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Epicuticular Lipid Accumulation on the Leaves of *Lycopersicon pennellii* (Corr.) D'Arcy and *Lycopersicon esculentum* Mill.

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JON F. FOBES*, J. BRIAN MUDD, AND MARGERY P. F. MARSDEN
ARCO Plant Cell Research Institute, Dublin, California 94568

ABSTRACT

A comparison was made of epicuticular lipid accumulation on leaves of *Lycopersicon pennellii* and *Lycopersicon esculentum* Mill. cv VF36 from 5 to 16 weeks of age. Epicuticular lipids were a small fraction of the leaf dry weight (0.16%) of 5-week-old 'VF36', and increased to only 0.96% of the leaf dry weight after an additional 12 weeks of growth. In contrast, leaves from 5-week-old and 17-week-old *L. pennellii* plants had, respectively, 0.94% and 19.9% of their total dry weight in epicuticular lipid. Lipid accumulation was not affected by drought stress. Leaf position appears to influence the amount of lipid on the leaf surface. A glycolipid appears to be exuded from the terminal cell of glandular trichomes found on the leaves, stems, peduncles, calyxes, and fruits of *L. pennellii*.

Native populations of *Lycopersicon pennellii* inhabit the extremely dry, lower, west slopes of the Central Peruvian Andes, from El Horador (Depto. Piura) in northern Perú to Camana (Depto. Arequipa) in southern Perú (6, 9). Since *L. pennellii* is interfertile in controlled pollinations with the cultivated tomato, it is commonly grouped with other wild species of tomato (7). Its autecology is such that oftentimes the only other plants in close proximity to plants of *L. pennellii* are species of cacti and bromeliads. Yu (11) and Rick (8) have shown that among tomato species, *L. pennellii* leaves have a unique and special ability to withstand desiccation, and that *L. pennellii* distinguishes itself from other *Lycopersicon* species, except *L. chilense*, in its ability to withstand conditions of extreme drought. These authors and others (4) have suggested that *L. pennellii* might effectively serve as valuable germplasm for the introgression of drought resistance into the cultivated tomato.

Both native and greenhouse populations of *L. pennellii* have a sticky exudate covering leaves, stems, peduncles, calyxes, and fruits, such that insects and dust often cover the above-ground plant surfaces. It was hypothesized that this sticky exudate might aid in the plant's ability to withstand extreme drought, particularly those conditions which would lead to desiccation of the leaves.

Very little has been reported on the epicuticular lipids (defined as those lipids extracted by a brief exposure to organic solvent such as chloroform [3]) found on tomato leaves. Ermakov (2) has assayed the total leaf lipid composition of *L. pennellii* and *L. esculentum* cv Gruntovy Gribosvsky. He discovered that *L. pennellii* leaves have a very high lipid concentration, and that major components of the total lipid composition are polar lipids. A thorough analysis of the epicuticular lipids of developing tomato fruit has been made (1). In young fruit epicuticular lipid was 6.7% of the cuticle weight, whereas in mature fruit it was only 1.7%. Of the epicuticular lipids in the young fruit, 94% was hydrocarbon. This percentage steadily decreased as the fruit matured. At fruit maturity, the epicuticular lipid was 29% hydrocarbon, 27% sterols, and 43% flavonoids.

In this study, epicuticular lipid accumulation over 12 weeks of growth was compared in *L. pennellii* and a cultivated tomato, *L. esculentum* cv VF36. The effect of drought stress on accumulation of *L. pennellii* leaf epicuticular lipids was analyzed. The anatomy of the plant was studied to relate morphological structure(s) to lipid production.

MATERIALS AND METHODS

Plant Materials. Seeds of *L. pennellii* (Corr.) D'Arcy from Atico, Arequipa, Perú (LA 716, PI 246502), and *L. esculentum* Mill. cv VF36 (LA 490) were obtained from Dr. Charles M. Rick, Tomato Genetics Stock Center, University of California, Davis. Seeds of both species were germinated in an incubator and subsequently transplanted to pots in either a greenhouse maintained at 27°C day/15°C night temperature, or a growth chamber maintained at 30°C day/15°C night, 40% day/95% night RH, >800 E m^{-2} s^{-1}, 14 h photoperiod.

