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# Natural Variability in Acyl Moieties of Sugar Esters Produced by Certain Tobacco and Other Solanaceae Species

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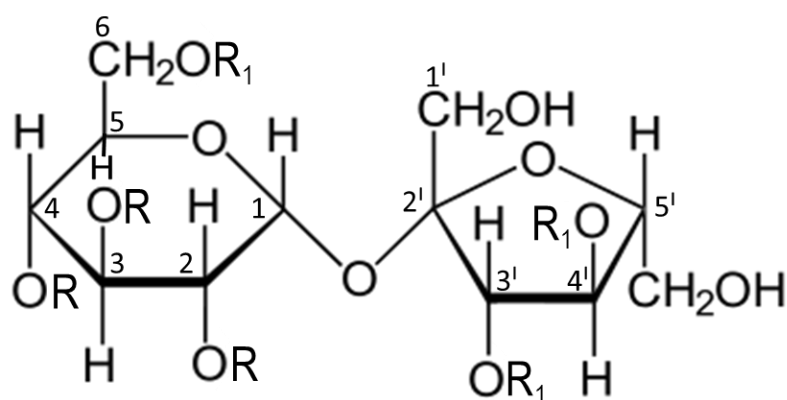
Natural variability in acyl moieties of sugar esters produced by certain tobacco and other Solanaceae species

Antoaneta B.M. Kroumova\*, Dave Zaitlin, George J. Wagner

Twenty different acyl groups were identified and quantified in 45 accessions, species, and cultivars. Three unusual acids were found in a few *Nicotiana* species: octanoate, 8-methyl nonanoate, and decanoate.

**(A)** General sucrose ester structure for tobacco cultivars, *N. obtusifolia*, *N. alata*, and *N. noctiflora*. Three acyl groups are esterified to carbons 2, 3 and 4 of the glucose moiety. R can be any acyl group with three to eight carbons in length. R<sub>1</sub> can be H or acetyl group. **(B)** The structures of straight, iso- and anteiso- branched carbon chains.

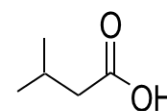
### A. General sucrose ester structure



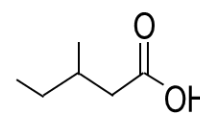
R<sub>1</sub> = H or acetyl group

R = branched- or straight-chain C<sub>3</sub> – C<sub>8</sub> acyl groups

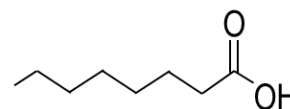
### B. Examples of carbon chain structure for R:



iso-chain: 3-methyl butyrate (C<sub>5</sub>)



anteiso-chain: 3-methyl valerate (C<sub>6</sub>)



straight-chain: octanoate (C<sub>8</sub>)

**Highlights:**

- Acyl moieties of Solanaceae sugar esters vary in carbon length and isomer structure.
- Octanoic acid is synthesized by *Nicotiana alata* and *N. × sanderae*.
- *N. hesperis* is exceptional in producing 8-methyl nonanoate and decanoate.
- Tobacco acyl groups have fewer carbons than those from non-tobacco species.
- Straight-chain acids are rare in sugar esters from *Nicotiana* species.

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Natural variability in acyl moieties of sugar esters produced by certain tobacco and other Solanaceae species

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4 **Abstract:**  
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6 A unique feature of glandular trichomes of plants in the botanical family *Solanaceae* is that they produce  
7 sugar esters (SE), chemicals that have been shown to possess insecticidal, antifungal, and antibacterial  
8 properties. Sugar esters of tobacco (*Nicotiana tabacum*) provide pest resistance, and are important flavor  
9 precursors in oriental tobacco cultivars. Acyl moieties of SEs in *Nicotiana* spp., petunia, and tomato are  
10 shown to vary with respect to carbon length and isomer structure (2 to 12 carbon chain length; *anteiso*-,  
11 *iso*-, and straight-chain). Sugar esters and their acyl groups could serve as a model to explore the basis of  
12 phenotypic diversity and adaptation to natural and agricultural environments. However, information on the  
13 diversity of acyl composition among species, cultivars, and accessions is lacking. Herein, described is the  
14 analysis of SE acyl groups found in 21 accessions of *N. obtusifolia* (desert tobacco), six of *N. occidentalis*  
15 subsp. *hesperis*, three of *N. alata*, two of *N. occidentalis*, four modern tobacco cultivars, five petunia  
16 hybrids, and one accession each of a primitive potato (*Solanum berthaultii*) and tomato (*Solanum pennellii*).  
17 A total of 20 different acyl groups was observed that were represented differently among cultivars, species,  
18 and accessions. In *Nicotiana* species, acetate and *iso*- and *anteiso*-branched acids prevailed. Straight-chain  
19 groups (2 to 8 carbons) were prominent in petunias, while octanoic acid was prominent in *N. alata* and *N.*  
20 × *sanderæ*. Two unexpected acyl groups, 8-methyl nonanoate and decanoate were found in *N.*  
21 *occidentalis* subsp. *hesperis*. Longer chain groups were found in the petunia, tomato, and potato species  
22 studied.  
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**Keywords:**

*Nicotiana* species

*Petunia hybrida*

Solanaceae

Glandular trichomes

Natural variability

Sugar esters

Acyl groups



## Introduction:

Plant trichomes are epidermal protuberances found on the surfaces of many plants. They play an important role in plant defense by providing protection against fungi, insects, herbivores, bacteria, and mechanical damage (Wagner et al., 2004). Trichomes vary in shape, function, and density. Some plants have both glandular and non-glandular types of trichomes, while others have strictly non-glandular ones (Wagner, 1991; Glass et al., 2012; Tissier, 2012). Species in the *Solanaceae* are well known for the presence of exudates produced by glandular trichomes (GTs) that contribute substantially to the diversity of plant surface chemistry (Wagner, 1999). Trichome exudates from this family can contain terpenoids, phenylpropanoids, flavonoid aglycones, and sugar esters (SEs) that are species- and cultivar-specific (Shepherd and Wagner, 2012). Sugar esters are produced by the GTs of this family. These natural products are non-volatile and are deposited on the leaf surface, comprising as much as 25% of the trichome exudate in some *Nicotiana tabacum* (*N. tabacum*) cultivars (Kelsey et al., 1984). In *Solanum pennellii* and some tobacco cultivars, they can accumulate to levels up to 20% and 17%, respectively, of leaf dry weight (Fobes et al., 1985, Wagner et al., 2004). Sugar esters have antiovipositional, antifeedant and toxic properties, and insecticidal and miticidal activity stronger than the insecticidal soap (Chortyk et al., 1997; Puterka et al., 2003). They also have inhibitory effects on tobacco seed germination and seedling growth (Peterson et al., 1998). Sugar esters have great potential as a new class of pesticides that degrade rapidly and are not known to be harmful to wildlife and other non-target organisms (<http://www.csrees.usda.gov/funding/sbir/success/avachem.pdf>). SEs also have potential as antibiotics, for cosmetic applications, and as food-grade sucrose esters (Chortyk et al., 1993; Hill and Rhode, 1999). Sugar esters consist of acyl moieties esterified to the hydroxyl groups of sugars (sucrose or glucose). Acyl groups vary in their length (from C<sub>2</sub> to C<sub>12</sub>) and backbone structure (*iso*-branched, *anteiso*-branched and straight carbon chain) (Severson et al., 1985; Burk et al., 1987). The purpose of this diversity is not clear. The pathway for biosynthesis of SEs is known for several species. In *N. benthamiana*, *N. gossei* and *N.*

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*glutinosa*, the alpha ketoacid elongation ( $\alpha$ -KAE) pathway produces medium-length, branched chain acids.

