

Amounts and Types of Epicuticular Leaf Waxes among Onion Accessions Selected for Reduced Damage by Onion Thrips

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ABSTRACT. Onion thrips (*Thrips tabaci*) is the main insect pest of onion (*Allium cepa*), and feeding damage routinely causes serious yield losses. Lower amounts of epicuticular waxes on onion leaves have been associated with fewer onion thrips and less feeding damage, and research is needed to assess the relationships between amounts and composition of epicuticular waxes and feeding damage by onion thrips. This study used gas chromatography mass spectroscopy to determine amounts and types of epicuticular waxes on the foliage of onion accessions that had been field-selected for lower damage from onion thrips. Hentriacontanone-16 (H16), octacontanol-1, and triacontanol-1 were the most prevalent waxes on the foliage of these selections. Amounts of H16 were significantly lower on selections visually classified as having glossy or semiglossy foliage. Semiglossy selections were identified with similar amounts of total epicuticular wax as waxy phenotypes, due primarily to lower amounts of H16 and higher amounts of other waxes. These semiglossy selections suffered significantly less feeding damage from onion thrips in a field evaluation, supporting the identification of unique wax profiles toward the development of thrips-resistant onion.

Feeding by onion thrips routinely causes severe damage to onion foliage and significantly reduces both bulb and seed yields (Diaz-Montano et al., 2010; Elmore, 1949; Fournier et al., 1995; Jones et al., 1934; Kendall and Capinera, 1987; Parrella and Lewis, 1997). Insecticides are commonly used to control onion thrips; however, populations have evolved resistance to pyrethroid and organophosphate insecticides (Herron et al., 2008; Shelton et al., 2003), reducing control options for growers. Management of onion thrips remains challenging due to the number of generations per season and large host range of the insect, as well as difficulty of sprays reaching between leaves.

Onion germplasm has been identified that suffers less damage by onion thrips. In field evaluations of plant introductions (PIs) from different regions of the world, Jones et al. (1934) reported that one accession of ‘White Persian’ onion had fewer onion thrips per plant. This accession was morphologically unique with light-green foliage, and subsequent research has documented that onion cultivars with lighter-green foliage support lower numbers of onion thrips and suffer less feeding damage (Boateng et al., 2014; Cramer et al., 2014; Damon et al.,

2014; Diaz-Montano et al., 2010; Jones et al., 1935). Light-green leaf color in onion has been associated with chemical composition of epicuticular waxes on foliage (Damon et al., 2014; Jones et al., 1944; Maughan and MacLeod, 1936; Molenaar, 1984). The epicuticular waxes in *Allium* are a mixture of long chain fatty acids (C20 to C30) and their derivatives (Rhee et al., 1998). H16 is the main epicuticular wax on leek (*Allium ampelorasum*) foliage and represents more than 15% of the total lipids. Damon et al. (2014) used gas chromatography–mass spectrometry (GCMS) to identify amounts and compositions of epicuticular waxes on the foliage of four onion inbreds with glossy (low wax), semiglossy (intermediate wax), and waxy (copious wax) phenotypes; these authors observed that lower thrip numbers and feeding damage were observed on onions with lower amounts of H16 and total epicuticular wax compared with susceptible phenotypes. In contrast, Njau et al. (2017) reported that total amounts of epicuticular wax were not correlated with onion thrips damage on onion in Tanzania. However Njau et al. (2017) extracted total epicuticular waxes from onion foliage and neither identified nor quantified these waxes. In our study, we used GCMS to measure the types and amounts of epicuticular waxes on foliage of diverse onion accessions that had been selected for reduced damage by onion thrips. We also assessed feeding damage by onion thrips to determine if specific wax profiles, as opposed to total amount of waxes, were associated with reduced damage by onion thrips.