Three experiments were performed. The first experiment compared epicuticular lipid accumulation in well-watered (~90% pot capacity) *L. pennellii* and *L. esculentum* over a 12-week period. Lipid accumulation was first measured on greenhouse-grown plants that were 38 d old and continued weekly until plants were 115 d old. The experiment was arranged in a randomized, complete block design, with four blocks (blocked with respect to seedling size), 12 plants per plot. An analysis of variance was performed.

Experiment two compared epicuticular lipid production on leaves of *L. pennellii* in two irrigation treatments (well-watered, ~90% pot capacity; reduced irrigation, ~50% pot capacity). Drought treatment was begun by withholding irrigation from 45-d-old, growth chamber-grown plants. The first epicuticular lipid extraction was made on the day the drought treatment was initiated, and was subsequently performed biweekly until plants were 115 d old (five additional harvests). Plants were arranged in a randomized, complete block design, with three blocks (blocked with respect to seedling size), two plots per block, and 18 plants per plot. An analysis of variance was performed.

Experiment three compared epicuticular lipid accumulation over the course of 181 d on *L. pennellii* leaves harvested at three positions within the plant canopy—top, middle, and basal. Leaves from the top position were rapidly expanding, young leaves. Leaves from the middle position were recently fully expanded or nearly fully expanded; while leaves from the basal portion of the plant were fully expanded and nonsenescent. A 5-g leaflet sample of each position was harvested from replicate plants, and the weight of epicuticular lipid/sample determined. Samples from six plants were taken; means and standard deviations were determined, and statistical significance of differences tested.

Estimation of Lipid Accumulation. Approximately 5 g of leaflets were excised from each plant for estimation of lipid amount and lipid analysis. Leaflets were sampled across position on the plant (*i.e.* age of leaf). Fresh weight was measured and total leaf surface area calculated for each sample. Area of a single leaf surface was measured with a LICOR 3100 area meter.

The excised leaves were gently swirled in 200 ml chloroform for 60 s; the chloroform-lipid solution was then decanted and filtered through Whatman No. 2 paper. The filtrate was rotary evaporated under vacuum, and the purified extract removed from the collection vessel with ~10 ml of chloroform. The remaining leaf material was dried at 70°C in a forced-air oven, and dry weight subsequently measured. When the leaves were washed with three successive aliquots of chloroform, the first wash removed 95% of the recovered lipid and the second removed the remaining 5%. Lipid extraction from halved leaves showed the reproducibility of lipid recovery to be ± 7.5%.

A 100-μl aliquot was removed from each lipid sample and placed in a tared, aluminum weight boat. The sample was dried at 100°C and weighed. Final weight of lipid from each sample was calculated from the aliquot. The lipid per plant sample was expressed as mg lipid/g dry weight and μg lipid/cm^2 leaf area. The calculation of sample dry weight included the dry weight of leaves after lipid extraction (g) plus the mg of extracted epicuticular lipid.

Data were analyzed by a linear regression model, regression lines fitted, and correlation coefficients calculated.

Lipid Analysis. For fractionation of lipid samples the methods of Silva Fernandes *et al.* (10) were followed. The chloroform was removed by rotary evaporation and the residue redissolved in hexane. The sample was applied to a column (13 × 1.1 cm) containing 10 g alumina (grade II) and eluted with: (a) 75 ml hexane, to give a fraction of hydrocarbons, (b) 50 ml hexane:diethylether (50:50) to give a fraction enriched in sterols, and (c) 100 ml methanol, to give a fraction enriched in polar lipids. Flavonoids, which were particularly obvious in the samples from *L. pennellii*, were adsorbed irreversibly at the top of the alumina column and were not recovered. The column fractions were taken to dryness in a rotary evaporator and redissolved in 4.0 ml $CHCl_3$. Duplicate 100 μl aliquots were dried in tared aluminum weight boats and weight of lipid determined by difference.

