lactone extracted from CGT on florets of Maximilian sunflower, *Helianthus maximiliani* Schrader, repelled *H. electellum* larvae, though repellency appeared to decrease for later instars (Rossiter et al. 1986). Tests with crude (i.e., mixed) CGT extracts or purified terpenoids from several *Helianthus* spp. incorporated into artificial diet also delayed larval growth and increased *H. electellum* mortality (Rogers et al. 1987). In addition to effects on *H. electellum*, extracts from CGT of wild sunflowers also showed repellency or toxicity to herbivores less closely associated with sunflower, including southern armyworm (*Spodoptera eridania* [Stoll] [Lepidoptera: Noctuidae]), migratory grasshopper (*Melanoplus sanguinipes* [F.] [Orthoptera: Acrididae]), and western corn rootworm (*Diabrotica virgifera virgifera* LeConte [Coleoptera: Chrysomelidae]) (Gershenson et al. 1985, Chou and Mullin 1993).

Subsequent research has revealed more opportunities for the use of glandular trichomes in sunflower breeding. A second type of sunflower glandular trichome, linear glandular trichomes (LGT), has been found to be ubiquitous in *Helianthus* spp., but with different on-plant distribution than CGT (Aschenbrenner et al. 2013); analysis of LGT extracts from cultivated *H. annuus* show a combination of sesquiterpenes and flavonoids, including several not previously described (Spring et al. 2015). Though it has been remarked that cultivated sunflowers (*Helianthus annuus* L.) are deficient in CGT (Gershenson 1984, Rossiter et al. 1986, Rogers et al. 1987), data show per floret densities of CGT in public inbred lines are often equal to or greater than those in wild germplasm (Prasifka 2015), and that while often differing in composition of terpenoids, CGT extracts from cultivated sunflowers maintain repellency or toxicity against *H. electellum* larvae (Prasifka et al. 2015). Genes that govern synthesis of sesquiterpene lactones in sunflower have been identified (Göpfert et al. 2009), as well as markers for genes that govern the abundance of CGT on florets (Gao et al. 2018). The apparent anti-herbivore effects of CGT in cultivated sunflowers and availability of genetic markers suggest that sunflower glandular trichomes could be enhanced by breeding without crosses to wild germplasm, greatly simplifying the process of developing host plant resistance.

Collectively, previous research projects with sunflower glandular trichomes show that: 1) the anti-herbivore effects of CGT may extend well beyond *H. electellum* (Gershenson et al. 1985, Chou and Mullin 1993), 2) CGT of cultivated sunflowers remain biologically active (Prasifka et al. 2015), and 3) breeding for enhanced CGT number or activity may be accomplished without recurrent phenotyping (i.e., using genetic markers; Gao et al. 2018). To extend work with sunflower CGT into a more realistic setting, recombinant inbred lines from a mapping population were tested for their ability to reduce seed damage in replicated field trials. Because *H. electellum* is a sporadic pest in the primary sunflower producing states of North Dakota and South Dakota, tests were conducted using natural infestations of the banded sunflower moth, *Cochylis hospes* Walsingham (Lepidoptera: Tortricidae). Though *C. hospes* has a habit of sequential floret- and seed-feeding like *H. electellum* (Charlet and Gross 1990), effects of CGT or extracts on *C. hospes* have not been previously studied, making this species a 'nontarget' pest for this sunflower resistance trait.

## Materials and Methods

Seeds from a biparental cultivated *H. annuus* population varying for density of CGT per floret (described by Gao et al. 2018) were planted in replicated field trials over 2 yr. Changes to methods between years were based on poor seed production (via self-pollination) of some entries and an effort to determine whether another putative resistance trait (pericarp strength; Prasifka et al. 2014) could help explain unexpected results from the first year of field trials.

### 2016

Partially inbred lines ( $F_{3,4}$ ) with per floret densities of glandular trichomes characterized as relatively low (mean  $\pm$  SD =  $3.1 \pm 3.0$  CGT/floret;  $n = 30$  lines) or high ( $213.6 \pm 43.7$ ;  $n = 30$  lines) were planted at North Dakota State University's Agronomy Seed Farm near Casselton, North Dakota in a randomized complete block design (RCBD) with five replicates. Single-row plots were 5.00 m long with 0.76 m between rows. After emergence, plots were thinned to 20–25 plants per plot. A planting date of 18 May was selected with the goal of maximizing oviposition by female *C. hospes*.

When plants were mature, heads from each plot were collected, processed, and rated for seed damage as detailed by Prasifka and Hulke (2016). In brief, subsamples from five threshed heads were pooled to create a 200 seed composite sample for each plot, with damage by banded moth larvae assessed based on the appearance of X-ray images.