Materials and Methods

PLANT MATERIAL. Fourteen onion accessions (Table 1) were assessed for epicuticular wax profiles and damage by onion

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Product names are necessary to report factually on available data; however, the U.S. Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Table 1. Origins and visually assessed foliage phenotypes of onion accessions evaluated for epicuticular waxes and feeding damage by onion thrips.

Accession ^z	Origin	Visual phenotype ^y	Avg score [mean ± SD (1–5 scale)] ^y
B9885	USDA	GL	1.0 ± 0.0
264320-1	Spain	SG	1.3 ± 0.6
B5351	USDA	SG	1.7 ± 1.2
264320-2	Spain	SG	1.7 ± 1.2
546101	Spain	SG	3.0 ± 0.0
546201	USA	SG	3.0 ± 0.0
546192	USA	SG	3.0 ± 0.0
288903	Hungary	WX	5.0 ± 0.0
264648	Germany	WX	5.0 ± 0.0
546096	USA	WX	5.0 ± 0.0
546106	USA	WX	5.0 ± 0.0
DH 2107	Cornell Univ.	WX	5.0 ± 0.0
22452 AI ^x	USDA	WX	5.0 ± 0.0
22452 AA ^x	USDA	WX	5.0 ± 0.0

^zSix-digit numbers are U.S. Department of Agriculture (USDA) plant introductions (PIs) selected in field trials for reduced damage by onion thrips (Boateng et al., 2014). Dashes indicate independent selections from PI 264320.

^yVisually classified phenotypes of glossy (GL), semiglossy (SG), or waxy (WX) foliage based on scores from 1 (glossy) to 5 (waxy) as shown in Fig. 1.

^xWaxy selections from the cross of DehyA × B5351 (Damon and Havey, 2014).

thrips. Nine of these accessions were from the U.S. Department of Agriculture germplasm collection and had been visually selected for less damage by onion thrips in field evaluations in Colorado (Boateng et al., 2014). Five control accessions were included: B9885 (PI 546303) is an inbred with glossy foliage originating from ‘White Persian’; B5351 is an inbred with semiglossy foliage selected from ‘Colorado #6’; DH2107 is a double haploid (DH) with waxy foliage; and 22452AI and 22452AA are waxy selections from the cross of DehyA × B5351 (Damon and Havey, 2014; Hyde et al., 2012; Molenaar, 1984).

In Jan. 2016, seeds of the 14 accessions were sown in a soilless medium (Metro-Mix; Sun Gro Horticulture, Agawam, MA) in 96-well trays in a greenhouse at the University of Wisconsin–Madison (UW) with growing conditions of 14-h days at 24 and 20 °C nights. Plants were watered daily and fertilized once per week with 20N–8.8P–16.6K water-soluble fertilizer (Peters Professional, Allentown, PA). At 72 d after seeding, individual plants were transferred to 10-cm-square pots with soilless mix in the greenhouse. Pots containing plants were moved outdoors at 120 d after seeding into coldframes at the UW Horticulture Research Farm (Arlington, WI), covered with a black net for 2 d, and arranged in a completely randomized design (CRD) with six replications. At 170 d after seeding, leaves were sampled for GCMS as described below (evaluation CF16). In 2017, the 14 accessions were planted in the greenhouse as described for 2016, pots were arranged in a CRD with six replications, and leaves were sampled for analysis by GCMS at 71 d after planting (evaluation GH17).

In 2017, 13 accessions (without 546201 due to lack of seed) were grown in the greenhouse as described above and transplanted in early June into field plots in a randomized complete block design (RCBD) at two locations on the UW Horticulture Research Farm (evaluations Arl17A and Arl17B). Fifteen plants per accession were transplanted into three blocks with 5 cm between plants and 10-cm separation between plots. Leaves were sampled for analysis by GCMS at 110 d after seeding.

EPICUTICULAR WAX EVALUATIONS.

