

CUTICULAR WAXES ON *ARABIDOPSIS THALIANA* CLOSE RELATIVES *THELLUNGIELLA HALOPHILA* AND *THELLUNGIELLA PARVULA*

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Thellungiella halophila and *Thellungiella parvula* have been recently reported to be close halophytic relatives of *Arabidopsis thaliana*. Crystallization patterns and chemical composition of stem and leaf cuticular waxes on *T. halophila*, *T. parvula*, and *A. thaliana* ecotype C24 were examined. Whereas the heavy glaucousness, total wax amounts, wax crystal structures, and wax chemical constituents on inflorescence stems of these species were similar, their leaf waxes differed significantly. *Arabidopsis thaliana* leaf surfaces were glossy, whereas *T. parvula* leaf surfaces were glaucous throughout development. By comparison, *T. halophila* leaf surfaces were glossy before flower initiation but glaucous after. Glaucousness resulted from the presence of densely distributed epicuticular wax crystals visible with electron microscopy. Glaucous leaves of both *T. halophila* and *T. parvula* produced 12.7- and 24.1-fold more total wax, respectively, than leaves of *A. thaliana*. Waxes on glossy leaves of *T. halophila* were greatly enriched in free acids relative to waxes on glossy leaves of *A. thaliana*. Glaucous leaves of *T. halophila* and *T. parvula* had similar wax composition and wax crystallization patterns as their respective stems, except total wax quantity was lower on leaves. As did glaucous stems, glaucous leaves produced relatively large amounts of secondary alcohols and ketones, constituents barely detectable on glossy leaves. Our results indicate that *T. halophila* and *T. parvula* may provide, via the application of genomics approaches recently developed for *A. thaliana*, valuable model systems for isolating genes involved in leaf wax synthesis and the regulation of leaf and stem wax specificity.

Keywords: *Arabidopsis thaliana*, *Thellungiella halophila*, *Thellungiella parvula*, cuticular waxes.

Introduction

Leaf cuticular waxes have been implicated in plant resistance to a variety of environmental stresses, including those caused by drought, pathogens, and phytophagous insects (Jenks and Ashworth 1999). Leaves, as the primary photosynthetic organs, comprise the primary biomass of most agronomic and horticultural crops and are often severely damaged by pests and other environmental stresses. Thus, development of new research approaches to identify genes involved in leaf wax synthesis could ultimately contribute to crop improvement.

Arabidopsis thaliana (L.) Heynhold is an important model plant system for molecular-genetic studies because it has unique attributes for laboratory-based research that include small size, short life cycle, the smallest-known genome of higher plants (125 Mb), facile transformation, and a completely sequenced genome. Mutagenesis approaches have recently been applied to *A. thaliana* as a means to elucidate gene involvement in cuticular wax biosynthesis (Koorneef et al. 1989; Jenks et al. 1995, 1996a, 1996b; Jenks and Ashworth 1999; Rashotte et al. 2001). Cuticular wax mutants, designated *eceriferum* (*cer*), were visually identified in mutagenized *A. thaliana* populations by their distinct reductions in glaucousness on inflorescence stems (Koorneef et al. 1989). These *cer* mutants generally show little, if any, significant variation

in their leaf wax characteristics, unlike *cer* stem waxes, for which dramatic variations have been reported (Jenks et al. 1995). Studies of *A. thaliana* ecotypes representing worldwide distribution revealed no glaucous leaf types in over 80 ecotypes (M. A. Jenks, unpublished data) and only little variation for wax composition in 40 ecotypes (Rashotte et al. 1997). As wild-type *A. thaliana* leaves are not glaucous, visual screening of mutagenized populations to find mutants having increased leaf glaucousness or glossiness due to alterations in cuticular waxes have had only little success in *Arabidopsis* (Jenks et al. 1996b; M. A. Jenks, unpublished data). Thus, mutagenesis of *A. thaliana* provides an ineffective method for uncovering new leaf wax mutants for use in studies to elucidate gene involvement in basic leaf wax synthesis.

