BIOLOGICAL ACTIVITY OF *Datura wrightii*
GLANDULAR TRICHOME EXUDATE AGAINST
*Manduca sexta* LARVAE

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Abstract—Natural populations of *Datura wrightii* in southern California consist of two distinctly different phenotypes. The leaves of one phenotype are densely covered with nonglandular trichomes and feel velvety. The other phenotype is covered with larger type IV glandular trichomes that excrete a sticky exudate. Neonate larvae of *M. sexta* reared on velvety leaves developed significantly faster than larvae on sticky leaves. Larvae on sticky leaves took 28% longer to reach the prepupal stage. Survival and pupal weight were not significantly different between the two groups. First instars of *M. sexta* had a significantly higher consumption rate on velvety leaves than on sticky leaves. Removal of the exudate from stickly leaves significantly increased larval consumption rates compared to unwashed controls. Female moths did not show an oviposition preference; both in the lab and in the field the two trichome phenotypes of *D. wrightii* received similar egg loads. Because there were no significant differences in other nutritional factors between the two plant phenotypes, we concluded that the exudate was responsible for the effect. We isolated a complex mixture of sugar esters (SE) as the biologically active compounds in the exudate of *D. wrightii*. The SE mixture was composed of glucose esterified with several combinations of straight chain C4–C9 acids. By comparing GC-MS spectra of synthetic SE with the SE extracted from *D. wrightii*, we identified one of the SE as 3′-O-hexanoyl glucose.

Key Words—Tobacco hornworm, Solanaceae, insect–plant interactions, glandular trichomes, exudate, resistance polymorphism, oviposition choice, acyl sugar esters, Lepidoptera, Sphingidae.

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INTRODUCTION

Glandular trichomes and their exudates confer insect resistance in several solanaceous plant species. They may form a physical barrier hampering feeding or movement of herbivores on the leaves, or a chemical defense that increases insect mortality (Tingey, 1981; Duffey, 1986). These mechanisms are active against a wide range of insect herbivores, such as aphids (Goffreda et al., 1989; Neal et al., 1990; Severson et al., 1994), whiteflies (Buta et al., 1993; Liedl et al., 1995), beetles (Neal et al., 1989; Yencho et al., 1994), and caterpillars (Thurston, 1970; Kennedy and Yamamoto, 1979; Dimock and Kennedy, 1983). Several chemical compounds involved in insect resistance have been isolated from glandular trichomes of solanaceous plants. The relatively small globular type VI (Luckwill, 1943) trichomes on tomato leaves produce a volatile compound, 2-tridecanone, that increases mortality of Manduca sexta and Helicoverpa zea larvae (Williams et al., 1980; Dimock and Kennedy, 1983). Type VI trichomes on tomato also contain phenolics and polyphenol oxidases (PPO) (Duffey, 1986; Steffens et al., 1990). Phenolics and PPO are stored in different compartments of the tetracellular tips of these trichomes. Rupture of the head by herbivores leads to enzymatic polymerization of the phenolics, resulting in entrapment of the insect (Duffey and Isman, 1981; Duffey, 1986). Moreover, several Solanaceae also possess type IV trichomes, which are longer than type VI trichomes and have ovoid glands on their tips. These glands excrete a sticky exudate that contains sugar esters (King and Calhoun, 1988; King et al., 1988; Goffreda et al., 1990; Liedl et al., 1995; Liu et al., 1996). Sugar esters either isolated from plants, or produced synthetically, significantly increase whitefly mortality (Buta et al., 1993; Chortyk et al., 1996) and reduce settling by aphids (Goffreda et al., 1989). Moreover, sugar esters have antagonistic effects on growth and survival of H. zea and Spodoptera exigua (Juvik et al., 1994).

Exudate-producing type IV trichomes are also found on leaves of Datura wrightii L. (Solanaceae) plants. However, not all individuals of this species possess type IV glandular trichomes; some plants are densely covered with nonglandular trichomes (van Dam et al., in press). All plants possess equally low numbers (~5% of total trichomes) of the tetraroded type VI trichomes. The appearances of plants with and without type IV glandular trichomes are distinctly different and the two phenotypes can be easily discriminated by eye and touch; plants with nonglandular trichomes feel velvety, while plants with glandular trichomes feel sticky. Only a few (less than 5%) intermediate types are found in the field. All southern Californian populations censused to date contained both velvety and sticky phenotypes. Trichome type in D. wrightii is determined by a single gene, with glandular trichomes being dominant (van Dam et al., in press).
Because trichomes are known to be involved in insect resistance, we hypothesize that insect herbivores play a role in the maintenance of this trichome polymorphism. Both nonglandular and glandular trichomes are involved in insect resistance (Tingey, 1981), but the production of exudate by sticky types may be an additional physical or chemical barrier for herbivores. Therefore, it is not unreasonable to expect that sticky types, overall, are more resistant to herbivores than velvety types.

One of the naturally occurring herbivores on *D. wrightii* in southern California and Arizona is *Manduca sexta* (Lepidoptera: Sphingidae), the tobacco hornworm (Casey, 1976). The larvae consume up to 3400 cm² of leaf area to reach the prepupal stage (Madden and Chamberlin, 1945), so grazing by *M. sexta* may constitute a significant selection pressure. Previous studies on Solanaceae, such as *Petunia*, tobacco, and tomato, indicated that *M. sexta* larvae are negatively affected by trichomes and their various exudates (Thurston, 1970; Kennedy and Yamamoto, 1979).