In petunia, this pathway is also involved in the synthesis of straight-chain acids, while in *Datura* and *S.*

*pennellii*, straight-chain acids appear to come from the fatty acid synthesis (FAS) pathway (Kroumova and

Wagner, 2003; Walters and Steffens 1990). The short length, branched-chain groups methyl propionate

(MePro)(4), and 2- and 3-methyl butyrate (2-MeBut(6), and 3-MeBut(7)) come from the pathways for

synthesis of the essential amino acids valine, *iso*-leucine and leucine and are products of the first step of

their degradations, and MePro(4) is the precursor for 8-methyl nonanoate (8-MeNon) (18) in *S. pennellii*

(Walters and Steffens 1990) (see Fig. 1).

Trichome-produced secondary metabolites in general, and sugar esters in particular, are attractive targets

for investigation for the purpose of breeding, engineering of herbivore resistance, resistance against *P.*

*infestans*, pesticide application, and selection for aroma and flavor (Holley et al. 1987; Glas et al. 2012;

Leffingwell, 1999). However, more knowledge about the natural production and function of SEs, and the

causes of natural variability observed among species and cultivars, needs to be gained.

From what is known about the extent of biodiversity of SE types (chemical diversity and regiospecificity of

acyl groups) found in the Solanaceae, it is clear that many individual SE structures containing short- and

medium-chain fatty acids are present in these plants. In the genus *Nicotiana*, structural and/or total acyl

identification of sugar esters has been reported for several *Nicotiana* species and *N. tabacum* varieties

(Kaburaki et al., 1969; Kallianos, 1976; Severson et al. 1984, 1985a, 1985b; Arrendale et al., 1990;

Matsuzaki et al., 1989, 1992; Ohya et al., 1994; Jia et al., 2013).

The purpose of the study was to analyze acyl group composition and acyl abundance diversity among some

species in the genus *Nicotiana* and among some cultivars of *N. tabacum*. For accuracy, the abundance of

acyl groups was expressed in molarity. Variability was found among species, cultivars, accessions, and even

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between different organs (leaves vs stems) of the same species. This appears to be another level of variability in addition to the structural diversity.

Herein described is the analysis of total sugar ester acyl composition in 21 accessions of *N. obtusifolia*, six of *N. occidentalis* subsp. *hesperis*, two of *N. occidentalis*, three each of *N. alata*, *N. × sanderae*, and *N. noctiflora*, four cultivars of modern tobacco, five petunia hybrids, and single accessions of the wild potato *S. berthaultii* and the wild tomato *S. pennellii*.

**2. Results**

2.1. Calculation of molarity of acyl groups on the basis of the peak areas

GC chromatograms obtained for butyl esters of SE acyl groups from *Nicotiana* species of interest contain numerous peaks, reflecting the diversity of the acyl groups synthesized. The peak areas were measured to assess the abundance of different compounds. In earlier publications related to acyl sugar composition, the authors sometimes postulated that the GC response of all compounds is the same (Chortyk et al., 1997, Peterson, 1998, and Kim et al., 2012). However, from previous liquid chromatography results it is known that there is a difference between responses, depending on chain length and degree of unsaturation (Kroumova and Wagner, 1995). Free acid GC response was also reported not to be representative of component levels (Severson et al., 1985).

In the current study, separation and identification of 8-12 acyl groups (as their butyl esters) per chromatogram was attained, and there abundances in moles were determined using linear regression curves created with standard acids. Each peak from the chromatogram, created with standard acids, was plotted as peak area versus moles of acid (Fig. S1). Each point on the graph was an average of five independent runs using five separate standard solutions. For each acyl butyl ester, a simple linear

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4 regression curve best fit the relationships of peak area to the molarity. The correlations were very strong,  
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6 showing  $R^2 > 0.995$  for all linear equations (Fig. S1).  
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9 As shown in Fig. S1, the absolute slope values of the lines vary greatly which means that when the  
10 molarity is equal, higher slope will give higher peak area or higher response, and vice versa. The least  
11 responsive was acetate (Ac) (**1**) (slope=1.5256), and the most responsive was laurate (Laur) (**20**) (slope =  
12 19.666). On the basis of these very different responses, it was not possible to compare Ac (**1**) and Laur (**20**)  
13 or, for example 5- and 6- methyl heptanoate (5-MeHept (**15**) and 6-MeHept (**16**)) and MePro (**4**). If area %  
14 (% of the highest) of the compounds in a chromatogram is calculated as a peak area, the wrong major peak  
15 could be assigned. This is especially true for Ac (**1**), as it shows modest peak abundance but very often  
16 happens to be a prominent product or the most abundant one. The only two acyl butyrates with very  
17 similar responses were 2-MeBut (**6**) and 5-methyl hexanoate (5-MeHex) (**13**). The results here are thus in  
18 agreement with earlier publications, where slopes of the calibration curves of free or butylated  
19 fatty acids having one to eight carbons were proportional to the carbon length (Severson et al.,  
20 1985a, Tangreman and Nagengast, 1996, Zhao et al., 2006, and Salanitro and Muirhead, 1975). For  
21  $C_2$  to  $C_5$  short chain fatty acids, the GC-MS data are supported by the paper of van Eijk et al.  
22 (2009), who used LC-MS to analyze free short chain fatty acids in blood samples.  
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43 Linear regression equations were used to convert the peak areas into moles and to then  
44 calculate the relative abundance of acyl groups in individual plants. The highest molarity was  
45 taken as 100% and the rest of the acids were expressed as a relative percentage of this.  
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## 53 2.2. Desert tobaccos from North America

54 Desert tobacco *N. obtusifolia* (formerly known as *N. trigonophylla* and *N. palmerii*), is native to arid  
55 regions of the southwestern United States (California, Arizona, New Mexico, and Texas) and northern  
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Mexico. It was established earlier that accession PI 555573 is a prolific producer of SEs (6.2 g of pure sugar ester per 50 plants) that are active against aphids and whiteflies (Jackson et al., 1998). This species can supply sugar esters for use as a biorational insecticide (relatively non-toxic to people and with few environmental side-effects) (Jackson et al., 1998). Sucrose esters of *N. obtusifolia* are predominant over glucose esters (Matsuzaki et al., 1989).

Acyl group analysis was done on the leaves of 21 accessions, in order to find the extent of variability or predictability among these accessions. Of particular interest were acyl groups, as they are very different from those synthesized by commercial tobacco cultivars, i.e. in order to eventually study the enzymes leading to this diversity. As seen from the data in Table 1, the acyl groups in all accessions consisted of Ac (**1**), MePro (**4**), But (**5**), 2-MeBut (**6**), 3-MeBut (**7**), 3- and 4 methyl valerate (3-MeVal (**9**) and 4-MeVal (**10**)), 4-methyl hexanoate (4-MeHex (**12**), 5-MeHex (**13**), and 5-MeHept (**15**).

The abundance of all these groups varied among the accessions, with the most variable being 5-MeHept (**15**) (from 0.5% of 2-MeBut (**6**) in ZS05-04, to 100% in DZ02-014, DZ02-019 and ZH04-8). High variability was observed with respect to the major acyl groups. Ac (**1**) was the most abundant in three accessions and 2-MeBut (**6**) - in five accessions. 4-MeHex (**12**) was the most abundant in all BBNP accessions and in accession 555573. 5-MeHex (**13**) was the major compound in four accessions, and 5-MeHept (**15**) – in three accessions.