Similarities between the growth habits and genomes of *A. thaliana* and many of its close relatives have revealed the potential of new genomics-based approaches recently developed for *A. thaliana* to identify and clone valuable new genes conferring traits not apparent in *A. thaliana* (Zhu 2001). A survey of species related to *Arabidopsis* reveals tremendous phenotypic diversity in traits like salt and drought adaptation (Al-Shehbaz and O’Kane 1995; R. A. Bressan and M. A. Jenks, unpublished data); life cycle (being either annual, biennial, or perennial); trichome branching pattern; flower color (white to lavender to purple); fibrous and nonfibrous flowers; the presence or absence of seed mucilage; photoperiod sensitivity affecting flowering time (Pollard et al. 2001); and the size and shape of leaves, stems, flowers, and fruits (Rollins 1993; Al-Shehbaz et al. 1999). Differences in native habitats of these *Arabidopsis* relatives also

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indicate yet unexploited variation in cold tolerance and other environmental stress resistances. Most interesting to us however is the dramatic variation in visible cuticular wax deposition on leaves among the relatives of *Arabidopsis*. Whereas *A. thaliana* has a glossy leaf surface throughout development, *Thellungiella halophila* (C. A. Meyer) O. E. Schultz and *Thellungiella parvula* (Schrenk) Al-Shehbaz and O'Kane (previously classified *Arabidopsis halophila* and *Arabidopsis parvula*, respectively; Al-Shehbaz 1988; Rollins 1993) produce highly glaucous leaf surface coatings (Al-Shehbaz and O'Kane 1995). The heavy leaf glaucousness of these *Thellungiella* species led us to hypothesize that these *A. thaliana* close relatives might provide model systems for applying mutagenesis approaches to uncover new mutants with glossy leaf surfaces, and, thereby, for identifying new leaf wax genes. Since differential leaf and stem wax alterations could be screened for simultaneously (as both leaves and stems of these *Thellungiella* species are glaucous), genes involved in leaf and stem differential regulation of wax synthesis might also be uncovered. Finally, *Thellungiella* has an extremely high drought tolerance relative to *Arabidopsis* (R. A. Bressan and M. A. Jenks, unpublished data), which indicates that wax genes associated with drought tolerance might likewise be identified.

Whether leaf waxes of *Arabidopsis* close relatives like *Thellungiella* possess characteristics that are useful for biochemical- and molecular-genetic dissection of cuticular wax metabolism has not been studied. In this study, we compare the structural and compositional properties of cuticular waxes on *T. halophila*, *T. parvula*, and *A. thaliana*, and we discuss how these *Thellungiella* species may provide a valuable tool for identifying new wax genes not easily accessible with the traditional mutagenesis approaches in *A. thaliana*.

Material and Methods

Plant Materials

Cuticular waxes from *Thellungiella halophila* (C. A. Meyer) O. E. Schulz, *Thellungiella parvula* (Schrenk) Al-Shehbaz and O'Kane, and *Arabidopsis thaliana* (L.) Heynhold ecotype C24 were extracted from plants grown under constant light (200 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and temperature (ca. 22°C) in growth chambers at Purdue University. *Arabidopsis thaliana* ecotype C24 and *T. parvula* induced bolting of the inflorescence at ca. 4 and 6 wk, respectively, under our conditions, whereas *T. halophila* required vernalization to induce flowering. *Thellungiella halophila* flowering (bolting) was induced by placing 8-wk-old rosettes in a cold room at 4°C for 4 wk before returning them to the growth room. Organs selected for wax analysis were chosen to best represent similar stages of development between these species.

Analysis of Epicuticular Wax Crystalline Morphology

Leaf samples were collected from the glossy leaves of flowering *A. thaliana* after 3 and 6 wk growth and from the glaucous leaves of *T. parvula* after 8 wk growth. *Thellungiella halophila* leaf samples were collected from glossy-leaved, 10-wk-old preflowering plants (without cold treatment); semiglaucous-leaved, 16-wk-old plants at early flowering stage; and glaucous-leaved, 20-wk-old plants at midflowering stage. Whereas *T. halophila* leaf samples were taken from the same leaves at 10 and 16 wk (i.e., excised from approximately the

same insertion points in the rosette), the 20-wk-old plant leaf samples were collected from leaves newly emerging from within the rosette after cold treatment. Stem samples of *A. thaliana* and *T. parvula* were collected from 6- and 8-wk-old flowering stems, respectively, at the middle of the third internode above the rosette. Stem samples of *T. halophila* were collected from similar locations on 20-wk-old midflowering stems. Three to four replicates of air-dried stem and abaxial and adaxial leaf samples from all three genotypes were mounted on aluminum stubs and, with six 30-s bursts from the sputter-coater, sputter-coated with gold palladium. Previous research has shown that air-dried samples coated in this way and viewed with an ambient temperature scanning electron microscope (SEM) are similar to specimens prepared with a low-temperature SEM with little evidence of artifacts in wax crystallization patterns observed (Jenks et al. 1992). Coated surfaces were viewed with a JEOL JSM-840 scanning electron microscope (JEOL, Tokyo) at 5 kV.