We have studied the biological activity of trichomes and exudate of *D. wrightii* on *M. sexta* performance. First, we assessed differences in development, feeding rates, and oviposition of *M. sexta* on velvety and sticky leaves. We evaluated the significance of the exudate indirectly by washing it off sticky leaves and comparing the feeding rate of neonate *M. sexta* larvae on these leaves with untreated leaves. Then, we assessed the biological activity of the exudate in isolation by adding leaf surface extracts to artificial diets and comparing the developmental rates of the larvae. Thus, we eliminated other factors, such as trichome morphology and nutritional factors as being responsible for the differences in growth rates. Finally, we analyzed the chemical composition of the active fraction and compared it with biologically active exudate compounds in other Solanaceae.

METHODS AND MATERIALS

Description of Species and Populations

*Datura wrightii* Regel (Solanaceae), is a self-compatible perennial species whose geographical distribution is western Texas to California and Mexico (Avery et al., 1959; Munz, 1973). *D. wrightii* is commonly found in sandy or gravelly dry places, such as river washes and slopes (Munz, 1973). One population in this study (UCR) is in an undisturbed site on the hills in and around the botanical garden at the University of California, Riverside campus. The Moreno Valley (MV) population is located 10 km southeast of the UCR population, near a residential area in a dry creek that is periodically tilled for weed control. Based on their appearance and differences in percentages of glandular
trichomes, plants with less than 15% glandular trichomes were designated as velvety, while plants with more than 85% glandular trichomes were designated as sticky (van Dam et al., in press).

_Manduca sexta_ (L.) (Lepidoptera: Sphingidae) is a naturally occurring herbivore on _D. wrightii_ plants throughout southern California and Arizona (Hodges, 1971; Casey, 1976). The geographic range of _M. sexta_ in North America stretches from southern California to Florida, with northern limits in southeastern New York (Hodges, 1971). The moths lay single eggs on the abaxial side of the leaves of _D. wrightii_ and other solanaceous plant species (Yamamoto et al., 1969; Hodges, 1971). The larvae go through five larval instars. In southern California, _M. sexta_ typically goes through two generations per season: the first lasts from June until August, the second, numerically larger generation, is from September until December (Oatman and Platner, 1978). Pupae of the second generation overwinter in the soil (Gilmore, 1938).

**M. sexta Rearing**

In 1994 a colony was started with _M. sexta_ larvae and eggs collected from _D. wrightii_ from several natural populations in and around Riverside. To prevent inbreeding, the colony was supplemented annually with new _M. sexta_ eggs and larvae, collected from natural populations between June and December. The larvae were reared individually on artificial diet (Hoffman et al., 1966) in a growth room (28°C const., 16L:8D). After the moths had emerged, they were transferred to a flight cage (1 × 1 × 1 m) for mating and oviposition. From October until April, the cage was placed in the greenhouse (20–30°C, ambient light conditions), while for the remainder of the year the cage was placed outside under a tree to protect it from excessive solar heat and radiation. The moths were provided with a 25% sucrose solution. The eggs were collected daily from leaves of sticky and velvety _D. wrightii_ plants placed in the cage and brought to the growth room. There they hatched within three to five days after collection.

**Performance of M. sexta Larvae on Velvety and Sticky Plants**

_Toxicity and Effect on Early Development._ Groups of 15 neonate _M. sexta_ larvae were placed in plastic boxes (16 × 30 × 8.5 cm) that contained either a sticky or a velvety branch, cut from plants grown at the Agricultural Operations experimental station at UCR (AgOps UCR). Plant type was initially determined by eye and touch and later confirmed by observation under a dissection microscope (25×). The branches were put in a Aqua-pic (Syndicate Sales Inc., Kokomo, Indiana) vial filled with water to prevent wilting. The boxes had ventilation holes in the sides, covered with brass screen, and were lined with a wet paper towel. Three boxes per trichome type were placed in the growth room
(conditions as above). After four days, the larvae were transferred to fresh branches cut from the same plants as previously. After six days the experiment was ended and the head capsule width and weight of each larva were measured. The numbers of larvae in each of the first three instars were noted. Differences in survival were analyzed with a t test, larval weight with a nested ANOVA (box nested in plant type; SAS, version 6.02, SAS Institute Inc., Cary, North Carolina), and the distribution of larvae over instars was analyzed with a G-test (BIOM statistical package, F. J. Rohlf, 1982).

**Long-Term Effect on Development.** To determine the effect of trichome type on larval development of *M. sexta*, branches were cut from neighboring sticky and velvety plants growing in the MV (three plants per type) and UCR (two plants per type) *D. wrightii* populations and placed in boxes as described above. Ten (MV plants) or 12 (UCR plants) neonate *M. sexta* larvae were placed on the leaves in each box. The boxes were placed in the colony room (conditions as above). The larvae fed ad libitum and the branches were replaced with freshly collected branches from the same plants twice a week or more frequently when necessary. When a larva reached prepupal (wandering) stage, the date was noted and the larva was transferred to a labeled cup filled with sterile vermiculite. Seven days after transfer, when the pupal case had hardened, the pupae were sexed and weighed. Differences in developmental time and pupal weights were analyzed with a mixed model ANOVA. Plant type was tested against the box within type error; sex and type × sex interaction were both tested against the sex × box within type interaction. Nonsignificant interactions were pooled with the error.