Considering the total acyl composition of the individual accessions, the *anteiso*-groups (2-MeBut **6**, 3-meVa **9**, 4-MeHex **12**, and 5-MeHept **15**) were prevalent. In ZH04-8 and 555573, these comprise 80.5% and 80.2%, respectively, of the total *anteiso*- and *iso*- groups. A strong presence of the medium length, branched-chain acids 4- and 5-MeHex (**12**) and (**13**), and 5-MeHept (**15**) was very characteristic for all the *N. obtusifolia* accessions. In all accessions, 6-MeHept (**16**) was either absent, or when present did not exceed 15% (15% of acetate (**1**) in DZ02-22), and hexanoate (Hex) (**11**) was detected in minute amounts in five accessions.

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4 2.3. Tobacco cultivars, ornamental varieties and wild species  
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6 Four cultivars of *N. tabacum* L: Tobacco introduction 1068 (TI 1068), line KDH 960; burley cultivars  
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8 Kentucky 14 (KY14) and TI 165; ornamental species *N. alata*, the ornamental hybrid *N. × sanderae* (*N.*  
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10 *alata* x *N. forgetiana*), were analyzed, together with the wild species *N. sylvestris*, and *N. noctiflora*. TI 1068  
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12 is a pale-yellow-type tobacco that synthesizes the highest amount of SE compared to the other cultivars  
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14 studied, and has been estimated to accumulate 56.6  $\mu\text{g cm}^{-2}$  sugar esters (Nielsen, 1989). TI 1068 has a high  
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16 density of secreting GTs - 54.5/ $\text{mm}^2$  versus, for example, 23/ $\text{mm}^2$  in KY14, (Johnson et al 1988). The  
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18 germplasm line KDH-960 was created as a source of genetic variation for use in studies of trichome  
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20 exudates and as a biosynthetic source for  $\alpha$ - and  $\beta$ -cembratriene-diols ( $\alpha$ - and  $\beta$ -CBT-diols) (Nielsen, 1989).  
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22 KY14 is an old commercial burley tobacco cultivar in which sugar esters reach 2 mg/g tissue at full maturity  
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24 (Leffingwell, 1999). TI 165 is an accession from Japan known as 'Hatano' and is classified as a cigar filler type  
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26 (Chaplin et al., 1982). TI 165 was previously reported to yield 9.9  $\mu\text{g}/\text{cm}^2$  SE (Johnson, 1984). *N. sylvestris*  
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28 is considered to be the maternal donor of *N. tabacum*, an allotetraploid species which was formed  
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30 ~200,000 years ago by interspecific hybridization with *N. tomentosiformis* (Leitch et al., 1998). *N. alata* is a  
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32 species with low drought tolerance and with large white, fragrant flowers, which is native in southern Brazil  
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34 to northeastern Argentina.  
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42 Diversity in acyl composition was observed between the tobacco cultivars. Ac (**1**) was a major acid in KY14  
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44 and KDH960, while 3-MeVal (**9**) was a predominant acyl group in TI 1068 and TI 165. Carbon chain length  
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46 also varied. The longest acids were 4-MeVal (**10**) in TI 1068, octanoate (Oct **17**) in KY 14, 5-MeHex (**13**) in TI  
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48 165, and 3-MeBut (**7**) in KDH 960.  
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53 KDH 960 has the smallest variety of acyl groups– only Ac (**1**) (100%), 3-MeBut (**7**) (66%), 2-MeBut (**6**)  
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55 (39%), MePro (**4**) (32%), and But (**5**) (19%) (of Ac **1**, respectively). KDH 960 has at least 2-3 times fewer acyl  
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57 groups than does TI 1068 (not shown). This cultivar was derived from parental lines TI 1068 and TI 1406  
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4 (Nielsen, 1989). The reduced amount of SEs compared to TI 1068 could be due to that fact that the TI 1406  
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6 parent lacks secreting GTs (Johnson et al., 1988).  
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9 The abundance of acyl groups in KY14 was found to be lower than in TI 1068. Exudate was collected from  
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11 40 cm<sup>2</sup> (vs 6.12 cm<sup>2</sup> for T.I. 1068) in order to be able to have enough material for reliable identification of  
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13 GC peaks. In KY14, Ac (**1**) is the most abundant group, followed by 2-MeBut (**6**), 3-MeBut (**7**), MePro (**4**), But  
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15 (**5**), 4-MeHex (**12**), 5-MeHex (**13**) and Oct (**17**) (Table 2). Among the tobacco cultivars, Oct (**17**) was  
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17 observed only in KY14.  
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21 The major acyl groups found in TI 165- 3-MeVal (**9**), Ac (**1**), 3-MeBut (**7**), and 2-MeBut (**6**), were identified  
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23 earlier (Severson et al., 1985a). In current work, however, the minor acids 4-MeVal (**10**) and 5-MeHex (**13**)  
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25 were present, while Val (**8**), Hex (**11**), Hept (**14**) and MeHept (**15** and **16**) were absent, in contrast to  
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27 Severson et al (1985a). No Val (**8**) or Hept (**14**) was found in any of the tobacco cultivars studied.  
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31 Sugar esters are in very low abundance in *N. sylvestris*, and they were not identified previously in this  
32  
33 species (Matsuzaki et al., 1989). Exudate was thus collected from 1,225 cm<sup>2</sup> of leaf material and it was  
34  
35 possible to recover five acyl groups which were in the order of abundance: Ac (**1**), 4-MeHex (**12**), But (**5**), 5-  
36  
37 MeHex (**13**) 2-MeBut (**6**), 3-MeVal (**9**) 3-MeBut (**7**), and 4-MeVal (**10**). The acyl moieties of SEs in *N.*  
38  
39 *sylvestris* are reported here for the first time. Due to the minuscule amounts of SEs recovered from *N.*  
40  
41 *sylvestris*, it can be assumed that the SEs in *N. tabacum* did not originate from this parent, but rather came  
42  
43 from *N. tomentosiformis* (Matsuzaki, 1989).  
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47  
48 *N. alata* produces a mix of glucose and sucrose esters (Matsuzaki et al., 1989). Comparison of the acyl  
49  
50 groups from three accessions of this species was carried out, as these accessions have unique compositions  
51  
52 consisting of Ac (**1**), 2-MeBut (**6**), Oct (**17**), 3-MeBut (**7**), MePro (**4**), But (**5**), 3-MeVal (**9**), and Hex (**11**). Oct  
53  
54 (**17**) and Hex (**11**) were not previously reported for *N. alata* (Severson et al., 1991). *N. alata* PI555474 has  
55  
56 lower abundance of Pro (**2**), 2-MeBut (**6**), and 3-MeVal (**9**), and higher abundance of MePro (**4**), Hex (**11**)  
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58 and Oct (**17**) comparatively to the other two accessions.  
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4 *N. alata* is one of the parents of the man-made hybrid *N. × sanderae* (*N. alata* × *N. forgetiana*). The type  
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6 of acids found in *N. × sanderae* is similar to that in *N. alata* (Table 2). The most abundant acyl groups are  
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8 Oct (**17**)(100%), 2-MeBut (**6**)(87% of **17**), and Ac (**1**) (62% of **17**).  
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10  
11 In *N. noctiflora*, the major acyl groups are 5-MeHex (**13**)(100%), followed by 3-MeBut (**7**) and Ac (**1**) (65  
12  
13 and 35% of 5-MeHex (**13**), respectively). The unique feature of the acyl composition is the predominance  
14  
15 of *iso*-branched-chain groups (84% *iso*-groups vs. 16% *anteiso* groups of the total branched-chain groups)  
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#### 18 2.4. Native *Nicotiana* species from Australia