Wax Extraction and Chemical Analysis of Cuticular Waxes

Wax samples were collected from the glossy leaves of flowering *A. thaliana* and the glaucous leaves of *T. parvula* after 6 and 8 wk growth, respectively. *Thellungiella halophila* waxes were extracted from glossy-leaved, 10-wk-old preflowering plants (without cold treatment); semiglaucous-leaved, 16-wk-old leaves at early flowering; and glaucous-leaved, 20-wk-old leaves at midflowering. Leaf wax extractions of *T. halophila* were made from the same leaves used in the wax crystalline morphology analysis described above. Stem samples of *A. thaliana* and *T. parvula* were collected from 6-wk-old flowering stems, and stem samples of *T. halophila* were collected from 20-wk-old flowering stems. Leaf or stem samples were inserted into a 20-mL standard glass scintillation vial, to which ca. 15 mL of gas-chromatograph-grade (GC-grade) hexane added. The tissues were agitated for 30 s, and the solvent was decanted into new scintillation vials. Tissues and vials were given a 1-s rinse with ca. 2 mL of hexane, and then the solution was decanted into the sample vial. The leaf extracts thus contained waxes from both abaxial and adaxial leaf surfaces and, like the stems, showed little or no coloration due to chlorophyll or other internal lipids.

Wax compositional analysis was according to Jenks et al. (1995). The hexane-soluble cuticular wax extracts were evaporated to dryness under a nitrogen stream, and the dried residue was prepared for gas chromatography by derivatization with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Derivatization was for 15 min at 100°C. After surplus BSTFA was evaporated under nitrogen, the sample was redissolved in hexane for analysis with a Hewlett-Packard 5890 series II GC equipped with a flame ionization detector. The GC was equipped with a 12 m \times 0.2-mm HP-1 capillary column with helium as the carrier gas. The GC was programmed with an initial temperature of 80°C and increased at 15°C min^{-1} to 260°C, where the temperature remained unchanged for 10 min. The temperature was then increased at 5°C min^{-1} to 320°C, where the temperature was held for 15 min. Quantification was based on flame ionization detector peak areas and the internal standard hexadecane. Specific correction factors were developed from external stan-

dards and applied to the peak areas of the free fatty acids, primary alcohols, and alkanes. For all other peaks, a factor of 1.03 was assigned (the average correction for nine standards at comparable concentrations). The total amount of cuticular wax was expressed per unit stem or leaf surface area. Stem surface areas were calculated as the surface area of a right circular cylinder, and leaf areas were determined with computer digitization. All values represent the average of three to four replicate plant samples \pm SE. Selected subsamples were used for injection in a GC-mass spectrometer (FinniganMAT/Thermospray, San Jose, Calif.) to produce electron ionization mass spectra that could be used to identify all components.

Results

SEM showed that *Thellungiella halophila* and *Thellungiella parvula* had higher wax crystal density over stem surfaces than *Arabidopsis thaliana*, with *T. parvula* possessing the most dense distribution and the largest wax crystals (fig. 1A–1C). The wax crystals on *T. halophila* stems appeared thicker in diameter than those of *A. thaliana*, while *T. parvula* stem wax crystals were even thicker and had larger horizontally oriented surface facets than the other two species (fig. 1A–1C). As with stems, leaf wax crystals differed significantly between these species (fig. 1D–1H). *Arabidopsis thaliana* ecotype C24 leaves remained glossy throughout development and produced no visible wax crystals on either abaxial (fig. 1D) or adaxial (not shown) surfaces after 3 and 6 wk growth. However, glossy *T. halophila* leaves at 10 wk had very small wax undulations and protruding wax structures detectable with SEM across the abaxial (fig. 1E) and adaxial (not shown) surfaces. After a cold treatment to induce bolting of the flowering stem, many leaves on 16-wk-old plants became slightly glaucous and began to produce a variety of rounded and irregular wax crystals that spread over the surfaces of both abaxial (fig. 1F) and adaxial (not shown) leaves. The presence of wax crystals on the 16-wk-old leaf surfaces, however, was highly variable, with some leaf samples nearly lacking wax crystals and others having crystals showing much patchiness over the surface (not shown). Scanning across the surfaces from the smooth to more crystalline areas of these patchy surfaces suggested that the leaf wax crystals of *T. halophila* formed on the surface of raised areas (not shown) and then arose from these areas predominantly as rectangular stumplike crystals that became more tall than wide with maturity. Once flowering began, rosette leaves on the 10- and 16-wk-old plants began to senesce. SEM revealed that leaves emerging after the cold treatment on the 20-wk-old *T. halophila* plants were much more glaucous than the most glaucous leaves formed before flowering and that they possessed even larger, more highly ornamented, and more densely distributed wax crystal structures on the abaxial (fig. 1G) and adaxial (not shown) surfaces. Whether cold treatment was a requirement for the induction of glaucousness was not determined. By comparison, *T. parvula* leaves were similarly glaucous from leaf emergence onward and, at 8 wk, possessed densely packed wax crystals similar to those on *T. parvula* stems, except that they had a higher proportion of the large, horizontal crystal surfaces (fig. 1H). No significant differences were evident between abaxial and adaxial surface wax crystal morphology on leaves of these species.