### 2017

Methods for planting (i.e., RCBD with replicates), harvesting and scoring of *C. hospes* seed damage were similar to 2016, except that poor production of self-pollinated seed from  $F_4$  plants reduced the number of lines evaluated in 2017. However, for the 2017 planting ( $F_{4,5}$ ) made on 19 May, estimated CGT densities (from Gao et al. 2018) for low (mean  $\pm$  SD =  $3.4 \pm 3.1$  CGT / floret;  $n = 27$  lines) and high ( $212.9 \pm 45.2$ ;  $n = 22$ ) groups remained distinct.

Because initial examination of 2016 data on seed damage did not suggest an association with CGT density, a secondary explanation, that differences in sunflower pericarp (hull) strength might help determine patterns of damage by *C. hospes* larvae, was tested. Like the CGT traits (density and chemical composition), prior studies of pericarp strength as a host plant resistance trait were related to *H. electellum* rather than *C. hospes* (Stafford et al. 1984, Prasifka

et al. 2014), but similarities in the feeding habits of the two pests suggested a plausible association. In brief, entries which sustained relatively high or low damage in 2016 ( $n = 10$  per group) were selected for evaluation of pericarp strength in 2017. Using methods described more completely by Prasifka et al. (2014), five plants per entry in the first planted replicate were tagged at the onset of bloom (stage R5.1; Schneiter and Miller 1981) and samples (a small wedge from each head) were collected 14 d later to evaluate pericarp strength in the lab by measuring the force (N) to penetrate the hull for each of five achenes per sample.

### Analysis

Data on seed damage by *C. hospes* larvae were converted to percentages. A model including fixed effects of year, group (low or high glandular trichome densities), entry (within group), and a random effect of replicate (within year) was used to test for the effect of glandular trichome density on the percent of seed damaged by *C. hospes* (SAS Institute Inc. 2016; Mixed Procedure). For pericarp strength data collected in 2017, a similar model including fixed effects of group (low or high damage in the previous year), entry (within group) and a random effect of replicate (i.e., each sampled head) was used to test whether the force required to penetrate pericarps differed among the least- and most-damaged lines. Lastly, because collection of pericarp strength data required tracking the first date of bloom for individual heads, a *t*-test was used to assess whether relative maturity (median from five heads per line) differed between the least- and most-damaged lines.

### Results

Damage to seeds by *C. hospes* larvae was not affected by year ( $F = 0.10$ ;  $df = 1, 8$ ;  $P = 0.758$ ), with mean percent seed damage across the trial of 7.71% (2016) and 7.94% (2017). Seed damage differed between low and high trichome density groups ( $F = 21.16$ ;  $df = 1, 476$ ;  $P < 0.001$ ), but with least-squared estimated means ( $\pm$ SE) for the percent of damaged seed for the high CGT group ( $8.60 \pm 0.40\%$ ) slightly greater than LS-mean for the low trichome group ( $7.05 \pm 0.40\%$ ). A significant effect of line within group was also found ( $F = 5.75$ ;  $df = 58, 476$ ;  $P < 0.001$ ). The percentages of seed damage to individual lines in the high and low CGT groups demonstrate this variation (Fig. 2).

Analysis of data collected in 2017 indicated that groups of lines that were least- or most-damaged by *C. hospes* in 2016 differed significantly in pericarp strength at 14 d after anthesis ( $F = 178.84$ ;  $df = 1, 8$ ;  $P < 0.001$ ), but that there also was significant variation within the groups ( $F = 20.46$ ;  $df = 18, 72$ ;  $P < 0.001$ ). As with the counterintuitive effect of CGT on damage by *C. hospes*, LS-means ( $\pm$ SE) for the force required to penetrate hulls was greater ( $3.29 \pm 0.04$  N) for the most-damaged lines compared to the least-damaged lines from 2016 ( $2.50 \pm 0.04$  N)(Supp Table 1 [online only]). Data on timing of bloom for 20 lines included in pericarp testing showed median date of anthesis differed significantly for the least- (2 August) and most-damaged (28 July) groups of lines ( $t = 5.75$ ,  $df = 18$ ,  $P < 0.001$ ).

### Discussion

Two years of field trials with sunflower lines which varied for CGT density failed to support the idea that CGT are a useful defense against larvae of *C. hospes*, a primary floret- and seed-feeding pest of sunflower in North America. Though groups of lines with low or

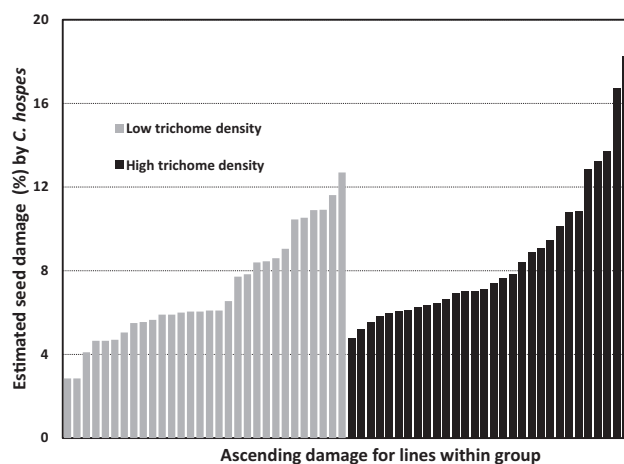


Fig. 2. Seed damage (least squares-estimated means) by *C. hospes* larvae to lines with low or high densities of capitulate glandular trichomes on florets in field trials in 2016–2017. Lines sorted in ascending order of damage within group. Low and high groups included 30 lines each in 2016, with 26 lines (low) and 23 lines (high) evaluated in 2017. Estimated standard errors for individual lines 1.25–1.74%.