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21 *N. occidentalis subsp. hesperis* is a species native to coastal Western Australia and offshore islands (Chase  
22  
23 et al., 2003). It produces mainly glucose esters and traces of sucrose esters in the leaves (Matsuzaki et al.,  
24  
25 1989). Six accessions were analyzed (Table 3). Leave trichomes did not produce abundant exudate and  
26  
27 material was collected from large leaf areas (426 cm<sup>2</sup>) to examine acyl groups (in contrast, exudate from  
28  
29 6.1 cm<sup>2</sup> of TI 1068 was sufficient for reliable peak identification analysis). In addition to leaf SE composition  
30  
31 stems and sepals were examined, as they possess abundant exudate-secreting trichomes. However, great  
32  
33 variability was observed in acyl abundance among accessions and less variability between organs of the  
34  
35 same accession (Table 3). Ac (**1**) was the major acid in stems of accessions 303765, 303763 and 303764,  
36  
37 and in the leaves of accessions 303765 and 303764, whereby 3-MeVal (**9**) was the major group detected in  
38  
39 303663, 303767, and 303721, while MePro (**4**) was major group isolated from leaves of 303763. The  
40  
41 abundance of 3-MeVal (**9**) varied among accessions from 6.4% (of Ac **1** in stems of 303765) to 100% in  
42  
43 303663, 303767 and 303721, and 4-MeVal (**10**) varied from 5% (of 3-MeVal **9**) in 303767 to ~75% (of Ac **1**)  
44  
45 in leaves of 303765 and stems of 303763. In accession 303721, 5-MeHex (**13**) was 77% in leaves and 3% in  
46  
47 stems (of 3-MeVal **9**). Very surprisingly, in four of the accessions (303663, 303763, 303765, and 303764)  
48  
49 synthesis of 8-MeNon (**18**) and decanoate (Dec **19**) was detected. To our knowledge, these have not been  
50  
51 previously reported to occur in the genus *Nicotiana*. Dramatic changes were found in the abundance of  
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53 these two acids during the growth and development of accession PI 303663: in 48 d-old plants, 8meNon  
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4 **(18)** was the major peak, and Dec **(19)** was abundant (not shown), while in 80d-old plants 8meNon **(18)**  
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6 accounted for 10% of 3-MeVal **(9)**, and Dec **(19)** was present in trace amounts (Table 3). Stem SEs from the  
7  
8 same accession had only trace amounts of both acids. The composition of all accessions was a mix of *iso*-  
9  
10 and *anteiso*-branched chain acids, but the *iso*-branched acids prevailed in 303765 and 303763, while  
11  
12 *anteiso*-branched prevailed in 303663, 303767, and 303764.  
13  
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15  
16 *N. occidentalis* is a short-lived, annual herb native to Australia. Sugar esters are represented only by  
17  
18 sucrose esters (Matsuzaki et al., 1989). Accession PI 555541 showed a very similar acyl group profile in  
19  
20 leaves and stems, and produced mainly 2-MeBut **(6)**(100%), Ac **(1)** (81%) and 3-MeVal **(9)** (63%) (of 2-  
21  
22 MeBut **6**). No carbon chain >6 carbons was detected. Accession PI 555689, in contrast, produces 7- and 8-  
23  
24 carbon chains, and variable amounts of acyl groups in the leaves, stems, and sepals. Ac **(1)** was the  
25  
26 predominant acyl group in all organs. 3-MeVal **(9)** was 66% (of Ac **1**) in the leaves, but was not found in  
27  
28 sepals. 5-MeHex **(13)** was 10% in leaves and 76% (of Ac **1**) in sepals. The major acids in leaves were Ac **(1)**  
29  
30 and 3-MeVal **(9)**, in stems - Ac **(1)** and 5-MeHept **(15)**, and in sepals - Ac **(1)**, 5-MeHex **(13)**, and 5-MeHept  
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32 **(15)**.  
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### 36 37 2.5. Petunia, *S. berthaultii*, and *S. pennellii*. 38

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40 Trichome glands in petunia produce a complex mixture of glucose and sucrose esters, and the  
41  
42 concentrations are known to vary among cultivars (Kays et al., 1994). Petunias SEs were shown to control  
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44 white fly infestation (Kays et al., 1994). Comparison of the five petunia hybrids analyzed here with data  
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46 from several cultivars reported in the literature (Son et al., 1994; Kays et al., 1994) resulted in great  
47  
48 variability being observed among the hybrids regarding abundance and occurrence of fatty acids (Table 4).  
49  
50 Malonate **(3)** predominated (100%) in the hybrid cultivars ‘Madness Red Picotee’, ‘Purple Tidal Wave’ and  
51  
52 ‘Pink Tidal Wave’ and was second in abundance in ‘Dreams Red Picotee’ (99% of 2-MeBut **6**) and ‘Purple  
53  
54 Tidal Wave’ (83% of Hex **11**). 2-MeBut **(6)** was the major group in ‘Dreams Red Picotee’. Hex **(11)** was the  
55  
56 dominant group in ‘Silver Tidal Wave’. 4-MeHex **(12)** was present only in ‘Pink’ and ‘Purple Tidal Wave’  
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4 (27% and 3% of malonate **3**, respectively). Hex (**11**) varied between the cultivars from 6% (of malonate **3**)  
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6  
7 in 'Purple Tidal Wave', to 100% in 'Silver Tidal Wave'. Hept (**14**) abundance varied from 14% (of 2-MeBut  
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9 **6**, in 'Dreams Red Picotee) to 36% (of Hex **11** in 'Silver Tidal Wave'), and Oct (**17**) varied from 4% (of Hex **11**)  
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11 in 'Silver Tidal Wave', to 32% (of malonate **3**) in 'Purple Tidal Wave'. Petunias SEs have a unique acyl  
12  
13 composition because of the predominance of straight carbon chains from C<sub>2</sub> to C<sub>8</sub> (Table 4). Malonate (**3**)  
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15 was not found in the other species studied here, which is in agreement with the previous reports  
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17 (Kays et al.,1994, Ohya et al., 1996, Chortyk et al, 1997) that malonate-containing SE is an unique  
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19 property of Petunia.  
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25 The wild Bolivian potato species *S. berthaultii* Hawkes synthesizes sucrose esters only in the type B tall  
26  
27 glandular trichomes (Neal et al., 1990). In *S. berthaultii* PI#498097, it was found that MePro (**4**) is a major  
28  
29 constituent, followed by Dec (**19**) and 2-MeBut (**6**)(81% and 77% of MePro **4**, respectively). Pro (**2**), But (**5**),  
30  
31 and Oct (**17**) were in minor abundance. In *S. berthaultii* accession PI #473340, King et al. (1986) also  
32  
33 observed MePro (**4**) and Dec (**19**) as major acids, but the minor ones were 8-MeNon (**18**) and 2-MeBut (**6**).  
34  
35 In accession PI# 473331, the major acids were MePro (**4**), 2-MeBut (**6**), and 8-MeNon (**18**), while Dec (**19**)  
36  
37 and Laur (**20**) were minor (Neal et al. 1990).  
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41 Glucose esters from the wild tomato *S. pennellii* have been studied extensively. The research here  
42  
43 confirmed the acyl composition previously reported in the literature (Walter and Steffens, 1990), in which  
44  
45 MePro (**4**) is the major acid, followed by 8-MeNon (**18**), 2-MeBut (**6**), and Dec (**19**). Of all species studied, *S.*  
46  
47 *pennellii* produces the longest carbon chains -C<sub>12</sub> (Laur **20**), and C<sub>11</sub> (9-methyl decanoate<sup>+</sup>) (Table 4).  
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### 54 **3. Discussion**

