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CHANGES IN EPICUTICULAR WAXES ON WILDTYPE AND *ECERIFERUM* MUTANTS IN *ARABIDOPSIS* DURING DEVELOPMENT

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Key Word Index—Epicuticular wax; development; *Arabidopsis thaliana*; Cruciferae; *eceriferum* mutant; leaf.

Abstract—We analysed the leaf epicuticular wax chemical constituents on wildtype and *eceriferum* (*cer*; *cer*1, *cer*3 and *cer*4) mutants of *Arabidopsis thaliana* at 7, 15, 25 and 40 days after germination. Changes during development are described for the partitioning of epicuticular waxes into the five major chemical classes; free carboxylic fatty acids, aldehydes, alkanes, primary alcohols and wax esters. Changes are described for cumulative total epicuticular wax loads per leaf area, percentages of total constituents in each of the five major chemical classes and chain length distributions for the two major chemical classes: alkanes and primary alcohols. Stem epicuticular waxes on wildtype and *cer* mutants were analysed at 25 and 40 days and found to be similar.

INTRODUCTION

Leaves are the major photosynthetic organs in most crop plants and often the first organs to be damaged by environmental stress. Previous studies showed how epicuticular waxes on leaves influenced plant resistances to environmental stresses including drought [1], fungal pathogens [2] and phytophagous insects [3–6].

Mutants with altered epicuticular waxes were previously identified in mutagenized plant populations using visual screens [7–11]. The *eceriferum* (*cer*) mutants in *Arabidopsis*, for instance, were identified based on altered visible reflectance of their stem surfaces [10]. Scanning electron microscopy showed that these *cer* mutants also had alterations in their stem epicuticular wax crystallization patterns [10]. Later studies showed that these *cer* mutants had altered epicuticular wax chemical constituents on stems [12] and most had altered epicuticular wax constituents on leaves [13].

In the absence of cloned genes, the epicuticular wax constituents on 25-day-old leaves and stems of wildtype and *cer* mutants in *Arabidopsis* were used to propose functions for *cer* gene products in hypothetical *Arabidopsis* epicuticular wax biosynthetic pathways [12–14]. Previous studies examined epicuticular wax constituents on stems of wildtype and *cer* mutants 21 and 42 days after germination [14]; however, changes in epicuticular waxes on leaves of wildtype and *cer* mutants during development have not been reported.

In the present study, we analysed the leaf epicuticular wax constituents on wildtype and three *cer* mutants were analysed at 25 and 40 days. The *cer*1, *cer*3 and *cer*4 mutants generated by T-DNA insertion [14] were selected for analysis because of the high probability that their mutations represent null alleles. The *cer*2 mutant had wild-type epicuticular waxes on leaves at 25 days and is therefore not considered in this report [13]. All alleles of the other 17 known *cer* mutants were generated by EMS or physical mutagens [10] and so it is yet unclear whether these alleles represent nulls or leaky mutations.

Arabidopsis is quickly becoming a model plant system to study biological aspects of epicuticular waxes [10, 12–14]. Therefore, our characterization of epicuticular waxes on wildtype and *cer* mutants in *Arabidopsis* during development provides useful information for future research to elucidate developmental aspects of epicuticular wax physiology, genetics and biosynthesis. For instance, this information may be useful for examining the role of epicuticular wax development in protecting plants from various environmental stresses, for analysing *cer* gene function during development in transgenic *Arabidopsis*, and elucidating both developmental and genetic regulation of the epicuticular wax biosynthetic pathway. Moreover, understanding how *cer* genes affect epicuticular wax development is important for future studies to engineer crop epicuticular waxes genetically.