**Consumption Rates.** Neonate larvae of *M. sexta* were placed singly on a *D. wrightii* leaf disc (18.5 mm diameter) that was placed with the abaxial side up on wet filter paper in a Petri dish (10 cm diameter). The plants used for this experiment were grown in the greenhouse from seeds obtained by selfing of plants grown at AgOps UCR. During the experiment, the survival of the larvae was monitored every 24 hr. After 72 hr, when the leaf discs started to show signs of decay, the larvae were removed and the leaf area consumed was measured on 1-mm graph paper. In the first experiment, the consumption by larvae on discs punched from four velvety plants was compared to that on discs from four sticky plants (10 larvae per treatment). These data were analyzed with a t test. In the second experiment, the discs were punched from four different sticky plants. A pair of discs were punched from the same leaf: one was washed with demineralized water to remove the exudate, while the other served as a control (10 larvae per treatment). The washed discs were carefully dried with a low-lint tissue (Kimberley-Clark, Atlanta, Georgia) and examined under a dissection microscope (25×) to confirm that the exudate droplets were indeed removed. The differences in consumption rates on washed and control discs of the same
leaf were analyzed with a paired $t$ test. In both cases, differences in the distribution of survival over time (one, two, or three days) were analyzed with a $G$ test.

_Oviposition Choice_. Oviposition activity by the moths peaks in the fall in southern California (Oatman and Platner, 1978). On September 13, 14, and 19, 1995, a total of 99 mature _D. wrightii_ plants in the UCR population were censused for the presence of _M. sexta_ eggs. Trichome type of the plants was assessed by sight and touch. Differences in egg distribution were analyzed with a $G$ test.

To study the oviposition choice of the moths under controlled conditions, we cut branches from adjacent velvety and sticky plants either in the MV population (November 15–December 3, 1995; 15 choice tests) or in the UCR population (June 12–24, 1996; 10 choice tests). Flowers, seed capsules, and the occasional wild _M. sexta_ egg were removed, and the branches were pruned to equal size and appearance. The branches were placed in individual 500-ml flasks filled with tap water. The phenotype of the plant was confirmed by observation under a dissection microscope. Two branches of a sticky plant and two branches of a velvety plant were placed 46 cm apart in the four corners of the _M. sexta_ cage. Sticky and velvety branches alternated, and every day the positions of the sticky and the velvety branches were exchanged. After one night in the cage, the branches were taken out and the number of eggs and the number of leaves on each branch were counted. The moths were supplied with fresh branches from a different set of plants. To correct for differences in plant size, we divided the number of eggs by the number of leaves on a branch. Overall differences in numbers of eggs per leaf on the two types were analyzed on log transformed data with a mixed model ANOVA (type × experiment, with day nested into experiment). Nonsignificant interactions were pooled with the error term.

*Identification of Active Compound*

To determine the cause of the difference in performance of _M. sexta_ on velvety and sticky plants, we carried out several analyses. First, we measured five general nutritional variables that may differ between the two plant phenotypes and are known to influence insect growth in general. Then we focused on the active compounds in the trichome exudates.

_Differences in Nutritional Parameters_. All solvents used for extraction and analysis were of certified ACS quality or better and were purchased from Fisher Scientific (Fair Lawn, New York) unless indicated otherwise. In November 1995, 100 leaves of seven sticky and seven velvety _D. wrightii_ plants grown at AgOps UCR were collected. The leaves were weighed, dried to constant weight at 70°C for 48 hr, and weighed again to assess percentage of water. The dried leaves were ground with a Wiley mill equipped with a 20-mesh sieve. The
samples were analyzed for total nitrogen by a Leco gas analyzer (Sweeney, 1989) at the DANR Analytical Laboratories, University of California, Davis. Soluble sugars and phenolic contents were determined with colorimetric methods: soluble sugars with anthrone reagent (Allen, 1974) and total phenolics with Folin-Denis reagent (Allen, 1974). Alkaloids were extracted from 125 mg leaf powder under reflux (2 x 1 hr) with 25 ml CHCl₃-NH₃OH (24:1) (Fliniaux et al., 1993). Before extraction, 0.375 mg anisodine in MeOH (Sigma, St. Louis, Missouri) was added to the leaf powder as an internal standard. The two extracts were combined and evaporated under reduced pressure. The alkaloids were recovered from the crude extract with 10.0 ml mobile HPLC phase. After filtration through a 0.45-μm Acrodisc PTFE filter (Gelman Sciences, Ann Arbor, Michigan), 20 μl of extract was injected on the HPLC. The method used for HPLC analysis was adapted from Fliniaux et al. (1993) with the following changes: flow rate: 1.2 ml/min, column: Beckman ODS 250 x 4.6 mm, 5-μm particle size (Beckman, Fullerton, California), mobile phase: 15% CH₃CN and 0.3% phosphoric acid in water, pH adjusted to 2.2 with triethylammonium. Retention times and UV spectra of peaks in the leaf extracts were compared with reference solutions of scopoline and hyoscyamine (Sigma) which have been described as the major alkaloids in related Datura species (Cheeke and Shull, 1985). Scopolamine and hyoscyamine also were the main alkaloids found in D. wrightii leaf extract; in addition we found a minor unidentified alkaloid with a UV spectrum similar to that of hyoscyamine. The quantities of these alkaloids were added to obtain total alkaloid contents.