Total stem cuticular wax amounts on *A. thaliana* ecotype

C24 differed very little from stem waxes of *T. halophila* and *T. parvula* (tables 1–3), even though the *T. halophila* and *T. parvula* stems had more dense and larger wax crystals in electron micrographs (fig. 1A–1C). Comparison of total stem wax class amounts revealed that the major difference in stem wax composition was that primary alcohols, secondary alcohols (14- and 15-nonacosanol and 15- and 16-hentriacontanol), and ketones (15-nonacosanol) were 1.8-, 1.3-, and 1.3-fold lower, respectively, and alkanes were 1.3-fold higher on *T. halophila* stems than on *A. thaliana* stems (tables 1, 2). Similarly, primary alcohols, secondary alcohols, and ketones were 2.5-, 4.3-, and 6.4-fold lower, respectively, and alkanes and esters were 1.6- and 2.6-fold higher on *T. parvula* stems than on *A. thaliana* stems (tables 1, 3). Thus, both *Thellungiella* species had elevated alkane amounts with notably lower primary alcohols, secondary alcohols, and ketones than *Arabidopsis*. Whereas the major primary alcohol homologue on stems was 28 carbons long in *A. thaliana* and *T. halophila*, the major primary alcohols on *T. parvula* stems were 24 and 26 carbons long (fig. 2). Interestingly, the primary alcohols on *T. halophila* stems had significantly higher amounts of the 24 carbon homologues and reduced 26, 28, and 30 carbon homologues relative to *A. thaliana* (fig. 2). Stem aldehydes on *T. parvula* differed from *A. thaliana* and *T. halophila* in that the 28 and 30 carbon aldehydes were much reduced (fig. 2). Relative acid and aldehyde chain length distributions on stems were comparable in all three species (fig. 2).

Leaf waxes on these species differed considerably, with glossy leaves of *A. thaliana* having 7.4-fold less wax than the glossy leaves of *T. halophila* and 12.6-fold less wax than the glaucous leaves of *T. halophila* (tables 1, 2). By comparison, *A. thaliana* leaves had 24.1-fold less total wax than the glaucous *T. parvula* leaves (tables 1, 3). Also notable was that total acid amounts on the glossy *T. halophila* leaves were higher than the amounts on the other leaves we examined (table 1–3). On the glaucous leaves of *T. halophila*, the acids comprised the least abundant wax class (table 2), and overall wax composition was similar to stem waxes of *T. halophila*, except that the quantity of most leaf wax constituents was lower than in stems (figs. 2, 3). The C_{31} homologue was the most abundant alkane on *T. halophila* glossy leaves (fig. 3). However, the more glaucous leaves had the C_{29} homologue as the most abundant alkane (similar to stem wax alkanes). Likewise, the glaucous leaves of *T. parvula* had the 29 carbon chain as the dominant alkane homologue (fig. 3). Unique among these species was *T. parvula*'s leaf primary alcohol profile, which had high relative amounts of the 24 and 26 carbon length homologues (fig. 3). The glaucous *T. halophila* and *T. parvula* leaves had greater amounts of esters than glossy *T. halophila* and *A. thaliana* leaves (table 1–3). Glaucous leaves also produced significant amounts of the C_{29} secondary alcohols and C_{29} ketones, a class of compounds not detected or detected only in trace amounts on glossy leaves (table 1–3). Analysis of *T. halophila* leaf waxes on 16-wk-old plants showed extremely high variations in their wax compositional profiles, variations that were apparently due to variation in the rate of development of the glaucous character (not shown).