RESULTS

Epicuticular waxes on wildtype during development

Table 1. Cumulative total loads* of the seven major classes of epicuticular wax constituents per leaf area ($\mu g\ dm^{-2}$) of the wildtype (WS) and *cer* mutants in *Arabidopsis* at 15, 25 and 40 days after germination

	Days after germination†		
	15	25	40
WS	125.8±5.3	122.8±7.3	78.8±4.4
*cer*1	28.3±1.6	36.1±5.5	35.7±5.3
*cer*3	44.0±2.1	37.6±1.1	40.0±2.0
*cer*4	77.7±5.9	98.7±5.5	69.4±5.3

*Cumulative total loads ± standard error based on three separate replicate bulked samples.

†Day 7 cumulative total loads per leaf area were not determined.

germination. The total epicuticular wax load in $\mu g\ dm^{-2}$ of leaf area was similar at 15 and 25 days and then decreased 1.6-fold between 25 and 40 days (Table 1). Leaves had not fully expanded at day 15. Total epicuticular wax load per leaf area at day 7 was not determined because the very large number of unexpanded leaves needed for a reasonable epicuticular wax sample made leaf area determination impractical. As a percentage of cumulative total leaf epicuticular wax load (from Table 1), free fatty acids decreased 4.5-fold between 7 and 15 days and then remained low through further development (Table 2). The percentages of leaf aldehydes and esters decreased between 7 and 15 days, remained constant between 15 and 25 days and then increased 2.1- and 9-fold, respectively, between 25 and 40 days (Table 2). In contrast, leaf alkane and primary alcohol (1-alcohol) percentages increased 1.4- and 1.9-fold, respectively, between 7 and 15 days, decreased 1.2- and increased 2.6-fold, respectively, between 15 and 25 days, and decreased 1.1- and 1.5-fold, respectively, between 25 and 40 days (Table 2). The amounts of secondary alcohols (2-alcohols) and ketones were extremely small and changed little during development on wildtype and *cer* mutants. Therefore, these leaf epicuticular wax constituents are not discussed further.

On wildtype stems, the epicuticular wax changed little between 25 and 40 days (data not shown; see ref. [13]). In general, the percentage of total stem free fatty acids, 1-alcohols, 2-alcohols and ketones decreased slightly, whereas total alkanes, aldehydes and esters increased slightly. These changes were very small and therefore not presented here.

The chain length distributions for fatty acid and aldehyde constituents changed little during develop-

Table 2. Percentages* of the free fatty acids (FFA), aldehydes (ALD), alkanes (ALK), primary alcohols (1-ALC) and esters (EST) from the cumulative total of the seven major epicuticular wax classes on leaves of wildtype (WS) and *cer* mutants in *Arabidopsis* at 7, 15, 25 and 40 days after germination

	Days after germination			
	7	15	25	40
WS				
FFA	21.3±0.8	4.7±0.1	3.7±0.4	5.9±0.7
ALD	10.1±0.1	2.1±0.1	2.5±0.4	5.2±0.3
ALK	59.4±0.5	80.1±1.3	64.6±1.2	58.9±0.8
1-ALC	5.4±0.2	10.2±1.2	26.3±0.7	18.2±0.7
EST	2.9±0.6	1.9±0.5	1.0±0.5	9.0±0.5
*cer*1				
FFA	33.7±2.0	11.6±0.4	10.3±1.6	9.8±0.5
ALD	27.2±1.7	6.2±0.2	7.3±0.8	17.2±0.8
ALK	16.2±0.9	19.5±1.2	13.1±1.5	27.5±2.6
1-ALC	11.9±0.9	54.6±1.2	64.9±4.9	30.0±2.3
EST	10.9±1.9	7.3±1.5	3.5±1.7	14.8±1.0
*cer*3				
FFA	12.0±0.6	6.5±0.5	4.8±0.8	7.6±0.9
ALD	10.2±0.5	2.6±0.7	5.7±0.3	14.6±0.6
ALK	60.0±0.9	58.7±1.8	53.9±0.8	46.2±0.7
1-ALC	6.6±0.2	29.0±1.1	33.4±0.8	21.1±0.6
EST	10.4±0.6	1.5±0.2	0.9±0.3	9.7±0.4
*cer*4				
FFA	14.4±1.3	6.1±0.7	3.9±1.3	5.4±0.4
ALD	17.6±0.7	3.0±0.2	2.9±0.3	7.9±0.7
ALK	57.2±0.2	83.0±1.4	86.3±2.8	72.0±1.1
1-ALC	5.3±0.1	4.3±1.1	3.3±0.5	5.3±0.5
EST	5.3±0.4	2.1±0.4	2.9±0.8	8.8±0.1