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56 A total of 20 acyl moieties were found in the sugar esters isolated from the different species, cultivars,  
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58 accessions, and hybrids of *Nicotiana*, *Petunia*, and *Solanum*. In all *N. obtusifolia* accessions, the strong  
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presence of 4- and 5-MeHex (**12**) and (**13**), and 5-MeHept (**15**) was very characteristic. 5-MeHex (**12**) was also abundant in *N. noctiflora* (Table 2) (Cutler et al., 1992). These medium length branched-chain groups were found in lesser amounts in *N. occidentalis*, *N. occidentalis* subsp. *hesperis* (Table 3), and in several other *Nicotiana* species (Severson et al, 1991, Ohia et al., 1994, Kroumova et al., 1993, Kroumova and Wagner, 2003, and Slocombe et al., 2008). Most of the species studied here have both *iso*-and *anteiso* branched carbon chains. However, *N. noctiflora* synthesizes mainly *iso*-branched-chain groups (Table 2), while *N. obtusifolia* accessions ZH04-8 and PI# 555573 have predominantly *anteiso* groups (Table 1). The *anteiso*- branched-chain acids 3-MeVal (**9**), 4-MeHex (**12**), and 5-MeHept (**15**) are the elongation products of 2-oxo-3-MeVal, while the *iso*-branched-chain acids 4-MeVal (**10**), 5-MeHex (**13**), and 6-MeHept (**15**) are elongation products of 2-oxo-4-MeVal. 2-oxo-3-MeVal and 2-oxo-4-MeVal are the intermediates for synthesis of Leu and Ile.

The acyl group composition of the SEs from KDH960 is unusual compared to the other studied species here because the carbon chains have no more than five carbons. Similarly, only short-chain groups were detected in some accessions of the wild tomato relative *S. habrochaites*. These accessions were found to lack specific acylsugar acyltransferases (ASAT3-like) that can add long acyl chains to the glucose moiety of SEs (Schilmiller et al., 2015).

The presence of Oct (**17**) in high levels, and of Hex (**11**), in leaves of *N. alata* and *N. × sanderae* was observed here for the first time. Severson et al. (1991) found only short-chain acids ( $\leq C_5$ ) in *N. alata*. Straight-chain acids were reported to be minor components in tobacco (Kaburaki et al., 1969 and Kallianos, 1976). Straight-chain acyl groups are rare in the genus *Nicotiana*. However, they have been found in other Solanaceae species such as *Petunia*, tomato, wild potato, and *Datura*. *Petunia* SEs are distinctive in having numerous straight-chain acids with two to eight carbons (**2**, **5**, **8**, **11**, **14**, and **17**) including malonate (**3**), while wild potato and tomato species have longer straight-chain acids (Dec **19** and Laur **20**) (Table 4). In *Petunia*, these acyl groups are synthesized via the  $\alpha$ -KAE pathway, while in *S. pennellii* and *Datura metel*,

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the FAS pathway is involved (Walter and Steffens, 1990, and Kroumova and Wagner, 2003). The origin of the straight-chain acids But (**5**), Hex (**11**), and Oct (**17**) in *N. hesperis*, *N. allata*, and *N. × sanderae* has not been studied, but either the  $\alpha$ -KAE (as in petunia) or FAS pathway (as in *Datura* or *S. pennellii*) are possibilities.

*N. occidentalis* subsp. *hesperis* accessions are unique in producing 8-MeNon (**18**) and Dec (**19**) (Table 3). This is the first report of their occurrence in any species in the genus *Nicotiana*. In *S. pennellii*, these groups are produced via FAS, which suggests the same possibility for *N. occidentalis* subsp. *hesperis*.

In our study, malonate (**3**) was found to be a major acid in several petunia hybrid cultivars. Earlier publications have shown the presence of malonate, but not in abundance (Ohya et al., 1996, and Chortyk et al., 1997). The peak abundance of malonate (**3**) in the GC chromatograms was not prominent, but due to the low malonate (**3**) response factor (as expected for three carbons) it is reflected in high nmol abundance. Why malonate (**3**) is found only in petunia SEs is unclear at present. *Petunia* species synthesize a lot of anthocyanins, which requires malonyl-CoA (Winkel-Shirley, 2001). A fraction of the activated malonate (malonyl-CoA) can be redirected to form esters with sugars.

There are several important questions that relate to the natural diversity: (1) why do some species synthesize only branched- or straight-chain acids, or both; (2) why in some species do *iso*- or *anteiso*- chains prevail; (3) why in tobacco are the acyl chain lengths reduced compared to other *Nicotiana* species; and (4) why does the desert tobacco (*N. obtusifolia*) synthesize seven- and eight-carbon branched-chain acids in abundance? To answer these questions, an exploration of the basis of phenotypic diversity and adaptation to natural and agricultural environments is required. Given the complexity of the acyl groups found in this study, much more may be learned about the possibilities for manipulating them so as to use SEs as biorationals to provide increased insect and bacterial resistance. Natural or modified sucrose esters may also prove useful as natural products in the food industry.

#### 4. Concluding Remarks

Significant variability in carbon chain length and branching was observed among sugar ester acyl groups in the Solanaceae species examined in this study, despite the fact that the longest chain length found was 12 carbons. The precise quantification of individual groups, using GC/MS and standard calibration curves, showed also variation in acyl abundance. Similar diversity among major acyl groups has been reported previously in other *Nicotiana* species (Severson et al., 1991; Chortyk et al., 1993). The major acyl groups in non-tobacco species can be short or medium-chain, while in tobacco they are only short-chain, with a prevalence of 3-MeVal (**9**) or 2- and 3-MeBut (**6** and **7**). The pathway for acyl group synthesis was confirmed earlier for several *Nicotiana* and other species in the Solanaceae (Kroumova and Wagner, 2003; Walters and Steffens 1990), but pathway(s) for straight-chain (**11**, **17**, and **19**) synthesis in *N. alata*, *N. sanderae*, *N. occidentalis* subsp. *hesperis*, and 8-MeNon (**18**) synthesis in *N. occidentalis* subsp. *hesperis* remain to be determined. Revealing the factors underlying interspecies diversity in sugar ester acyl groups will contribute to our ability to improve some plant properties related to defense against abiotic and biotic stress, and to improve the synthetic capacity for industrial applications.