Test for Volatile Toxicity of Sticky Leaf Surface Extract. A crude chloroform extract was prepared from sticky leaves (1120 g) collected in a population close to the UCR campus (CC population, see van Dam and Hare, 1998) and bioassayed as described in Kennedy et al. (1981). A CHCl₃ solution of the extract was applied to five Whatman filter paper circles (4.5 cm diam.), so that each contained 2.5 mg/cm² of the extract, and placed in individual glass Petri dishes (5 cm diam.). Another five filter paper circles were treated with solvent only. The CHCl₃ was evaporated at room temperature prior to placing three neonate larvae on the filter paper. The dishes were sealed with parafilm. Surviving larvae were counted after 24 hr.

Extraction and Purification of Type VI Trichome Exudate. The first part of the extraction and purification procedure is a modified version of the sugar ester isolation procedure described in Severson et al. (1994). Young branches were cut from 25 sticky plants growing at AgOps UCR on October 10, 1996. The leaves [652.2 g fresh weight (FW)] were soaked for 1 hr in 3 liters CHCl₃ (Figure 1). The extract was filtered over Na₂SO₄ to remove water and the CHCl₃ was removed by rotary evaporation and an airstream. The yield of this brown residue of the crude extract (coded CS) was 5.794 g or 0.89% of the leaf FW. A sample of 25 velvety plants (240.7 g FW) was extracted likewise in 1.2 liters
CHCl₃, yielding 0.388 g of a brown–green gum (coded CV, 0.16% FW). Both extracts were analyzed for the presence of sugar esters (SE) on TLC (see below). Because the CV extract appeared to be inactive against M. sexta larvae and to contain no compounds consistent with Rₚ values for SE, further purification and analysis was carried out with the CS extract only.

The CS residue was suspended in 100 ml CH₃CN and placed in an ultrasonic bath for 10 min to dissolve all material. A portion (25 ml) of the CH₃CN solution was kept for analysis, and 75 ml was partitioned three times against hexane in a separatory funnel (140 ml and 2 × 100 ml hexane, respectively). In the last separation, 70 ml CH₃CN was added. The CH₃CN fraction was removed in vacuo, which resulted in 1.238 g of a light brown gum (code AS for acetonitrile solubles). The AS residue then was dissolved in 50 ml dichloromethane (CH₂Cl₂), 40 ml of which was partitioned two times against 20 ml of 1 N tartaric acid solution, and three times against 15 ml water. The tartaric acid and
water layers with alkaloids and other water-soluble compounds were combined and kept at -20°C. Evaporation of the CH₂Cl₂ resulted in a light brown residue with the alkaloid-free SE (AFSE) (Severson et al., 1994), weighing 0.792 g (0.202% FW, corrected for aliquots taken).

To obtain sufficient material for testing, an additional 700.2 g of sticky branches were collected on October 17, 1996. They were extracted as described above and yielded 5.250 g CS (0.75% of leaf FW) and eventually 0.663 g (0.095% FW) of AFSE residue. The two AFSE fractions were combined before testing.

 Procedures Used for SE Analysis

**TLC Procedure.** Between 1 and 10 µl of test solution was applied with a glass microcapillary to 2.5 × 7.5 cm TLC plates (MK6F Si-gel, 60 Å, 205 µm layer, Whatman, Clifton, New York). The presence of SE on the plates after elution with CHCl₃-MeOH (9:1) was tentatively detected as described in King and Calhoun (1988).

**Hydrolysis of SE and Identification of Sugar Moiety.** Dried samples of purified plant extracts were dissolved in 2 ml MeOH, and 25 µl was vortexed with 25 µl 0.1 M NaOH in 1 ml microcentrifuge tubes (Goffreda et al., 1990). After 10 min at room temperature, 2 µl of the hydrolyzed sample were applied to a cellulose TLC plate (13245 Cellulose, Kodak, Rochester, New York) and developed twice in the top layer of a BuOH-acetic acid-H₂O (4:1:5) mixture (Harborne, 1973). After air drying, the plates were sprayed with 2.5 g aniline hydrogen phthalate in 100 ml BuOH-acetone-H₂O (49:49:2) and heated for 10 min at 105°C (Harborne, 1973). The sugars were identified by comparison with reference solutions of glucose and fructose. Glucose and fructose could clearly be discriminated based on their Rₚ values (0.77 and 0.81 in this system, respectively) and a distinct difference in color (brown for glucose vs. yellow for fructose) (Harborne, 1973).

**Derivatization and GC-MS Analysis of SE.** The SE in the AFSE fraction were characterized by GC and GC-MS analysis of their trimethylsilyl (TMS) derivatives. Between 1 and 10 µg of oven dried (50°C) sample were combined with 1.0 ml dimethylformamide (DMF) and 100 µl N, O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) in 4 ml Reacti-Vials (Pierce, Rockford, Illinois). The vials were closed, vortexed, and heated to 60°C for 15 min (Pierce Silylation Sample Reagent Kit Instruction Booklet; Pierce). Samples of 1 µl were injected (splitless) on a 30-m × 0.32-mm-ID DB-5 column, film thickness 0.25 µm (J&W Scientific, Folsom, California) in an HP 5890 GC connected to an HP 3392A integrator (Hewlett Packard). Injector temperature: 250°C; FID detection; detector temperature: 300°C, column pressure: 50 kPa. The initial temperature of 150°C was maintained for 1 min, after which the temperature was increased to 300°C at 10°C/min. The
final temperature was maintained for 10 min. GS-MS (El, 70 eV) analysis was performed on an HP 5890 GC coupled to an HP 5989 A mass spectrometer, with a scan range of 40–650 Da at 2 scans/sec. Apart from the temperature program rate (6°C/min), the conditions and column were the same as for GC analysis.