Discussion

Leaf cuticular wax quantity, composition, and morphology of *Arabidopsis thaliana* ecotype C24 were similar to those of

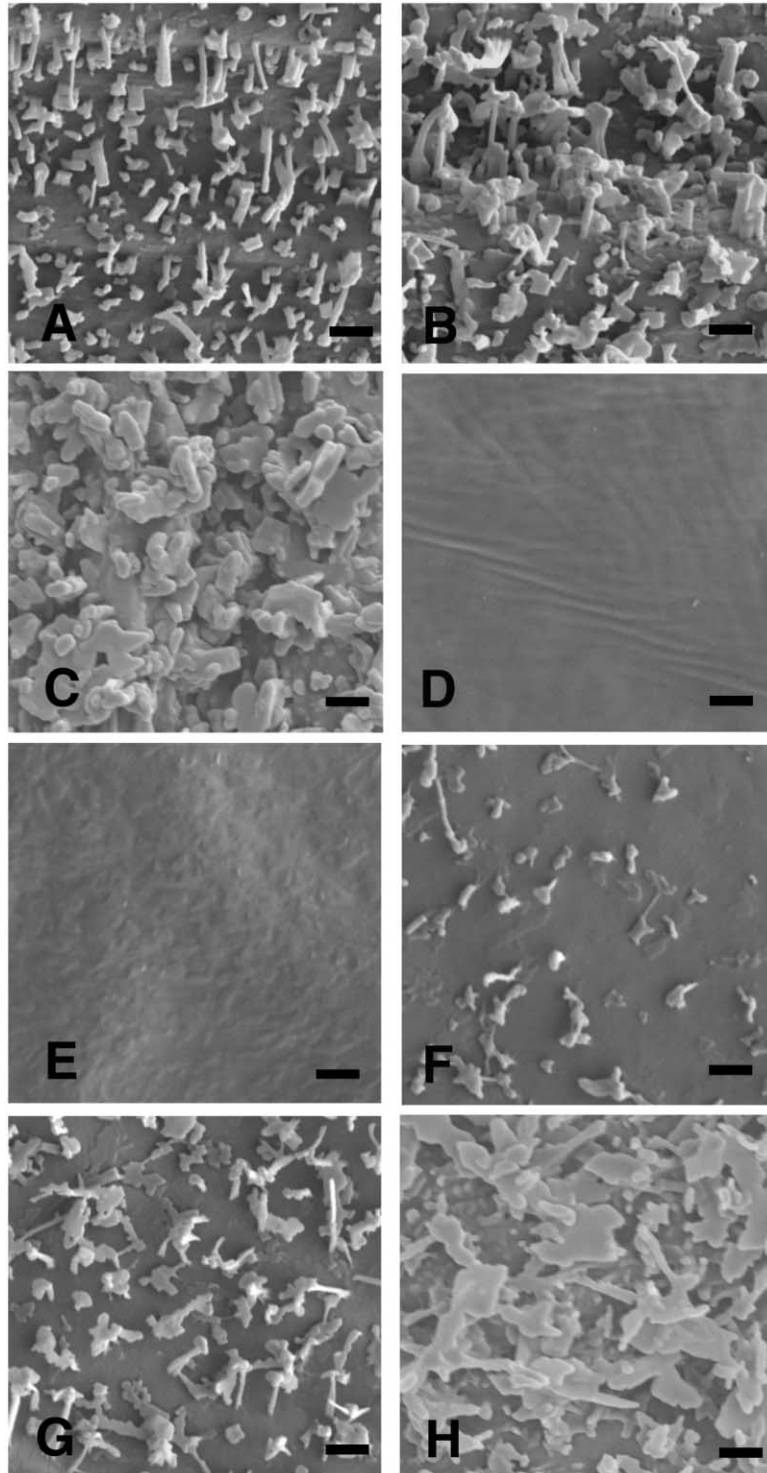


Fig. 1 SEM images of wax crystal morphology of (A) *Arabidopsis thaliana* ecotype C24 stems, (B) *Thellungiella halophila* stems, (C) *Thellungiella parvula* stems, (D) *A. thaliana* ecotype C24 abaxial leaf, (E) *T. halophila* glossy abaxial leaf, (F) representative *T. halophila* abaxial leaf with a semiglaucous coating, and (G) *T. halophila* abaxial leaf with heavy glaucous coating. H, *T. parvula* abaxial leaf. Bar = 2 μm .