Lipid composition was determined by TLC on silica gel G (Whatman) using the solvent mixture of hexane/diethylether/acetic acid, 40/10/1. Compounds were detected by: (a) exposure to iodine vapors, (b) spraying with orcinol spray to detect sugars, and (c) spraying with ferric chloride spray (0.05% $FeCl_3 \cdot 6H_2O$ in water/sulfuric acid/acetic acid, 90/0.5/5), to detect sterols (red color on heating the plate).

The major components of epicuticular lipids from VF36 were hydrocarbons and sterols. The major compounds of epicuticular lipids from *L. pennellii* were hydrocarbons, polar lipids, and flavonoids.

Scanning Electron Micrography. Fresh wet leaf material was examined and photographed very rapidly in a scanning electron microscope at 5 kv, in order to prevent specimen degradation.

RESULTS

The accumulation of epicuticular lipids on leaves of *L. pennellii* and VF36 plants is graphically compared in Figure 1. As can be readily observed. *L. pennellii* produces up to 20× the amount of epicuticular lipid by the completion of the experiment. The results were the same, whether expressed on either a dry weight or total leaf surface area basis. By the end of experiment 1, epicuticular lipids made up 19.9 ± 2.7% of the leaf dry weight. Accumulation of epicuticular lipid on the leaves of *L. pennellii* was linear, with an average weekly addition of 16.1 mg lipid/g dry weight of leaf.

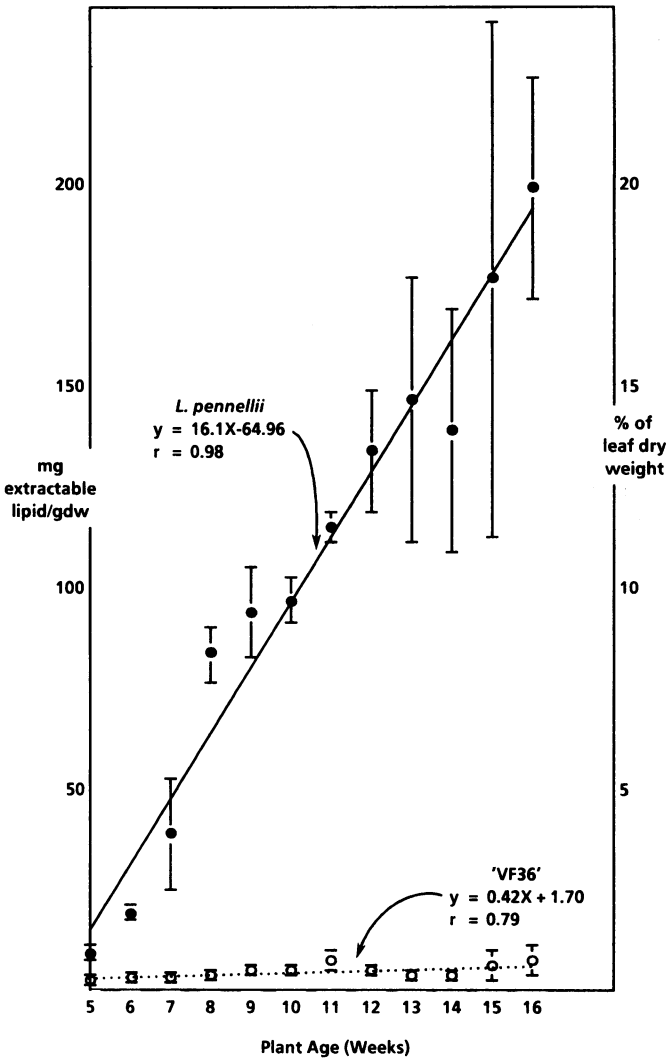

FIG. 1. Epicuticular lipid accumulation on leaves of *L. pennellii* and *L. esculentum* cv VF36.