#### 5. Experimental

##### 5.1. General experimental procedure

##### 5.1.1. Preparation of standard curves of butyl esters

The thirteen most-often observed acids in tobacco sugar esters were selected to make standard curves of peak area response to the molarity of the acid. Acetic acid (**1**) was from Thermo Fisher Scientific Inc.; MePro (**4**), But (**5**), 2- and 3-MeBut (**6**) and (**7**), Val (**8**), 3-MeVal (**9**) and Oct (**17**) were obtained from Sigma-Aldrich Co. LLC.; 4- and 5-MeHex (**13**), 5- and 6-MeHept (**15**) and (**16**) were from Narchem™ Corporation, USA, and 8-MeNon (**18**) was from Chemica Alta LTD, Canada. The standards were converted to butyl esters in order to increase detection sensitivity by gas chromatography. To determine the retention time and mass spectrum of individual butyl esters on the chromatogram, they were prepared separately. An equal

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4 volume (2  $\mu\text{L}$ ) of each standard acid was added to of 1 M KOH (500  $\mu\text{L}$ ) in MeOH : H<sub>2</sub>O (80:20, v/v) (to  
5  
6 mimic the SE saponification method, see below) and allowed to stand overnight. Samples were dried  
7  
8 under a N<sub>2</sub> stream at 40°C; each residue was then dissolved in *n*-BuOH (0.5 mL) containing 3 drops of conc.  
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10 H<sub>2</sub>SO<sub>4</sub> and the mixture was heated at 110°C for 1 hour. The level of meniscus of the reaction mix was  
11  
12 monitored before and after the reaction to ensure no change in volume. After cooling, hexane (2 mL) was  
13  
14 added to the reaction mix and the organic phase was partitioned with H<sub>2</sub>O until the H<sub>2</sub>O reached a pH of  
15  
16 7.0 by litmus paper (modified method of Severson et al., 1985). The hexane phase, containing the butyl  
17  
18 esters, was adjusted to 1 mL and an aliquot injected into the GC. To make calibration curves for the butyl  
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20 esters, a mixture of 19 authentic standard acids (2  $\mu\text{L}$  each) was derivatized as above followed by  
21  
22 preparation of six dilution stocks. The procedure was repeated four times to create four additional stocks.  
23  
24 The standard curve for malonate (**3**) was prepared separately to monitor for its degradation to Ac (**1**) during  
25  
26 the derivatization. GC/MS chromatograms showed no evidence for malonate (**3**) degradation. The butyl  
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28 esters were separated and analyzed by GC-MS (HP6890 GC equipped with HP5973 MS and automatic  
29  
30 sampler injector), using a 30.0 m Agilent capillary column (19091J-413) with 0.25  $\mu\text{m}$  film thickness and  
31  
32 0.25 mm diameter. The GC-MS approach was applied in order to resolve *iso*- and *anteiso*- isomers of  
33  
34 branched acyl chains. He was used as carrier gas at 1.8 mL/min constant flow compensation, and the  
35  
36 injection temperature was 250°C. The oven program for running the butyl ester derivatives was as follows:  
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38 initial temperature 90°C for 3.0 min; rate - 3°C/min until 160°C, hold 2.0 min; rate - 15°C/min until 250°C  
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40 with a hold for 5.0 min. Total run time was 39.33 min. The butyl esters on the chromatogram were  
41  
42 identified according to their retention times and MS profile.

### 3.1.2. Preparation of butyl ester derivatives of SE acyl groups and GC-MS analysis

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54 Collection of leaf exudates was performed essentially as described by Severson et al. (1985). Abundance  
55  
56 of SEs varied greatly among species. For plants with abundant sugar esters, two leaf discs (= 6.12 cm<sup>2</sup>) were  
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58 washed for 1 min with CH<sub>3</sub>CN, with these washes vacuum-evaporated to dryness, dissolved in CHCl<sub>3</sub> (1 mL),  
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transferred to 1.5 mL GC vials and dried. Where SEs were scarce (e.g. *N. sylvestris*), in order to obtain sufficient amount for analysis, whole leaves were washed with CH<sub>3</sub>CN for 1 min, then traced on uniform paper, and the image was cut out and weighed to determine the leaf area in cm<sup>2</sup>. The CH<sub>3</sub>CN washes were vacuum-evaporated to dryness and dissolved in CHCl<sub>3</sub> (10 mL). Polar impurities were removed by double partitioning with H<sub>2</sub>O. CHCl<sub>3</sub> fractions were evaporated to dryness in GC vials (1.5 mL). Further saponification, derivatization, GC-MS analysis and acyl identification were done as described in 3.1.1.

3.2. Plant seed sources, germination, and plant growth

Seeds of the desert tobacco, *N. obtusifolia* accessions DZ02-12 to -22, ZH04-08, ZS05-04, and ZS05-05 were collected by D. Zaitlin. Accessions DZ02-12 to -22 were from southern Arizona; ZH04-08 was from Starr County, TX; ZS05-04 was from the Anza-Borrego Desert State Park, San Diego County, CA; ZS05-05 was collected along highway S2, just west of the Anza-Borrego Desert State Park. BBNP-8 to -12 are collections from Big Bend National Park in West Texas, and were the gift of Mr. Joe Sirotnak, BBNP botanist, in 2002. Accessions PI# 555543 and PI# 555573 were originally obtained from the USDA Tobacco Collection, and reportedly originated from Arizona and Mexico, respectively (Heist et al. 2004).

*N. tabacum* varieties TI 1068, TI 165, KY14, and accessions of *N. sylvestris*, and *N. noctiflora* were obtained from the KTRDC seed collection. *N. alata* accessions TW 7 (PI# 42334), TW 8 (PI# 555474), and TW 9 (PI# 555473) were originally received from the Germplasm Resources Information Network (GRIN) and are maintained in the KTRDC seed collection. *N. × sanderae* accessions were purchased from Plant World Seeds ([www.plant-world-seeds.com](http://www.plant-world-seeds.com)). *N. occidentalis* subsp. *hesperis* accessions AusTRCF 303663, 303763, 303721, 303765, 303767, and 303764 were obtained from the Australian Tropical Crops and Forages Germplasm Centre, Queensland, Australia. *N. occidentalis* TW 91 accessions PI#555541 and PI#555689 were of Australian origin, but were received from GRIN.

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4 Petunia 'Madness Red Picotee' and Petunia 'Dreams Red Picotee' were purchased from Ball Seeds  
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6 (www.ballhort.com; US patent 5,659,623). Petunia 'Silver Tidal Wave' and 'Pink Tidal Wave' were  
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8 purchased from Park Seed, Inc. *Solanum berthaultii*, (PI# 498097) and *S. pennellii* (PI# 246502) were  
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10 obtained from GRIN.  
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14 For all species and accessions, seeds were sown on Pro-mix medium (Premier Horticulture Inc., Canada) in  
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16 6-inch pots and covered with plastic until germination occurred in a growth chamber at 22.8°C with a 16/8h  
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18 light/dark regime and 23% relative humidity. After germination, the plastic was removed and plants were  
19  
20 allowed to grow under the same conditions. The seeds of *N. hesperis*, with the exception of PI# 303663,  
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22 failed to germinate on soil, so they were sterilized with EtOH for 1 min, followed by 20% NaOCl for 15 min,  
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24 and grown on MS medium (Murashige and Skoog, 1962) in petri dishes. About ten days later, the seed  
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26 testa was scratched with a sterile needle under microscope and returned to *in vitro* conditions to allow  
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28 moisture and nutrition penetration. One month later seedlings were transferred to soil in pots and grown  
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30 under fluorescent lighting.  
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38  
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46  
47 Genebank for supplying seeds of *N. occidentalis* and *N. occidentalis* var. *Hesperis*. We thank Dr. Socrates  
48  
49 Canete (KTRDC, University of Kentucky) for the helpful discussion regarding GC-MS detection sensitivity.  
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### 56 **Appendix A. Supplementary data**