Table 1
Cuticular Waxes on Stems and Leaves of
Arabidopsis thaliana Ecotype C24

	Stem	Leaf
Total load	2906.9 ± 139.4	105.8 ± 11.9
Acids	28.6 ± 5.6	4.4 ± 0.3
Aldehydes	104.2 ± 10.2	5.6 ± 0.2
1° alcohols	318.7 ± 13.5	10.9 ± 1.1
Alkanes	1390.6 ± 110.5	53.0 ± 6.5
2° alcohols	111.8 ± 23.1	0
Ketones	726.9 ± 59.4	0
Esters	118.7 ± 2.1	2.6 ± 1.3
Unknowns	182.5 ± 7.6	29.3 ± 3.6

Note. Values represent cuticular wax loads in $\mu\text{g}/\text{dm}^2 \pm$ SE of leaf and stem area.

the Landsberg *erecta* and Wassilewskija ecotypes (Jenks et al. 1995). Leaves of *Thellungiella halophila* and *Thellungiella parvula* had total wax quantities that were between 7.4- and 24.1-fold higher than those of the *A. thaliana* C24 ecotype examined in this study. The amount of leaf waxes on *T. parvula* was nearly as high as the amount of stem waxes on C24 and other previously reported *A. thaliana* ecotypes (Rashotte et al. 1997). Leaf wax amounts on *Thellungiella* are thus more similar to stem wax amounts on these species, with *A. thaliana*, by comparison, producing a relatively low amount of total leaf waxes. Whereas the glossy leaves of *A. thaliana* had smooth surfaces throughout development (as visualized by SEM), the glossy leaves of 10-wk-old *T. halophila* plants had a few relatively small wax crystals on their leaf surfaces. The semiglaucous leaves of 16-wk-old *T. halophila* plants and the highly glaucous leaves of 20-wk-old *T. halophila* possessed abundant epicuticular wax crystals. The wax crystalline morphology of the most glaucous leaves of *T. halophila* was similar to that of its stems, except that leaves displayed a higher proportion of the more horizontally oriented and flattened wax crystals. The wax composition of glossy *A. thaliana* leaves was dominated by alkanes with the next most abundant wax class being primary alcohols. In contrast, the wax composition of glossy leaves on 10-wk-old pre-flowering *T. halophila* plants was dominated by acids, with the next most abundant wax class being alkanes. Compared to the leaves on 10-wk-old *T. halophila*, leaves on 20-wk-old *T. halophila* that emerged after the plants were exposed to 4 wk of cold (4°C) temperatures were greatly reduced in acids, increased in C₂₉ alkane and total esters, and possessed significant amounts of C₂₉ secondary alcohols and C₂₉ ketones. The wax composition of these *T. halophila* glaucous leaves now resembled *T. halophila* stems, except that nearly all wax constituents occurred in lower amounts. Further studies are needed to determine what role cold treatment and other environmental stimuli may play in the induction of *T. halophila* leaf glaucousness and wax compositional changes reported here.

Wax crystalline morphology and composition of the glaucous leaves of *T. parvula* resembled those of 20-wk-old plants of *T. halophila*, except that *T. parvula* leaves had higher wax crystal density, larger wax crystals, and possessed more of the horizontally oriented and flattened wax crystals. Glaucous leaves of *T. parvula* also had notably lower C₂₈ and C₃₀ aldehydes and primary alcohols than glaucous leaves of *T. hal-*

Table 2

Cuticular Waxes on Stems and Leaves of *Thellungiella halophila*

	Stem	Glossy leaf	Glaucous leaf
Total load	3100.5 ± 47.3	779.5 ± 59.2	1338.3 ± 127.2
Acids	40.9 ± 3.6	654.9 ± 48.8	16.4 ± 2.8
Aldehydes	108.1 ± 3.4	4.4 ± 0.4	40.8 ± 0.5
1° alcohols	176.4 ± 14.5	17.7 ± 1.6	69.3 ± 6.2
Alkanes	1767.4 ± 3.4	64.6 ± 7.2	761.8 ± 80.3
2° alcohols	86.3 ± 12.7	0	52.4 ± 13.2
Ketones	576.8 ± 16.0	0	216.2 ± 17.2
Esters	117.3 ± 6.9	9.2 ± 0.3	96.7 ± 17.7
Unknowns	227.2 ± 41.9	28.7 ± 3.7	84.8 ± 2.9

Note. Values represent cuticular wax loads in $\mu\text{g}/\text{dm}^2 \pm$ SE of leaf and stem area.

ophila and *A. thaliana*. It is unclear whether *T. parvula* converts a larger portion of the C₂₈ and C₃₀ aldehyde pool into alkanes than primary alcohols or whether some other mechanism explains these compositional differences. As *T. parvula*'s leaf surfaces were glaucous very early after leaf emergence, it is reasonable to speculate that the *T. parvula* wax profiles reported here for 8-wk-old plants may have been formed even earlier in leaf development.