*Percentage of cumulative total epicuticular wax constituents ± standard error based on three separate replicate bulked samples. Total secondary alcohols and ketones were also included in ment (data not shown; see ref. [13]). However, the two major epicuticular wax constituent classes on wildtype leaves, alkanes and 1-alcohols changed dramatically during development. Alkanes became increasingly longer from 7 to 25 days, but then decreased slightly in chain length distribution between 25 and 40 days (Fig. 1). In contrast, the leaf 1-alcohols became increasingly shorter from 7 to 25 days, but then increased in chain length distribution between 25 and 40 days (Fig. 2). The major 1-alcohol was the C_{30} constituent at 7 days, changing to the C_{28} 1-alcohol from 15 to 40 days (Fig. 2). Wax ester chain length distributions were not determined.

On the stems, the chain length distribution for alkanes changed little between 25 and 40 days (data not shown; see ref. [13]). The 1-alcohol chain length

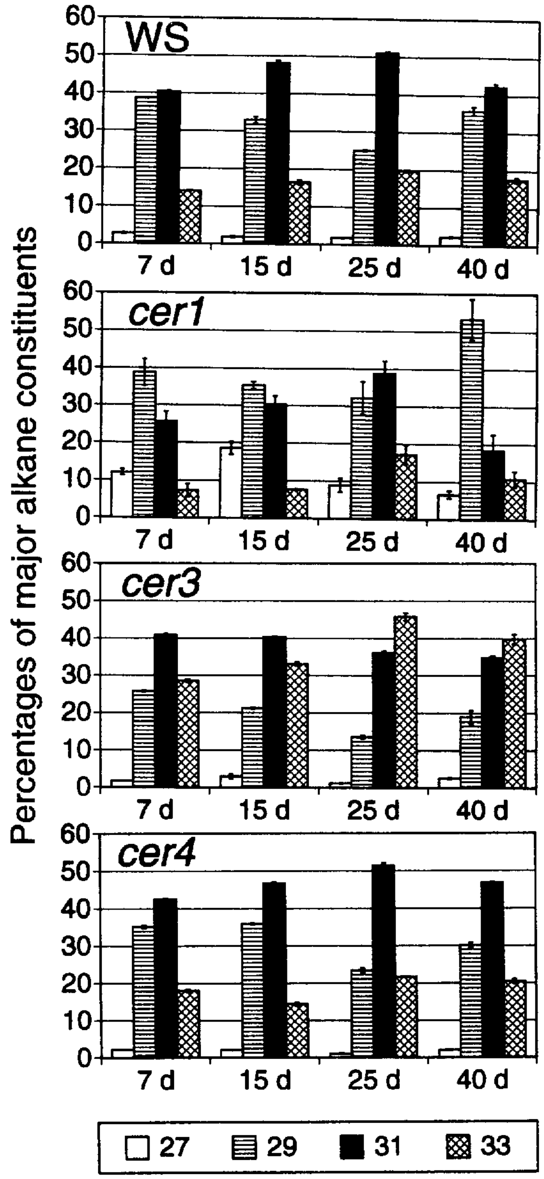

Fig. 1. Chain length distribution for major alkanes on wildtype (WS) and *cer* mutant leaves of *Arabidopsis* on days 7, 15, 25 and 40. Percentages were calculated based on the cumulative total of all leaf alkanes (including even and odd chain length alkanes not shown). Bar equals standard error based on three replicate bulked samples. Numbers in box represent alkane chain lengths. Absolute amounts of individual