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58 Supplementary Fig. S1.  
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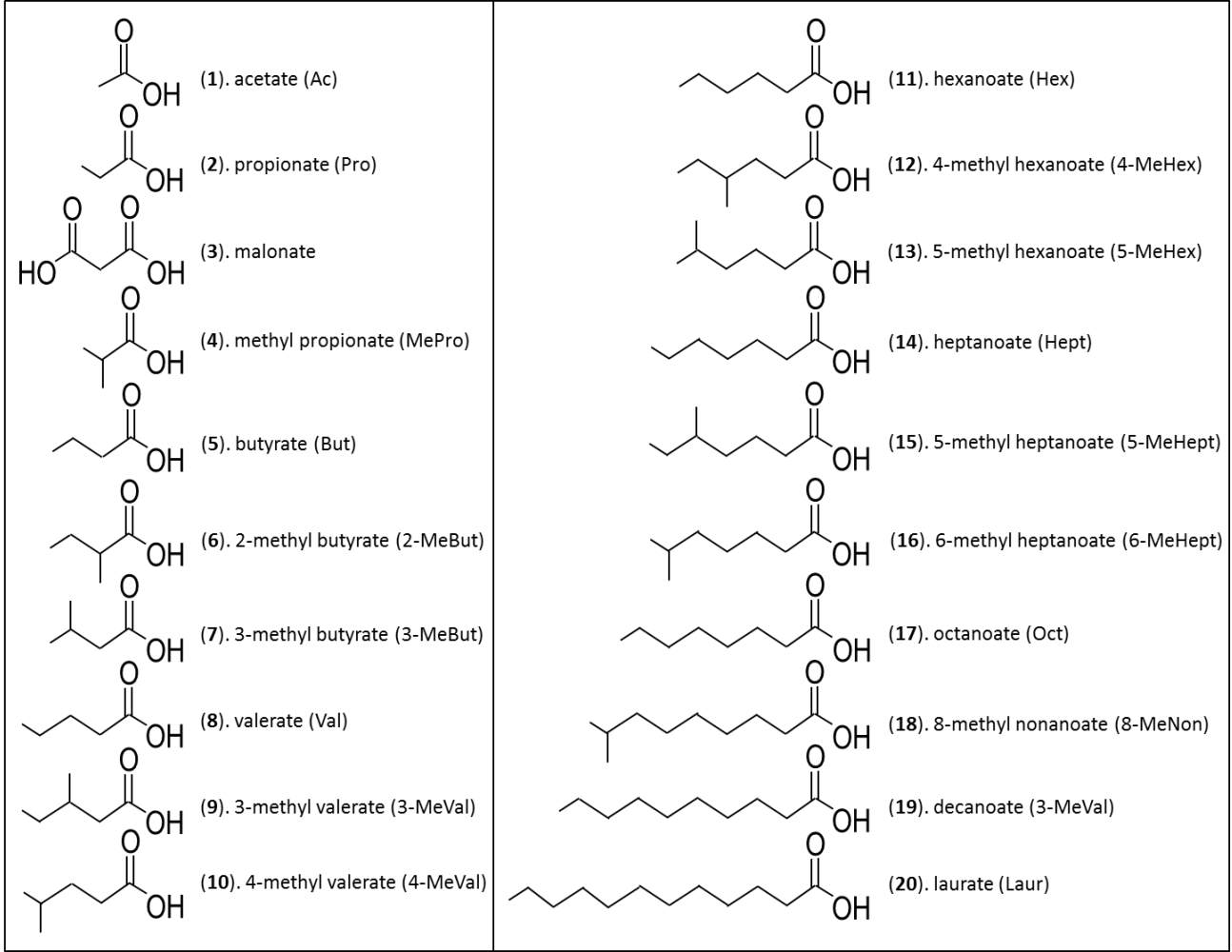
**Figure captions:**

**Figure 1.** Structure of all acyl groups detected.

A designated number and an abbreviation for each group are given in parenthesis.

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**Fig. 1**



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**Table 1.** Relative abundance of acyl groups in the accessions of *Nicotiana obtusifolia*.

Acyl group	DZ02-012	DZ02-013	DZ02-014	DZ02-015	DZ02-016	DZ02-017	DZ02-018	DZ02-019	DZ02-020	DZ02-021	DZ02-022
	mol % of highest										
Ac(1)	75.5	68.8	72.0	19.1	94.5	45.5	<u>100</u>	4.7	87.4	<u>100</u>	<u>100</u>
MePro(4)	24.3	27.4	21.5	10.3	37.1	29.1	22.3	7.0	24.7	27.2	38.0
But(5)	8.4	9.2	14.6	3.3	6.1	7.0	11.6	-	7.0	7.5	9.6
2-MeBut(6)	77.1	69.1	56.7	63.8	78.5	<u>100.0</u>	86.1	42.8	<u>100</u>	93.2	66.8.0
3-MeBut(7)	7.0	7.7	6.4	4.4	9.1	14.0	11.2	4.8	5.4	10.9	12.9
3-MeVal(9)	10.3	13.0	15.4	5.1	9.9	9.7	14.1	10.0	5.9	8.7	16.6
4-MeVal(10)	9.6	9.7	8.7	10.2	9.0	8.9	9.0	13.7	6.7	9.8	6.6
Hex(11)	2.4	<sup>b</sup>	-	-	-	-	-	-	-	-	-
4-MeHex(12)	70.7	56.8	90.7	61.5	47.6	54.2	73.6	48.7	45.2	53.2	36.3
5-MeHex(13)	<u>100</u> <sup>a</sup>	<u>100</u>	86.9	<u>100</u>	<u>100</u>	89.5	81.0	78.0	55.8	92.4	69.4
5-MeHept(15)	60.8	71.6	<u>100</u>	14.7	55.8	50.7	66.7	<u>100</u>	12.5	45.1	82.3
6-MeHept(16)	7.2	12.3	8.2	tr <sup>c</sup>	7.7	6.2	7.3	11.6	2.2	5.4	14.5
Acyl group	BBNP-8	BBNP-9	BBN P-10	BBNP-11	BBNP-12	ZH04-8	ZS05-04	ZS05-05	55557-3	55554-3	
	mol % of highest										
Ac(1)	66.07	66.1	79.3	64.5	71.1	75.3	93.5	86.1	51.5	37.5	
MePro(4)	24.3	17.7	18.3	16.4	18.0	18.3	15.9	22.3	12.0	27.9	
But(5)	16.2	9.6	7.6	11.5	5.5	13.1	5.9	5.2	4.2	3.8	
2-MeBut(6)	85.5	83.0	98.1	64.5	86.8	57.8	<u>100</u>	<u>100</u>	41.9	<u>100</u>	
3-MeBut(7)	7.7	3.7	8.8	7.3	6.8	8.7	4.9	8.8	3.8	11.1	
3-MeVal(9)	24.7	13.2	17.4	25.8	16.1	36.6	2.6	4.0	18.9	6.5	
4-MeVal(10)	9.7	8.4	10.4	8.3	10.7	7.0	6.3	6.1	5.4	6.6	
Hex(11)	-	2.4	2.6	-	1.9	-	-	-	0.7	-	
4-MeHex(12)	<u>100</u>	<u>100</u>	<u>100</u>	<u>100</u>	<u>100</u>	75.3	20.3	25.5	<u>100</u>	26.1	
5-MeHex(13)	63.06	78.3	84.1	49.1	80.9	27.0	63.6	56.9	28.8	51.5	
5-MeHept(15)	73.6	54.7	60.2	71.7	55.0	<u>100</u>	0.5	3.9	47.2	23.9	
6-MeHept(16)	tr	4.6	4.1	4.06	3.1	4.4	-	-	1.3	2.9	

<sup>a</sup> major acid is underlined; <sup>b</sup> not found; <sup>c</sup> white rows-straight chain; light-grey rows - iso chains; dark-grey – anteiso chains

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**Table 2.** Relative abundance of acyl groups in tobacco cultivars, ornamental varieties, and wild tobaccos.