Saponification of SE and Identification of Free Acids. A 30-mg sample of AFSE was saponified following the exact procedure described in Buta et al. (1993). The free acids were identified by comparison with reference samples on GC and GC-MS. We used the same GC conditions as for the TMS derivatives of the SE, except for the temperature program. Initial column temperature was set to 50°C for 3 min, followed by a 10°C/min increase until 250°C, which was maintained for 10 min. The identity of the acids was confirmed with GC-MS analysis (El 70 eV, 40–400 Da, 1.7 scans/sec) on an HP 5890 GC coupled to a 5970 B Mass Selective Detector equipped with a 25-m x 0.2-mm Ultra 2 column (Hewlett Packard). The temperature program was 40°C for 1 min, increasing by 10°C/min to 250°C. Identities were confirmed by comparison of retention times and mass spectra with those of authentic standards.

Synthesis of Reference Sugar Esters. To synthesize hexanoyl and heptanoyl reference SE, we followed the exact procedure described by Chortyk et al. (1996), with β-D-glucose (ICN, Cleveland, Ohio) and either capryl (hexanoyl) chloride or heptanoyl chloride (Sigma) as reagents. The synthetic SE were derivatized as described above and analyzed on GC and GC-MS. This procedure resulted in a mixture of mono-, di-, and triacyl sugar esters, which can be detected as three separate groups on GC (Chortyk et al., 1996). Their retention times and fragmentation patterns were used as references for the AFSE extract of sticky D. wrightii plants.

Biological Activity of Extracts

General Procedures. Figure 1 summarizes the extracts tested on M. sexta larvae. In all cases, the extracts were first weighed to calculate the original leaf concentration, based on the yield of residue per gram fresh weight. Corrections were made for aliquots taken for chemical analysis during the purification process. The residues were dissolved in 4 ml CH₂Cl₂ before they were added to a weighed amount of warm (50°C) standard M. sexta rearing diet. The diets with the extracts were heated and stirred on a hot plate until the CH₂Cl₂ had evaporated. To adjust for losses during the purification and diet preparation, the test concentrations in the diets were prepared at concentrations four to eight times the original leaf content. Small cubes (ca. 125 mm³) of diet were placed in 28-ml clear polystyrene cups with a lid. Neonate larvae were individually placed on the diet, and the lids were sealed with parafilm. The diet was replaced every
other day, so the larvae could eat ad libitum. The number of surviving larvae in each group was counted daily, and at the end of the experiment, the head capsule width of the larvae was measured under a dissection microscope.

*Experiment 1: CV and CS.* We mixed 0.158 g CV with 12 g diet (eight times more concentrated than original), and 0.779 g CS with 15 g diet (5.8 times concentrated). The experiment started with 15 larvae per group and was ended after eight days.

*Experiment 2. CV, CS, and AFSE.* We mixed 0.329 g CV with 40 g diet (5.1 times more concentrated), and 0.824 g CS extract with 20 g diet (4.6 times more concentrated). An 0.174-g aliquot of the AFSE fraction was taken and mixed with 13.5 g diet (4.7 times more concentrated than on the plant, corrected for aliquots taken). The experiment started with 15 neonate larvae in each group and was ended after seven days.

**RESULTS**

*Performance of M. sexta Larvae on Velvety and Sticky Plants*

*Toxicity and Effect on Early Development.* The development of *M. sexta* on sticky leaves was significantly delayed: after six days, the majority of larvae in this group was still in their second instar, while most larvae reared on velvety leaves were third instars (Table 1; G test, \( G_2 = 54.171, P < 0.001 \)). The larvae feeding on sticky leaves also had a considerably lower body weight (Table 1, nested ANOVA, \( F_{1,68} = 42.70, P < 0.001 \), with no significant effect of box within plant type). However, survival rates of neonate larvae feeding on sticky leaves were not significantly different from those on velvety leaves (Table 1; \( t_4 = 1.99, P = 0.12 \)).

*Long-Term Effects on Development.* Larvae of both sexes developed significantly slower when reared on sticky leaves [mixed ANOVA, sex effect \( F_{1,42} \)]

| Table 1. Average Survival, Weights (SE), and Number of *Manduca sexta* Larvae per Instar after Six Days on Nonglandular or Glandular *Datura wrightii* Leaves |
|---------------------------------|----------|------------|------------|------------|
| **Plant type**                  | **Survival (%)** | **Larval weight (g)** | **First instars** | **Second instars** | **Third instars** |
| Nonglandular                    | 84.5 (2.3) | 47.0 (2.61) | 0          | 6          | 32          |
| Glandular                       | 80.0 (0.0) | 24.6 (2.12) | 2          | 32         | 2           |
Female *M. sexta* reached greater pupal weights than males, but the absence of a significant sex \times type interaction indicated that both sexes responded similarly to plant type. On average, larvae on sticky leaves took 22.2 ± 0.4 days to reach the prepupal stage, which is 28% longer than larvae on velvety leaves (17.3 ± 0.5 days). Plant type, however, did not have a significant effect on pupal weight. As before, larvae reared on velvety and sticky leaves had comparable survival rates (48.7 ± 5.6% on sticky vs. 49.3 ± 9.1% on velvety; *t* = 0.145, *P* = 0.89). For both developmental time and pupal weight there was a significant effect of box within type (mixed ANOVA, *F*<sub>8,42</sub> > 4.0, *P* < 0.001 for both variables). Because the boxes each contained leaves of a different plant, this may indicate variation in the nutritional value of individual plants within type.