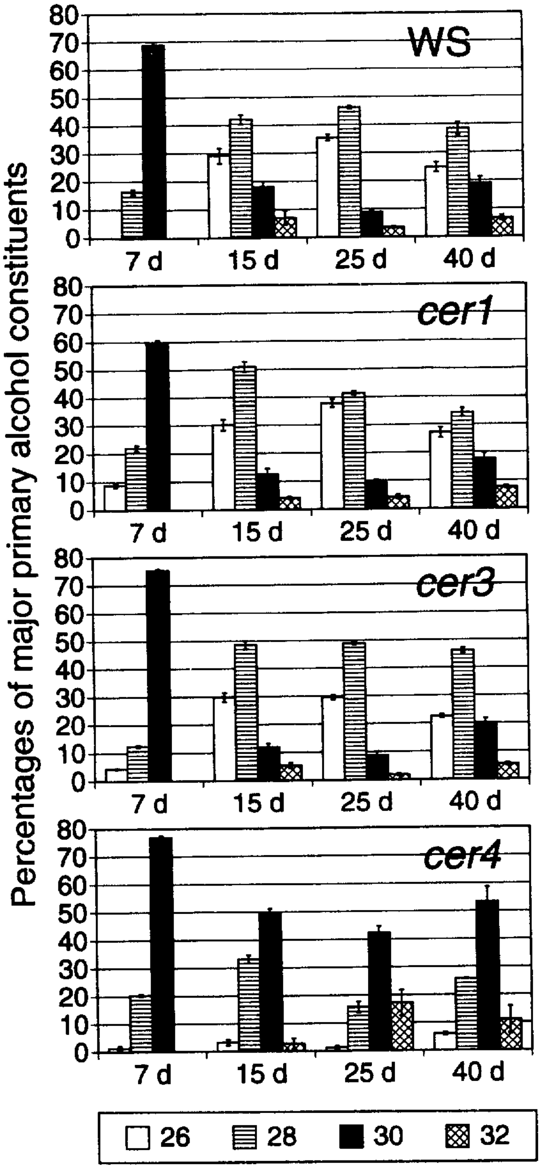

Fig. 2. Chain length distribution for major primary alcohols on wildtype (WS) and *cer* mutant leaves of *Arabidopsis* on days 7, 15, 25 and 40. Percentages were calculated based on the cumulative total of all leaf primary alcohols (including even and odd chain length primary alcohols not shown). Bar equals standard error based on three replicate bulked samples. Numbers in box represent primary alcohol chain lengths. Absolute amounts of individual primary alcohol chain lengths

distribution decreased just slightly between 25 and 40 days (data not shown).

cer1 *Epicuticular wax development*

The *cer*1, *cer*3 and *cer*4 mutants had leaf and stem morphologies during development that were visually identical to wildtype.

The total epicuticular wax load per *cer*1 leaf area changed little between 15 and 40 days (Table 1). While the absolute percentages were often different, the amounts of free acids, aldehydes and esters on *cer*1 leaves changed during development similarly to those on wildtype (Table 2). Unlike wildtype, the percentages of alkanes on the *cer*1 leaf changed little during development, except between 25 and 40 days where the percentage of *cer*1 leaf alkanes increased 2.1-fold (Table 2). The *cer*1 leaf 1-alcohol percentages increased 4.6-fold between 7 and 15 days, increased 1.2-fold between 15 and 25 days, and decreased 2.2-fold between 25 and 40 days (Table 2).

On the stems, the acids and esters increased, and the aldehydes, 1-alcohols, 2-alcohols and ketones decreased more on *cer*1 than on wildtype between 25 and 40 days. However, these changes were very small so data are not presented.