Two samples from each treatment in the experiment described by Figure 1 were analyzed by chromatography. The results demonstrate that the major component of the epicuticular lipid of *L. pennellii* is a glycolipid; the determination of the structure will be reported in a separate publication (B. A. Burke, personal communication). The percentage of distribution of the lipids did not change significantly as a function of the age of the plants. In *L. pennellii* the glycolipid and hydrocarbon were 86 and 9%, respectively, of the epicuticular lipid. In VF36 the hydrocarbon and terpenoid were 82 and 18%, respectively, of the epicuticular lipid.

Figure 2a compares epicuticular lipid production by well-watered and drought-stressed *L. pennellii* plants. Two weeks after irrigation was reduced in the dry treatment, *L. pennellii* plants had a significantly higher (60% increase) lipid accumulation than well-watered plants. By the 4th week, however, drought-stressed and well-watered *L. pennellii* plants had accumulated a similar amount of epicuticular lipid (~80 mg/g dry weight). This trend continued to 8 weeks after drought stress was applied, at which time the well-watered *L. pennellii* plants had a significantly higher lipid content (80% increase). Lipid accumulation on leaves was reduced at week 4 in the drought stress treatment, while it continued to increase at the rate of 13.75 mg lipid/g dry weight in the well-watered treatment.

Drought-stressed *L. pennellii* plants did not significantly in-

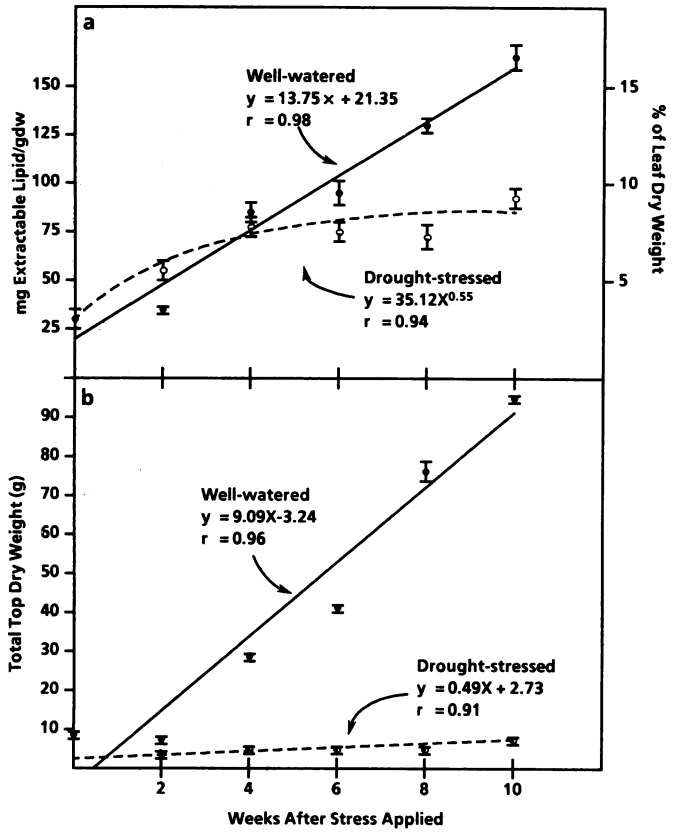

FIG. 2. Epicuticular lipid accumulation (a) and total top dry weight (b) on drought-stressed and unstressed *L. pennellii* leaves. Results of analysis of variance: (a) moisture levels, $F = 41.80$ ($P = 0.05$); harvests, $F = 63.72$ ($P = 0.001$); moisture × harvest, $F = 26.12$ ($P = 0.001$). $LSD_{0.05} = 16.17$, $LSD_{0.01} = 22.15$, $LSD_{0.001} = 30.19$. (b) Moisture levels, $F = 1354.13$ ($P = 0.001$); harvests, $F = 294.65$ ($P = 0.001$); moisture × harvest, $F = 245.89$ ($P = 0.001$). $LSD_{0.05} = 4.61$, $LSD_{0.01} = 6.31$, $LSD_{0.001} = 8.60$.