**Consumption Rates.** In the first experiment, neonate larvae on velvety leaf discs consumed significantly more leaf area (63.6 ± 18.2 mm²) than those on sticky leaf discs (4.7 ± 1.5 mm²; *t* = 3.066, *P* = 0.0067). In the second experiment, the larvae fed significantly more on those leaf discs whose exudate was removed (16.2 ± 2.3 mm² on washed discs vs. 6.0 mm² on unwashed discs; paired *t* test, *t* = 3.38, *P* = 0.008), indicating that the exudate is at least partly responsible for the lower feeding rates on sticky plants. In neither experiment were survival rates of neonate larvae on leaves with and without exudate significantly different (*G* test on survival classes, *G*<sub>2</sub> < 3 and *P* > 0.05 in both cases).

**Oviposition Choice.** There was no significant difference in egg distribution over velvety (24 with eggs, 43 without) and sticky (15 with, 17 without) plants in the UCR population (*G* test: *G*<sub>1</sub> = 1.09, *P* = 0.29). In the cage experiments, there was also no significant difference in the numbers of eggs per leaf between the two plant types (0.17 ± 0.06 eggs per leaf on velvety vs. 0.16 ± 0.2 on sticky in 1995, and 0.85 ± 0.18 on velvety and 0.64 ± 0.8 on sticky in 1996, respectively; *F*<sub>1,23</sub> = 0.07, *P* = 0.80). We found a significant difference in numbers of eggs between the two experiments (*F*<sub>1,48</sub> = 312.27, *P* < 0.001) and between test days within the experiments (*F*<sub>22,48</sub> = 11.29, *P* < 0.001), which is probably due to variable numbers of female moths available in the colony. The absence of a significant interaction between plant type and experiment, however, indicates that the moths were consistent in their lack of preference.

**Identification of Active Compounds**

**Differences in Nutritional Value of Plants.** None of the internal leaf characteristics differed significantly between the two types of *D. wrightii* (Table 2). This reduces the probability that differences in growth rates of the larvae are caused by differences between the phenotypes other than trichome morphology.
TABLE 2. CONSTITUENTS OF LEAVES OF GLANDULAR AND NONGLANDULAR

* * *

Datura wrightii Plants Grown at UCR in 1995*

<table>
<thead>
<tr>
<th></th>
<th>Nonglandular plants</th>
<th>Glandular plants</th>
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<tbody>
<tr>
<td>Water (%)</td>
<td>86.9 (0.1)</td>
<td>85.7 (1.5)</td>
</tr>
<tr>
<td>Nitrogen (% DW)</td>
<td>4.02 (0.11)</td>
<td>4.31 (0.14)</td>
</tr>
<tr>
<td>Soluble sugars (% DW)</td>
<td>6.58 (0.37)</td>
<td>6.97 (0.66)</td>
</tr>
<tr>
<td>Total alkaloids (mg/g DW)</td>
<td>4.79 (1.11)</td>
<td>6.12 (0.88)</td>
</tr>
<tr>
<td>Total phenolics (mg/g DW)</td>
<td>27.01 (1.49)</td>
<td>26.18 (1.83)</td>
</tr>
</tbody>
</table>

*None of the plant constituents was significantly different between types (t test, P > 0.1 for all tests). Values in parentheses are standard errors.

These results, together with the lower consumption rates on sticky leaves, indicate that the glandular trichomes, and more specifically the exudate, are responsible for the adverse effects on the developmental rate of M. sexta larvae reared on sticky leaves. Therefore, we isolated exudate from sticky leaves to test its biological activity in M. sexta larvae and to identify the chemical compounds responsible for the activity.

Volatile Toxicity of Sticky Exudate Extract. Larvae confined to Petri dishes with surface extract had exactly the same survival rates as larvae in the control treatment (93.5 ± 6.5%), although the concentration of the D. wrightii extract was over 50 times the LD₅₀ reported for crude extract from tomato leaves (44.2 μg/cm²) (Williams et al., 1980). This indicates that trichomes of D. wrightii do not produce volatiles, e.g., 2-tridecanone, in sufficient quantities to be lethal to first-instar M. sexta larvae. Therefore, we pursued extraction and detection procedures for biologically active, nonvolatile compounds of type VI trichomes. We focused on sugar esters, because they are known for their antiherbivore activity (Buta et al., 1993; Liedl et al., 1995) and have been extracted from a closely related plant species, D. metel (King and Calhoun, 1988).