Similarly to wildtype, *cer*1 leaf alkanes became increasingly longer from 7 to 25 days and then decreased in chain length distribution between 25 and 40 days (Fig. 1). However, the alkane chain length distribution decreased more in *cer*1 than in wildtype between 25 and 40 days (Fig. 1). The major *cer*1 alkane was a C_{29} carbon at 7, 15 and 40 days and a C_{31} carbon at 25 days, whereas the major wild-type alkane was a C_{31} carbon throughout development (Fig. 1). The 1-alcohol development on *cer*1 leaves was similar to that on wildtype, becoming increasingly shorter from 7 to 25 days, but then increasing slightly in chain length distribution between 25 and 40 days (Fig. 2).

On the stems, the chain length distribution for *cer*1 alkanes was similar to that for wildtype at both 25 and 40 days (data not shown; see ref. [13]). The *cer*1 1-alcohols were mostly the C_{30} carbon chain whereas in the wildtype it was the C_{28} constituent (data not shown; see ref. [13]). In contrast to wildtype, 1-alcohol chain length distribution on *cer*1 stems increased just slightly between 25 and 40 days (data not shown).

cer3 *Epicuticular wax development*

The total epicuticular wax load per *cer*3 leaf area decreased 1.2-fold between 15 and 25 days and then changed little between 25 and 40 days (Table 1). While the absolute percentages were often different, the changes in free acids, aldehydes and esters on *cer*3 leaves during development were similar to those on wildtype (Table 2). However, in contrast to the wildtype, the percentage of *cer*3 leaf 1-alcohols increased 4.2-fold between 7 and 15 days, increased 1.2-fold between 15 and 25 days, and then decreased 1.6-fold between 25 and 40 days (Table 2). The total increase for *cer*3 1-alcohols was greater between 7 and 15 days than between 15 and 25 days as in wildtype (Table 2).

On stems, the percentage of each epicuticular wax constituent class changed little on *cer*3 between 25 and 40 days (data not shown; see ref. [13]).

Similarly to wildtype, *cer*3 alkanes became relatively longer from 7 to 25 days and then decreased slightly in chain length distribution between 25 and 40 days (Fig. 1). However, the major *cer*3 leaf alkane at 7 and 15 days was C_{31} and at 25 and 40 days C_{33}, whereas the major wildtype alkane was always C_{31} (Fig. 1). The *cer*3 1-alcohol development on leaves was similar to that of wildtype, becoming increasingly shorter from 7 to 25 days and then increasing slightly in chain length distribution between 25 and 40 days (Fig. 2).

On the stems, the chain length distributions for *cer*3 alkanes and 1-alcohols were longer than those for wildtype at 25 and 40 days (data not shown; see ref. [13]). The major 1-alcohol was C_{30} on *cer*3, but C_{28} on wildtype (data not shown). Otherwise, *cer*3 epicuticular wax constituents changed little on stems between 25 and 40 days (data not shown; see ref. [13]).

cer4 *Epicuticular wax development*

The total epicuticular wax load per *cer*4 leaf area increased 1.3-fold between 15 and 25 days and then decreased 1.4-fold between 25 and 40 days (Table 1). The relative changes in *cer*4 free acids, aldehydes and esters during these developmental time points were similar to those in wildtype (Table 2). The *cer*4 alkane percentages increased 1.5-fold between 7 and 15 days and decreased 1.2-fold between 25 and 40 days (Table 2). During development, the percentage of 1-alcohols on *cer*4 leaves changed little from the day 7 values where the *cer*4 and wildtype 1-alcohol percentages were similar (Table 2).

On the stems, the relative changes in the percentages of all epicuticular wax constituent classes were similar on *cer*4 and wildtype at 25 and 40 days (data not shown; see ref. [13]).

In contrast to wildtype, *cer*4 leaf alkane chain lengths changed little between 7 and 15 days, became longer between 15 and 25 days and then decreased in chain length distribution between 25 and 40 days (Fig. 1). The *cer*4 1-alcohol chain length distribution was similar to that of wildtype at day 7. However, the *cer*4 1-alcohol chain length did not change during development similarly to that of wildtype (Fig. 2).