high CGT density differed in the percentage of seeds damaged by the caterpillars, the putative plant defense of high trichome density corresponded to slightly more, rather than less, insect damage. A test of a secondary explanation, that strength of sunflower hulls could help determine the patterns of seed damage among tested lines, produced similarly negative results; there were differences in pericarp strength between the least- or most-damaged groups of lines, but not in the direction expected.

The negative results of field trials contradict positive data from previous laboratory-based tests, which have indicated that CGT or CGT extracts can cause mortality, delayed development or reduced feeding for both sunflower specialists and generalist herbivores (Gershenson et al. 1985, Rossiter et al. 1986, Chou and Mullin 1993, Prasifka et al. 2015). Prior field and laboratory tests also have supported the idea that greater pericarp strength is a useful trait to reduce seed-feeding by sunflower insects (Rogers and Kreitner 1983, Prasifka et al. 2014). Explanations as to how such promising plant defense traits might prove less useful in a field environment include avoidance (e.g., choice to feed away from CGT or on less-developed achenes) and reduced susceptibility as larvae grow (Rossiter et al. 1986). Observations that CGT may be removed by bees foraging for pollen (Sammaturo et al. 1985) also suggest field exposures for lepidopteran larvae may be lower than expected. But testing large numbers of lines ( $n = 49$ – $60$  for 2 yr of CGT tests), lines with common parentage (to reduce other confounding genetic differences), high replication ( $n = 5$  in each of 2 yr) and doing so under natural conditions represent improvements over earlier laboratory and field tests on the effects of CGT, and indicate that any true value of these specific traits in limiting sunflower insect pests may be less than previously believed.

Because both CGT and pericarp strength are putative resistance mechanisms supported by previous research with *H. electellum* and not *C. hospes*, it is also possible that testing these traits against a ‘nontarget’ pest explains the negative results of field trials. Similar field tests with *H. electellum* might support CGT or pericarp strength as host plant resistance trait, but such trials have not been conducted because *H. electellum* populations are sporadic in the primary sunflower-growing area of the United States; in places where *H. electellum* populations are sufficient (e.g., Texas, Kansas),

their numbers may be so great as to overwhelm any effects in such a trial. It is also possible that glandular trichome effects on *C. hospes* exist, but for LGT rather than CGT; the density of linear glandular trichomes is greatest on the involucre bracts (phyllaries; Aschenbrenner et al. 2013), the location of oviposition and larval hatch for *C. hospes* (while *H. electellum* oviposit among and within florets, closer to CGT).

Data collected during the 2017 season also suggest time of bloom ( $\approx$ date anthesis begins) as one additional variable that may have influenced seed damage by *C. hospes*. In general, a later date of anthesis (produced by later planting or longer relative maturity) should produce less seed damage by *C. hospes* and more by the red sunflower seed weevil, *Smicronyx fulvus* LeConte (Coleoptera: Curculionidae) (Oseto et al. 1989). But pheromone trapping data across North Dakota show moderate-to-high adult moth populations sustained for 4–5 wk (i.e., covering the prebloom period of oviposition for all 20 lines with data on bloom timing in 2017; <https://www.ag.ndsu.edu/ndipm/ipm-survey-archives/sunflower-insect-trap-archives>), meaning that small differences (<7 d) in maturity are unlikely to have strong effects on damage to entries in field trials. Previous tests of inbred lines and hybrids in North Dakota where notes on time of bloom were carefully taken have not shown small differences in maturity to be important (Prasifka and Hulke 2016), but also have shown large differences in damage to lines with similar dates of bloom ( $\pm 1$  d). Though the average difference between lines in the least- or most-damaged groups was only  $\approx 5$  d, the pattern of lines with later bloom and lower amounts of damage by *C. hospes* indicate data on flowering time should be collected for all lines tested as a covariate in subsequent analyses.

Though results from field trials did not support two specific plant traits as sources of resistance to *C. hospes*, field trials have shown differences in susceptibility to *C. hospes* (Prasifka and Hulke 2016, Prasifka 2019) and *H. electellum* (Wilson and McClurg 1987) that are not accounted for by differences in maturity. Identification of sunflower inbred lines with lower susceptibility to seed-feeding pests alone can be helpful for breeding, as damage to an inbred parent is correlated to resulting hybrids (Prasifka 2019), but continued efforts to understand the causes of reduced susceptibility to *C. hospes* and other pests improve the odds that host plant resistance can be useful in cultivated sunflowers.

## Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

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