crease top dry weight after water was witheld (Fig. 2b). Therefore, in the first 4 weeks after the imposition of stress, leaves of *L. pennellii* plants accumulated a significantly higher concentration of epicuticular lipid, while not accumulating significant dry weight.

Lipid accumulation in well-watered *L. pennellii* plants was linear over the course of the second experiment. The weekly rate of accumulation was lower in this experiment than in the first experiment: 13.8 mg/g dry weight *versus* 16.1 mg/g dry weight.

Experiment 3 tested the effect of leaf position (ontogeny) on epicuticular-lipid accumulation over the course of 181 d. Top, middle, and basal leaves from unstressed *L. pennellii* plants were analyzed. The results (Table I) show similar levels for top and middle leaves. Basal leaves, however, contain significantly lower amounts of epicuticular lipid. By the conclusion of the experiment, basal leaves had, on average, only 55% of the lipid that had accumulated on top and middle leaves. Standardized areas (7.5 mm^2) of the adaxial and abaxial leaf surfaces of top, middle, and basal leaves were photographed and the trichomes in the area counted. For adaxial surfaces of top, middle, and basal leaves the mean counts (six replications) were 179 ± 16.8, 136 ± 4.6, and 33 ± 8.4, respectively. For the abaxial surfaces, the counts were 222 ± 22.9, 207 ± 11.1, and 42 ± 7.7, respectively.

L. pennellii has glandular trichomes (Fig. 3) that exude substances from the terminal cell of the trichome. These glandular trichomes are absent in *L. esculentum* cv VF36. When droplets are removed with a capillary pipet, they reform after 1 h. The droplets have been demonstrated by TLC and IR spectroscopy to contain glycolipid, but not hydrocarbon. The droplets can be stained with Sudan IV, indicative of their lipid content. We interpret Figure 3 to suggest that these glandular trichomes exude the glycolipid which is finally deposited on the leaf surface.

DISCUSSION

The epicuticular lipids, which in *L. pennellii* plants amount to 25% of their dry weight, have no obvious metabolic role in the plants. It is known that the sticky exudate produced by *L. pennellii* plants is primarily a glycolipid, containing both hydrophilic and hydrophobic moities. It therefore has properties that could aid the plant's need to retain water. The polarity of the lipid may act to reduce surface tension of adsorbed dew water. This may provide for the adsorption of a greater proportion of the condensed water by the leaves.

Georgieva and Achkova (5) observed that *L. pennellii* plants have special, glandular trichomes (their *type d* trichomes) on both upper and lower leaf surfaces. These trichomes possess a bulbous tip, and are also found on stem, fruit and peduncle surfaces. During much of the day, the leaf of *L. pennellii* plants glisten; droplets of the glycolipid have collected on the tips of the trichomes. The density of the trichomes, and of the droplets of glycolipid, is such that a thickened boundary layer of high RH might hypothetically be created, producing a reduced vapor pressure gradient for water loss.

Maximum lipid accumulation in *L. pennellii* cannot be attained by imposition of drought stress. The amount of accumulated lipid did not significantly increase past the 4th week of drought imposition. Those *L. pennellii* plants that were not stressed, accumulated epicuticular lipid at a linear rate throughout the course of experiment 2 (Fig. 2a).