On the stem, the chain length distribution for *cer*4 alkanes was similar to that for wildtype at 25 and 40 days (data not shown; see ref. [13]). Like *cer*1 stems, the major *cer*4 stem 1-alcohol was the C_{30} chain tribution decreased just slightly between 25 and 40 days (data not shown).

DISCUSSION

Previous studies have shown that changes in the total amounts and chain length distributions of leaf epicuticular wax constituents during leaf development can be highly variable and species dependent [5, 15–25]. On wild-type *Arabidopsis*, the percentages of free fatty acids and aldehydes on leaves decreased during leaf expansion and early maturation and then increased late in development. By comparison, leaf fatty acid percentages decreased continuously during leaf development in *Coffea arabica* [15], whereas the leaf acids on *Sorghum bicolor* [5], *Fagus sylvatica* [16] and *Tilia tomentosa* [17] increased continuously during leaf development. Similarly to *Arabidopsis*, leaf aldehydes increased early and decreased late in leaf development of *S. bicolor* [5] and *F. sylvatica* [16].

Leaf alkane percentages on *Arabidopsis*, *F. sylvatica* [16] and various citrus [21] increased early and then decreased late in leaf development similarly to the *Arabidopsis* 1-alcohols. In contrast, leaf alkane percentages on *T. tomentosa* [17], *C. arabica* [15], *Triticum aestivum* [20], *Triticum durum* [20] and *S. bicolor* [5] increased continuously during leaf development.

Arabidopsis leaf 1-alcohol percentages increased early and decreased late in leaf development similarly to those of *Quercus robur* [18], *Vaccinium ashei* Reade cv. Bluegem [19], *T. tomentosa* [17], *T. aestivum* [20], *T. durum* [20] and *S. bicolor* variety IS1082 [5]. By comparison, leaf 1-alcohols of *F. sylvatica* [16] decreased early and increased late in leaf development whereas leaf 1-alcohols of *C. arabica* [15] and *S. bicolor* var. CSH-1 [5] decreased continuously during leaf development. The development of leaf 1-alcohols on numerous citrus species were variable [21].

The major constituent class on wildtype *Arabidopsis* leaves, the alkanes, became increasingly longer up to 25 days and then decreased in chain length distribution between 25 and 40 days. By comparison, the alkane chain length distribution increased throughout development of *C. arabica* [15], *Nicotiana tobacum* [22], *T. tomentosa* [17], *S. bicolor* [5] and all but one of eight species of *Khaya* [23]. In contrast, the chain length distribution of alkanes on *T. aestivum* decreased continuously during development [20]. The decrease in *Arabidopsis* leaf alkane chain length distribution late in development was similar to that observed for *Solandra grandiflora* [24] and *Rhododendron fortunei* cv. Admiral Piet Hain [25].

The second largest constituent class on *Arabidopsis* leaves, the 1-alcohols, became increasingly shorter up to 25 days and then increased in chain length distribution between 25 and 40 days. Similarly, *T. aestivum* during early leaf development in *C. arabica* [15], *F. sylvatica* [16] and *S. bicolor* [5], while chain lengths of 1-alcohols on leaves of *T. tomentosa* changed little during development [17].

Further studies are needed to associate more closely the above mentioned changes in leaf epicuticular wax development with other physiological changes in leaf maturation.

Previous studies with *Arabidopsis* showed that stem waxes of wildtype, *cer*1, *cer*3 and *cer*4 changed dramatically between 21 and 42 days [14]. By comparison, our measurements showed little change in wildtype and *cer* mutant epicuticular waxes between 25 and 40 days. It is unclear whether developmental or environmental factors caused these differences. For a more detailed explanation of the stem epicuticular wax constituents and comparisons between leaf and stem epicuticular waxes on wildtype and *cer* mutants in *Arabidopsis* see ref. [13].