Stem wax amounts on *A. thaliana* ecotype C24 were at the upper end of the range for wax amounts on *A. thaliana* ecotypes (Rashotte et al. 1997). The total stem wax quantity on *T. halophila* and *T. parvula* was slightly higher than *A. thaliana*, although differences were not significant at $P < 0.05$. As do their leaves, stems of *T. halophila* and *T. parvula* had larger and more dense wax structures than *A. thaliana* and a wax composition that had significantly higher amounts of alkanes than *A. thaliana* as well as lower amounts of primary alcohols, secondary alcohols, and ketones. Overall, for both leaves and stems, our results indicate a positive correlation between wax crystal morphology and total wax amount, which suggests that, among these three species, organs with the highest wax amounts had the most dense and largest wax crystals (cf. total loads with micrographs of fig. 1). Further studies are needed, however, to determine what role these differences in total wax quantity and wax composition play in explaining the different wax crystal morphologies evident among the leaves and stems of these species.

The difference in leaf waxes among the species we examined,

Table 3

Cuticular Waxes on Stems and Leaves of *Thellungiella parvula*

	Stem	Glaucous leaf
Total load	3290.5 ± 179.7	2548.8 ± 123.3
Acids	54.8 ± 3.5	40.6 ± 6.7
Aldehydes	32.8 ± 2.1	55.1 ± 6.1
1° alcohols	128.3 ± 10.2	252.4 ± 45.1
Alkanes	2164.6 ± 139.1	1530.0 ± 144.0
2° alcohols	26.2 ± 2.1	36.6 ± 1.4
Ketones	112.7 ± 6.9	154.3 ± 12.7
Esters	304.9 ± 17.6	285.0 ± 45.8
Unknowns	466.1 ± 19.6	194.7 ± 19.9

Note. Values represent cuticular wax loads in $\mu\text{g}/\text{dm}^2 \pm$ SE of leaf and stem area.

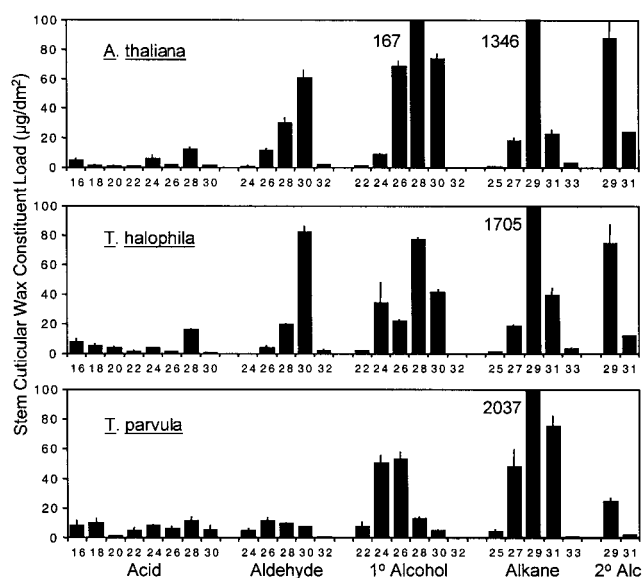


Fig. 2 Cuticular wax composition of stems of *Arabidopsis thaliana* ecotype C24, *Thellungiella halophila*, and *Thellungiella parvula*. Values represent cuticular wax load in $\mu\text{g}/\text{dm}^2$ of stem area \pm SE. Chemical classes and chain lengths are labeled on the horizontal axis. Where cuticular wax constituent amount was off the scale, a number designating actual value is next to the bar. $2^\circ\text{Alc} = 2^\circ$ Alcohol. Ketones were all 29 carbons, and ester chain lengths were not determined (see tables 1–3 for total quantities).