Biological Activity and Chemical Composition of Type IV Trichome Exudate. After eight days, most larvae on the diet with crude extract of sticky D. wrightii plants (CS) were still in their first instar and thus developed significantly slower than larvae on the diet with velvety leaf (CV) extract, which were mostly second and third instars (G₂ = 8.89, P = 0.0117). TLC analysis of the crude extracts revealed that the CS extract showed two major charred spots (Rj = 0.48 and 0.69) in the Rj region typical of SE (King and Calhoun, 1988), while the CV extract showed no charred spots at all. Analysis of hydrolyzed aliquots of the extracts indicated that the sugar moiety of the SE in the CS extract consisted of glucose. In the hydrolyzed CV extract, no monosaccharides
were detected on TLC, which is consistent with the above finding that there are no SE present on the leaf surfaces of velvety plants. These results suggested that the presence of SE in the CS extract plays a role in the difference in development rates of *M. sexta* larvae on diet with CS vs. diet with CV. Therefore, only the CS extract was subjected to further analysis.

The purified extract (AFSE) had a significant deleterious effect on the development of *M. sexta* larvae (*G*₂ = 6.84, *P* = 0.0327). After seven days, the instar distribution of larvae on AFSE diet (six second and eight third instars) was not significantly different from that on CS extract (six second and six third instars; *G*₁ = 0.133, *P* = 0.715), while larvae on CV (one second and 12 third instars) developed significantly faster than on either CS (*G*₁ = 5.96, *P* = 0.014) or AFSE diet (*G*₁ = 4.73, *P* = 0.029). The differences in larval survival (87–93%) were small and did not indicate a toxic effect of SE.

To identify the acyl side chains of the SE, the AFSE extract was saponified. GC-MS analysis showed that hexanoic acid was the most abundant residue (57%), followed by heptanoic (19%), nonanoic (13%), and octanoic (9%), acid residues. Pentanoic acid was only a minor compound (2%). GC-MS analysis of the derivatized AFSE fraction showed that the extract contained at least 13 peaks that contained SE (Figure 2). Although mass spectra of sugar esters cannot

![Figure 2. GC trace of trimethylsilyl-derivatized alkaloid-free sugar ester (AFSE) extract from glandular *Datura wrightii* plants.](image-url)
reveal the exact stereochemistry, MS data can adequately determine the position and the size of the acyl groups (Patouraux-Promé and Promé, 1984; Severson et al., 1994). Detailed analysis of the mass spectra revealed that peaks 1 and 2 were monohexanoyl esters (MW = 566), while the mass spectra of peaks 3–7 all showed signals for hexanoyl (m/z 99) or heptanoyl (m/z 113) side chains. Peaks 8 and 9 were the only two peaks whose mass spectra contained strong signals for octanoyl (m/z 127) side chains in addition to hexanoyl signals. Peaks 10–13 all showed a strong m/z 141, indicating the presence of nonanoyl side chains, in addition to hexanoyl or heptanoyl signals. The presence of a strong m/z 273 ion (ion 14 in Patouraux-Promé and Promé, 1984) indicated that the nonanoyl side chain in peaks 10–13 probably was substituted at the 6′ position. Unfortunately, the parent ion for TMS derivatives of SE is generally not observed (Severson et al., 1994).

Additional evidence for the presence of SE in the AFSE extract of *D. wrightii* was provided by comparison with synthetic SE. Comparison of retention times and mass spectra with the GC-MS data of synthetic hexanoyl and heptanoyl glucose esters revealed that peak 1 in the AFSE extract had exactly the same retention time (29.548 min), molecular weight (MW = 566), and mass spectrum (Figure 3) as one of the monoacyl esters in the hexanoyl-glucose ester mixture. The presence of a strong m/z 243 signal (Figure 3) (F1 ion, Patouraux-Promé and Promé, 1984) indicated that the glucose was acylated at either the 2′ or 3′ position. However, in the case of a 2′ acylated monohexanoyl SE, we would have expected a strong m/z 316 signal (ion 13, Patouraux-Promé and Promé, 1984). Because the m/z 316 signal was completely absent from the MS, we identified peak 1 as 3′-O-hexanoyl glucose, derivatized with four trimethylsilyl groups (Figure 3). The position of the hexanoyl group was further confirmed by the low m/z 218/217 rate, which excludes 4′-acylation (Patouraux-Promé and Promé, 1984), and the presence of a strong m/z 132 signal (Figure 3), which is absent in MS from 6′-acylated SE (Patouraux-Promé and Promé, 1984).

We conclude that the complex mixture of SE in the exudate produced by type IV glandular trichomes on *D. wrightii* is responsible for the differences in performance of *M. sexta* on sticky and velvety leaves. The mixture is composed of glucose esterified with several combinations of C₆–C₉ acids.

**DISCUSSION**

Exudate of sticky *D. wrightii* phenotypes contains a mixture of sugar esters that significantly reduces the developmental rate of *M. sexta* larvae, one of its naturally occurring herbivores. The chemical structures of the SE from *D. wrightii* are similar to those of SE in the exudate of the closely related species
Fig. 3. Mass spectrum and molecular formula of 3'-O-hexanoyl glucose derivatized with TMS.

*D. metel* (L.) (King and Calhoun, 1988). In both species, the SE consist of a glucose, substituted with straight chain acid groups. This is unique in comparison with SE from other Solanaceae, which generally produce SE with branched acid side chains (King et al., 1988; Walters and Steffens, 1990; Severson et al., 1994).