Previous studies have set forth possible functions for *CER*1, *CER*3 and *CER*4 gene products [12–14, 26]. McNevin *et al.* [14] proposed that the *CER*1 gene product may be involved in decarbonylation of alkanes since, in the *cer*1 mutant, alkanes were reduced by proportion more than any other epicuticular wax constituent class. However, because *cer*1 had reductions in nearly all epicuticular wax classes on leaves and stems, Jenks *et al.* [13] proposed that the *CER*1 gene product may be involved in a substrate transfer function. The *cer*3 mutant had reduced acids and aldehydes and increased chain length distributions of major epicuticular wax constituents and it was proposed that the *CER*3 gene product may affect release of products from a putative elongase compartment [13]. The *cer*4 mutant was deficient in 1-alcohols and it was suggested that the *CER*4 gene product may be associated with a reductase enzyme in 1-alcohol production [12–14].

The epicuticular wax constituents on *cer*1 leaves were altered at each time period of 7, 15, 25 and 40 days, suggesting that the *CER*1 gene product is required for epicuticular wax production throughout leaf development. The relative changes in epicuticular waxes on *cer*1 leaves were similar to those on wildtype except that the chain length distribution of alkanes decreased more between 25 and 40 days in *cer*1 than in wildtype. Since the 1-alcohol chain lengths on *cer*1 leaves did not decrease as alkanes on *cer*1 leaves between 25 and 40 days, it is unclear whether this *cer*1 alkane chain length reduction reflects reduced elongation processes or an inability to convert alkane precursors.

Like *cer*1, the relative changes in epicuticular waxes on *cer*3 leaves were similar to those on wildtype except that the alkane and 1-alcohol chain length distributions were longer for *cer*3 during each developmental period. Therefore, the *CER*3 gene product, like *CER*1, most likely plays an important role in epicuticular wax production during all stages of leaf development.

occurred in 1-alcohol development wherein the major 1-alcohol changed from the C_{30} constituent at 7 days to the C_{26} and C_{28} constituents from 15 to 40 days. This normal developmental transition in 1-alcohol production, however, did not occur in *cer*4 leaves. The *cer*4 1-alcohols remained essentially unchanged from the day-7 1-alcohols throughout development. These results suggest that 1-alcohol biosynthesis may be under different regulation early and late in leaf development.

Our characterization of epicuticular waxes on wild-type and three *cer* mutants in *Arabidopsis* during development provides useful information for future studies to elucidate developmental aspects of epicuticular wax physiology, genetics and biosynthesis.

EXPERIMENTAL

Plant material. We used the *A. thaliana* ecotype Wassilewskija along with the T-DNA-induced *cer* mutants from ref. [14]. Plants were grown in a controlled environment chamber at 22° and 16 hr photoperiod (*ca* 240 μmol m^{-2} sec^{-1}; 75–95% RH).

Epicuticular wax analysis. The hexane soluble surface lipids (designated epicuticular wax) were bulk extracted from adult leaves of 7-, 15-, 25- and 40-day-old plants and stems of 25- and 40-day-old plants by immersing tissues in hexane for 30 sec. Three separate replicate bulks of leaf and stem tissues for each line and each tissue age were used in calculating standard errors. Derivatization, GC, MS and quantification of identified epicuticular wax constituents, based on correction factors developed using one int. standard and calibration curves of 18 ext. standards, were as according to ref. [14].

Chemicals identified as epicuticular wax constituents on Arabidopsis. Tetradecanoic, hexadecanoic, octadecanoic, eicosanoic, docosanoic, tetracosanoic, hexacosanoic, octacosanoic and triacontanoic acids as C_{14}, C_{16}, C_{18}, C_{20}, C_{22}, C_{24}, C_{26}, C_{28} and C_{30} free fatty acids; *n*-pentacosane, *n*-heptacosane, *n*-nonacosane, *n*-hentriacosane and *n*-tritriacontane as C_{25}, C_{27}, C_{29}, C_{31} and C_{33} alkanes; 1-tetracosanol, 1-hexacosanol, 1-octacosanol, 1-triacontanol and 1-dotriacontanol as C_{24}, C_{26}, C_{28}, C_{30} and C_{32} primary alcohols; tetracosanal, hexacosanol, octacosanal and triacontanal as C_{24}, C_{26}, C_{28} and C_{30} aldehydes; 13- and 14-heptacosanol, 14- and 15-nonacosanol, 15- and 16-hentriacontanol as C_{27}, C_{29} and C_{31} secondary alcohols; and 15-nonacosanone as C_{29} ketone.