Drought-stressed *L. pennellii* plants, however, did not significantly increase top dry weight after the imposition of stress (Fig. 2b). Research by Cohen *et al.* (personal communication) has shown that plants of *L. pennellii* respond to osmotic stress by greatly reducing top growth almost immediately following the imposition of stress. It is interesting to note that *L. pennellii* plants did accumulate highly significant amounts of epicuticular lipid in the first 2 weeks after imposition of the drought-stress treatment, and a significantly greater amount of lipid in the subsequent 2 weeks (Fig. 2a). *L. pennellii* plants accumulated an

Table I. *Effect of Leaf Position on Epicuticular Lipid Accumulation*

Leaf Position	Plant Age (d)							
	48		59		83		181	
	mg/g dry wt	*μg/cm²*	*mg/g dry wt*	*μg/cm²*	*mg/g dry wt*	*μg/cm²*	*mg/g dry wt*	*μg/cm²*
Top	28.32	53.63	67.91	143.21	79.29	375.25	255.41	569.24
Middle	26.33	69.50	52.39	143.07	77.10	459.00	287.28	747.25
Basal	17.48	49.18	47.11	146.01	59.96	436.31	155.90	489.35
$LSD_{.05}$	5.06	16.28	8.69	26.53	13.36	94.64	59.60	157.73
$LSD_{.01}$	7.03	22.60	12.19	37.20	18.48	130.87	82.42	218.92

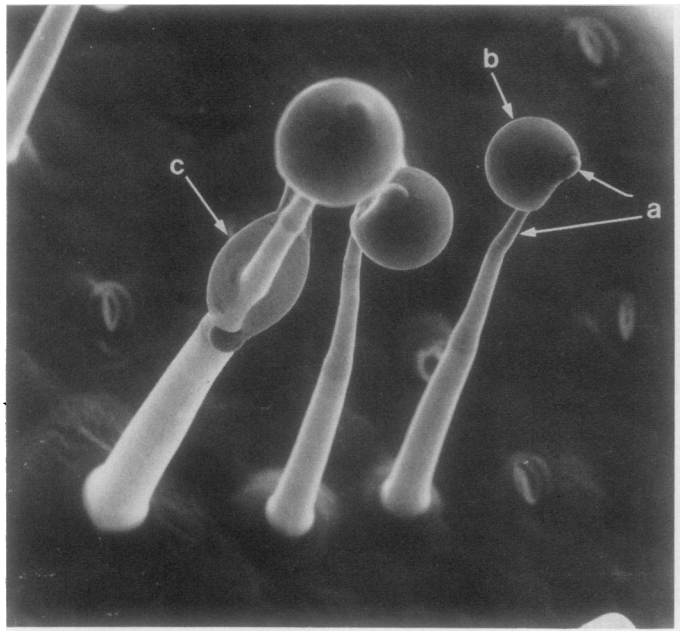

FIG. 3. Scanning electron micrograph of glandular hairs found on *L. pennellii* leaf surface. a, Terminal cell of glandular hair; b, exuded droplet of glucolipid; c, glucolipid flowing down surface of glandular hair.

additional 151% of epicuticular lipid in the 4 weeks after drought stress was applied. During this period, the plant was also responding to the stress treatment by drastically reducing dry matter accumulation.

Bottom leaves of *L. pennellii* plants had significantly less accumulated epicuticular lipid as that of the top and middle leaves (Table I). This difference can be accounted for by the density of trichomes on the leaves: bottom leaves have significantly fewer trichomes than either the top or middle leaves.

Epicuticular lipids make up a remarkably large fraction of the total dry weight of *L. pennellii* leaves. The ecological role of these compounds is not known, yet can be hypothesized to be part of the functional mechanism(s) leading to drought resistance in native habitats. The chemical identification of the compounds, the study of their biosynthesis, and the actual role of these compounds in or on the plant has yet to be fully investigated. *L. pennellii* is interfertile with the cultivated tomato, so it provides a logical means by which to study the genetic control of epicuticular lipid biosynthesis in segregating generations. If indeed the lipids play an important role in the expression of drought resistance in the wild tomato species, *L. pennellii* might serve as a source to introgress that character into the cultivated tomato.

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