Acyl group	TI				<i>N. alata</i> <i>N. alata</i> <i>N. alata</i>						
	TI 1068	KY14	165	KDH 960	<i>N.sylvestris</i>	PI 555474	PI 42334	PI 555473	<i>N. x sanderae</i>	<i>N. noctiflora</i>	
	mol % of highest										
Ac(1)*	78.7	<u>100</u>	69.7	<u>100</u>	<u>100</u>	<u>100</u>	<u>100</u>	<u>100</u>	89.7	61.9	35.0
Pro(2)	1.8	2.3	9.2	tr <sup>a</sup>	-	tr	11.7	12.8	1.6	tr	
MePro(4)	13.3	10.9	19.9	31.9	-	83.9	30.5	34.0	11.0	5.4	
But(5)	3.7	3.1	17.8	18.5	10.3	37.2	24.8	27.2	10.5	1.6	
2-MeBut(6)	28.8	59.7	28.4	38.7	7.4	58.8	88.4	<u>100</u>	87.0	2.4	
3-MeBut(7)	29.1	44.2	35.0	65.5	1.6	36.3	30.5	17.2	2.5	64.6	
3-MeVal(9)	<u>100</u> <sup>a</sup>	tr	<u>100</u>	-	2.9	tr	22.3	24.8	5.5	16.0	
4-MeVal(10)	5.4		11.8	-	1.5	tr	tr	tr	-	4.8	
Hex(11)	- <sup>b</sup>	-	-	-	-	43.6	15.5	16.1	10.0		
4-MeHex(12)	tr <sup>c</sup>	5.2	-	-	24.2	-	-	-	-	2.2	
5-MeHex(13)	-	tr	9.6	-	7.7	-	-	-	-	<u>100</u>	
5-MeHept(15)	-	-	-	-	-	-	-	-	-	13.6	
6-MeHept(16)	-	-	-	-	-	-	-	-	-	9.7	
Oct(17)	-	5.6	-	-	-	87.4	57.0	59.4	<u>100</u>	-	

<sup>a</sup> traces; <sup>b</sup> not found; \*white rows - straight chain; light grey rows - iso chains; dark grey rows – anteiso chains

**Table 3.** Relative abundance of acyl groups in *Nicotiana* species from Australia.

Acyl group	<i>Nicotiana occidentalis</i> subsp. <i>hesperis</i>									<i>Nicotiana occidentalis</i>			
	303663 leaves	303767 leaves	303765, leaves	303765, stem	303721, leaves	303721, stems+sepals	303763, leaves	303763, stems	303764, leaves+stems	555541 leaves+stems	555689 leaves	555689 stems	555689 sepals
	mol % of highest									mol% of highest			
Ac(1)*	52.6	52.5	<u>100</u>	<u>100</u>	86.9	91.6	55.6	<u>100</u>	<u>100</u>	80.5	<u>100</u>	<u>100</u>	<u>100</u>
Pro(2)	- <sup>b</sup>	4.3	7.1	1.5	-	0.3	22.1	0.3	0.52	0.64	9.6	18.3	3.7
MePro(4)	26.4	11.1	80.4	55.8	65.5	22.0	<u>100</u>	55.7	10.4	16.3	25.6	43.4	33.5
But(5)	8.1	2.9	11.8	0.8	-	1.7	28.7	1.9	0.9	1.1	13.4	27.8	5.2
2-MeBut(6)	25.5	32.1	30.1	7.5	51.6	61.3	22.0	8.6	67.1	<u>100</u>	21.5	15.0	18.7
3-MeBut(7)	15.2	8.2	39.0	15.5	45.6	20.7	23.2	18.5	14.8	7.2	30.8	8.7	5.2
Val(8)	-	-	-	-	-	2.1	-	0.5	1.1	0.7	9.0	-	-
3-MeVal(9)	<u>100</u> <sup>a</sup>	<u>100</u>	23.0	6.4	<u>100</u>	<u>100</u>	7.9	8.0	86.9	62.6	66.3	16.5	0.0
4-MeVal(10)	10.5	5.3	73.8	61.6	34.5	16.9	53.2	74.8	6.6	0.9	7.8	-	5.4
Hex(11)	-	-	10.8	0.6	-	2.8	-	1.1	-	-	-	-	-
4-MeHex(12)	tr <sup>c</sup>	1.2	14.8	4.7	tr	4.1	5.0	5.8	2.5	-	-	17.9	9.2
5-MeHex(13)	tr	tr	54.7	30.2	77.1	2.8	18.5	33.6	tr	-	10.2	27.2	75.8
Hept(14)	-	-	11.1	0.4	-	1.9	-	1.0	-	-	-	-	-
5-MeHept(15)	-	-	-	-	-	-	-	-	-	-	19.8	65.1	65.3
6-MeHept(16)	-	-	-	-	-	-	-	-	-	-	-	11.0	9.1
8-MeNon(18)	9.7	-	20.6	tr	-	-	34.6	-	2.0	-	-	-	-
Dec(19)	tr	-	17.3	0.3	-	0.4	13.7	0.5	1.6	-	-	-	-

<sup>a</sup> traces, <sup>b</sup> not found; \*white rows - straight chain; light grey rows - iso chains; dark grey – anteiso chains

**Table 4.** Relative abundance of acyl groups in *Petunia*, *S. bertaultii*, and *S. Pennellii*.

Acyl group	P.Madness Red Picotee	P. Dreams Red Picotee	P. Silver Tidal Wave	P.Purple Tidal Wave	Petunia Pink Tidal Wave	Kays et al.,1994	<i>S.berthaultii</i> al.,1990	Neal et al.,1990	<i>S.pennellii</i> †
	mol % of highest								
Ac(1)*	48.7	39.5	19.0	33.3	<u>28.4</u>	38.0	tr	-	1.9
Pro(2)	7.7	2.7	3.3	8.9	9.3	-	4.3	1.2	0.8
Malonate(3)	<u>100</u> <sup>a</sup>	98.5	82.7	<u>100</u>	<u>100</u>	-	-	-	-
MePro(4)	14.4	7.3	2.8	17.15	18.1	-	<u>100</u>	<u>100</u>	<u>100</u>
But(5)	18.0	6.5	6.5	10.7	12.7	4.0	4.3	2.3	1.0
2-MeBut(6)	77.2	<u>100</u>	44.3	92.1	31.2	<u>100</u>	76.5	62.2	43.4
3-MeBut(7)	15.0	18.3	5.4	14.2	21.8	12	24.8	-	12.3
Val(8)	18.2	7.6	14.9	7.8	9.2	7.0	-	-	-
3-MeVal(9)	12.9	5.2	3.2	5.1	83.4	-	-	-	5.1
4-MeVal(10)	14.9	35.4	7.6	2.1	27.3	32	-	-	tr <sup>c</sup>
Hex(11)	61.7	36.2	<u>100</u>	6.0	23.9	37.0	-	-	-
4-MeHex(12)	- <sup>b</sup>	-	-	3.1	27.0	5	-	-	-
5MeHex(13)	-	-	2.3	2.9	-	10	-	-	-
Hept(14)	23.2	13.5	35.9	15.3	15.7	21.0	-	-	-
6MeHept(16)	-	-	-	-	-	-	-	-	0.9
Oct(17)	8.7	3.8	4.4	32.4	5.7	5.0	3.5	-	-
8-MeNon(18)	-	-	-	-	-	-	-	47.9	58.9
Dec(19)	-	-	-	-	-	-	80.6	4.4	17.6
Laur(20)	-	-	-	-	-	-	-	4.4	6.2

<sup>a</sup> major acid is underlined; <sup>b</sup> traces; <sup>c</sup> not found; \*white rows-straight chain; ligh- grey rows - iso chains; dark-grey – anteiso chains; †Non and 9-MeDec were observed in trace amounts in *S. pennellii*.

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