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REFERENCES

1. Jordan, W. R., Shouse, P. J., Blum, A., Miller, F. R. and Monk, R. C. (1984) *Crop Sci.* **24**, 1168.
2. Jenks, M. A., Joly, R. J., Peters, P. J., Rich, P. J., Axtell, J. D. and Ashworth, E. A. (1994) *Plant Physiol.* **105**, 1239.
3. Eigenbrode, S. D. and Espelie, K. E. (1995) *Annu. Rev. Entomol.* **40**, 117.
4. Atkin, D. S. J. and Hamilton, R. J. (1982) *J. Nat. Prod.* **45**, 694.
5. Atkin, D. S. J. and Hamilton, R. J. (1982) *J. Nat. Prod.* **45**, 697.
6. Edwards, P. B. (1982) *Aust. J. Ecol.* **7**, 347.
7. Macey, M. J. K. and Barber, H. N. (1970) *Phytochemistry* **9**, 5.
8. Macey, M. J. K. and Barber, H. N. (1970) *Phytochemistry* **9**, 13.
9. Lundqvist, U. and Lundqvist, A. (1988) *Hereditis* **108**, 1.
10. Koornneef, M., Hanhart, C. J. and Thiel F. (1989) *J. Hered.* **80**, 118.
11. Jenks, M. A., Rich, P. J., Peters, P. J., Axtell, J. D. and Ashworth, E. N. (1992) *Int. J. Plant. Sci.* **153**, 311.
12. Hannoufa, A., McNevin, J. P. and Lemieux, B. (1993) *Phytochemistry* **33**, 851.
13. Jenks, M. A., Tuttle, H. A., Eigenbrode, S. D. and Feldmann, K. A. (1995) *Plant Physiol.* **108**, 369.
14. McNevin, J. P., Woodward, W., Hannoufa, A., Feldmann, K. A. and Lemieux, B. (1993) *Genome* **36**, 610.
15. Stocker, H. and Wanner, H. (1975) *Phytochemistry* **14**, 1919.
16. Prasad, R. B. N. and Gulz, P. G. (1990) *Z. Naturforsch.* **45**, 805.
17. Gulz, P. G., Muller, E. and Prasad, R. B. N. (1991) *Phytochemistry* **30**, 769.
18. Gulz, P. G. and Muller, E. (1992) *Z. Naturforsch.* **47**, 800.
19. Freeman, B., Albrigo, L. G. and Biggs, R. H. (1979) *J. Am. Soc. Hort. Sci.* **104**, 398.
20. Tulloch, A. P. (1973) *Phytochemistry* **12**, 2225.
21. Freeman, B., Albrigo, L. G. and Biggs, R. H. (1979) *J. Am. Soc. Hort. Sci.* **104**, 801.
22. Chang, S. Y. and Grunwald, C. (1976) *Phytochemistry* **15**, 961.
23. Faboya, O. O. P., Okogun, J. I. and Goddard, D. R. (1980) *Phytochemistry* **19**, 2462.
24. Herbin, G. A. and Robins, P. A. (1969) *Phytochemistry* **8**, 1985.
25. Salasoo, I. (1983) *Phytochemistry* **22**, 461.
26. Lemieux, B., Koorneef, M. and Feldmann, K. A. (1994) in *Arabidopsis* (Meyerowitz, E. M. and Somerville, C. R., eds), pp. 1031. Cold Springs Harbor Laboratory Press, NY.