especially *T. halophila*'s and *T. parvula*'s very glaucous leaf surfaces with wax constituent profiles similar to those of stems, clearly delimits the wax phenotypes of these *Thellungiella* and *Arabidopsis* species. Besides these clear taxonomic differences, our results indicate that *Arabidopsis* close relatives *T. halophila* and *T. parvula* possess characteristics that suggest that they may be useful for biochemical- and molecular-genetic dissection of cuticular wax metabolism. As we discussed above, the leaves of these *Thellungiella* species produce a heavy glaucous coating. Thus, mutagenesis of *Thellungiella* to induce loss-of-function mutations would likely generate many glossy-leaved mutants that would be easily identified with visual screens of mutagenized populations. By comparison, leaf wax mutants of *Arabidopsis* are currently difficult to identify because mutations affecting leaf wax synthesis are generally not visible to the eye (Jenks et al. 1996b; M. A. Jenks, unpublished data). Mutagenesis of *Thellungiella* may thereby provide a means to identify genes whose normal function is, primarily, leaf wax synthesis. Genes involved in differential regulation of leaf and stem pathways may also be identified by isolating *Thellungiella* mutants that have altered glaucousness of leaves only, stems only, or of both organs. Of all the wax mutants described, only *cer2* (Negruk et al. 1996; Xia 1996), *cer3* (Hannoufa et al. 1996), and *gl15* (Moose and Sisco 1996) have, thus far, been associated with genes that encode putative regulatory proteins.

To date, we have not found *A. thaliana* mutants completely lacking waxes or having a reduction in the cuticle membrane like the *bm2* mutant in *Sorghum bicolor* L. Moench (Jenks et al. 1994). Perhaps severe wax and cuticle mutations in *A. thaliana* are lethal due to an inability to restrict water loss

leading to dehydration death under the normal growth conditions used for screening. *Thellungiella halophila* and *T. parvula* have a more elevated drought tolerance than *A. thaliana* (R. A. Bressan and M. A. Jenks, unpublished). Thus, like the drought-tolerant *S. bicolor bm2* mutant that survives severe mutation-induced wax and cuticle reduction, these more drought-tolerant *Thellungiella* species may, likewise, survive more severe alteration of their cuticular layers than *A. thaliana*. A mutagenesis approach could, thereby, reveal many additional and more physiologically significant wax mutants in *Thellungiella* than *Arabidopsis*. T-DNA-insertion mutagenized populations of *Thellungiella* have been developed within our research group, and these populations will be screened for this new class of wax mutants. The T-DNA tags should provide an efficient means of cloning newly identified wax genes (Jenks and Feldmann 1996). Finally, we should note that, in addition to *T. halophila* and *T. parvula*, many other species within the Brassicaceae that suggest adaptation to extreme environments have been found in native habitats (Rollins 1993). The usefulness of these biological materials for molecular genetic dissection has not been evaluated.

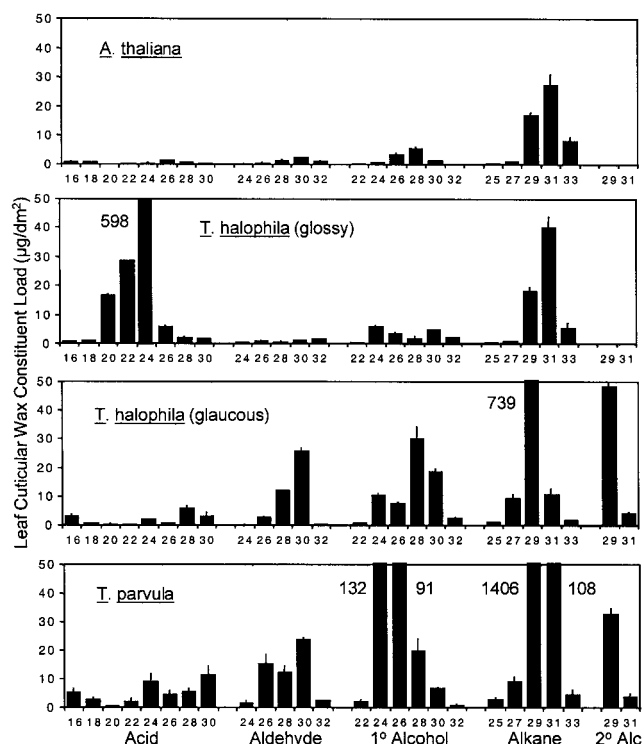


Fig. 3 Cuticular wax composition of leaves of *Arabidopsis thaliana* ecotype C24, *Thellungiella halophila*, and *Thellungiella parvula*. Values represent cuticular wax load in $\mu\text{g}/\text{dm}^2$ of leaf area \pm SE. Chemical classes and chain lengths are labeled on the horizontal axis. Where cuticular wax constituent amount was off the scale, a number designating actual value is next to the bar. $2^\circ\text{Alc} = 2^\circ$ Alcohol. Ketones were all 29 carbons, and ester chain lengths were not determined (see tables 1–3 for total quantities).

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