The effects of SE in *D. wrightii* on *M. sexta* are similar to those described for SE from wild tomato species. Both *H. zea* and *S. exigua* larvae developed significantly slower on diets with SE, while their survival was not affected. Unlike *M. sexta*, their pupal weights were lower when fed on diets with SE (Juvik et al., 1994). Although we showed that trichome IV exudate alone significantly affects *M. sexta* growth, we cannot exclude that other factors, such as size differences between glandular and nonglandular trichomes, play an additional role. The presence of type VI glandular trichomes with PPOs and phenolics, which are present in low densities on both phenotypes of *D. wrightii*, may have a synergistic effect on type IV trichome-based resistance on sticky plants (Duffey, 1986; Neal et al., 1990).

Exudates with SE can act both as a physical and a chemical barrier to insect herbivores (Duffey, 1986). The exudate may reduce feeding activity by entrapping the insects or gumming the mouth parts, or it may act as an antifeedant
(Juvik et al., 1994; Duffey, 1986). When the insects do feed, the SE in the exudate may also negatively affect the metabolism of the insect, resulting in lower growth rates (Appel and Martin, 1992). Physical entrapment of *M. sexta* larvae does not seem to be the main mode of action of *D. wrightii* exudate, because the effects of SE on growth rates were preserved after incorporation in artificial diet. This, together with the reduced feeding rates on leaves with exudate, suggest that the SE in *D. wrightii* act as antifeedants and possibly also as metabolic toxins. This combination of biological activities has also been proposed as the mechanism behind the effects for orally ingested nicotine and other secondary metabolites on third instars of *M. sexta* (Appel and Martin, 1992).

Female moths did not choose to oviposit more frequently on the phenotype that maximizes the developmental rate of their offspring. It is not uncommon to find a poor relationship between oviposition preference and offspring performance in Lepidoptera (Thompson, 1988). For example, *H. zea* oviposition was even positively correlated with SE presence, despite a significantly lower larval performance on diets and leaves with SE (Juvik et al., 1994). As for many other moths (Juvik et al., 1994), oviposition is a crucial step that greatly determines the fitness of *M. sexta*, because the larvae normally do not leave the plant on which they hatch (McFadden, 1968). This raises the question why *M. sexta* moths do not oviposit more frequently on velvety plants than on sticky plants.

It has been argued that moths oviposit on suboptimal hosts because they provide enemy-free space (Thompson, 1988; Juvik et al., 1994; Berdegue et al., 1996). Glandular trichomes and their exudate have indeed been reported to reduce parasitization rates of *M. sexta* eggs on tobacco (Rabb and Bradley, 1968). However, the overall parasitization rates for *M. sexta* eggs and larvae in southern California are low (0–14.1%) (Oatman et al., 1983; N. M. van Dam and J. D. Hare, personal observations for seasons 1994–1997), which makes it unlikely that the search for enemy-free space motivates oviposition choices of *M. sexta* on *D. wrightii* phenotypes.

The results of our experiments indicate that, overall, sticky phenotypes are more resistant to *M. sexta*. The larvae of *M. sexta* are voracious herbivores that consume large amounts of leaves to complete their life cycle (Madden and Chamberlin, 1945) and occasionally completely defoliate *D. wrightii* plants (J. D. Hare, personal observation). The production of exudate by glandular trichomes significantly reduces the consumption and developmental rates of neonate larvae on sticky leaves. However, larvae on both phenotypes eventually attain similar pupal weights, which suggests that they ingest similar amounts of leaf biomass. Total herbivory on sticky plants may still be reduced if the longer developmental time on these phenotypes leads to a reduction of *M. sexta* numbers over time. The slow developmental rate may not only lead to a slower population growth within one season, but also increase the probability that the
larvae are exposed to poor climactic conditions at the end of their growth season (Juvik et al., 1994). Because D. wrightii is a perennial plant, long-term effects on herbivore numbers can indeed result in lifetime benefit gains for sticky phenotypes.

The net fitness costs or benefits for sticky and velvety phenotypes will not only depend on M. sexta resistance, but also on other factors influencing selection for trichome type. Glandular trichome production and exudate may be costly compared to the production of nonglandular trichomes (N. M. van Dam and J. D. Hare, unpublished data). Allocation costs (sensu Simms, 1992) may occur when limited resources are shunted into the production of defenses rather than into the primary metabolism. The production of SE in tomato not only requires glucose molecules, but also amino acids for the synthesis of the acid side chains (Walters and Steffens, 1990). Moreover, the production of SE solution by type IV glandular trichomes may be costly if it diverts a portion of the water resources of sticky plants, especially in the arid natural environment of D. wrightii (Lauter and Munns, 1986).

Additionally, there may be ecological costs (Simms, 1992) involved with glandular trichome production when, for example, resistance against one herbivore leads to susceptibility for another. In the wild, D. wrightii is attacked by a suite of herbivores of different orders, among which are whiteflies (Homoptera: Aleyrodidae) and Tepiocoris notatus (Heteroptera: Miridae). The same exudate that makes sticky plants less suitable for M. sexta and whitefly also renders them more attractive to T. notatus (van Dam and Hare, 1998). Hence, it is not yet possible to predict, on the basis of the interaction with one herbivore alone, which of the two phenotypes is the most fit. The quantification of the costs and benefits involved with glandular trichome and exudate production are the subject of our current studies.

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REFERENCES

Datura EXUDATE ACTIVITY AGAINST Manduca LARVAE