For evaluation of waxes using GCMS from plants in the CF16 and GH17 evaluations, two samples were taken from the middle section of the fourth leaf from each of six plants per accession. For Arl17A and Arl17B evaluations, two samples were taken from the middle section of the fourth leaf from each of two randomly chosen plants per accession from each of three blocks. Each sample was weighed and docosane (Sigma, St. Louis, MO) dissolved in high-performance liquid chromatography (HPLC) reagent grade chloroform (Thermo Fisher Scientific, Waltham, MA) was added as an internal standard at a rate of 100 µg·g⁻¹ fresh weight. Each leaf section was submerged into HPLC-grade chloroform and removed after 1 min. Chloroform was evaporated in a fume hood for ≈5 d. Dried extracts were dissolved in 500 µL chloroform, followed by

addition of 600 µL acetonitrile (HPLC grade, Thermo Fisher Scientific) and derivatization with 210 µL N,O bis(trimethylsilyl) tri-fluoroacetamide (BSTFA) (1% trimethyl-chlorosilane, HPLC/GC grade; Sigma) for 30 min at 80 °C. The GCMS instrument (QP2010; Shimadzu, Santa Clara CA) had a capillary column (SH-Rxi-5Sil MS, 30 m long; 0.30 mm i.d.; dim.f = 0.25 µm), and on-column injection at 250 °C and column oven temperature 150 °C constant for 10 min, ramp 10 °C·min⁻¹ to 300 °C, constant for 10 min. Helium gas was the carrier at a flow rate of 1.0 mL·min⁻¹ with primary pressure of 700 kPa. Tandem MS was equipped with a detector (GCMS-QP2010, Shimadzu) with ion source range (35 to 600 m·z⁻¹) for identification of the wax components. The detection MS interface and ion source temperatures were 290 and 260 °C, respectively, and a split ratio of 20. Amounts of individual waxes are reported as peak areas per leaf fresh weight after adjusting to the internal docosane standard.

ONION THRIPS EVALUATIONS. In 2016 and 2017, seeds from the 14 accessions (Table 1) were planted in a greenhouse as described. At ≈105 d after sowing, plants were moved outside and covered with black nets for 2 d. Fifteen plants per accession were then transplanted into three blocks at 5 cm distance between plants and a 10 cm separation between plots in a RCBD at the UW Horticulture Research Farm. Plots were not sprayed with pesticides and natural populations of onion thrips colonized plots from adjacent sources. Three evaluators visually classified the foliage type of each accession using the scale of 1 = glossy, 3 = semiglossy, and 5 = waxy foliage (Fig. 1). Plants were scored when feeding damage by onion thrips was severe on waxy DH2107. Four evaluators independently scored damage on each replicated plot using a severity scale of 1 to 9, where 1 = no damage and 9 = severe damage.

DATA ANALYSIS. Peak areas for each wax component were averaged from the two leaf samples from each plant, yielding six observations corresponding to the six plants per accession from the CF16 and GH17 plantings and two observations per

block from the Arl17A and Arl17B plantings. Statistical analyses were performed in R studio (R Foundation for Statistical Computing, Vienna, Austria). Accessions were considered as fixed and evaluations, accession-by-evaluation interaction, and blocks as random variables. Analysis of variance for fixed effects used the anova function and for the mixed model analyses the Lmer package with function lmer. Significant differences for wax composition among accessions were tested using the package emmeans and Tukey's honestly significant differences test at $P = 0.05$. R package multcompView with function cld was used to extract and display pairwise comparisons of estimated marginal means using parameters adjust="tukey". The model used to estimate accession means in the CF16 and GH17 evaluations was $y_{ijl} = \mu + g_i + t_j + g_{tj} + \epsilon_{ijl}$ where μ is the overall mean, g_i is the fixed effect of the i^{th} onion accession, t_j is the random effect of the j^{th} evaluation, g_{tj} is the random effect of the ij^{th} accession by evaluation, and ϵ_{ijl} are residuals. For the Arl17A and Arl17B evaluations, the model was $y_{ijkl} = \mu + g_i + t_j + g_{tj} + \beta_k + \epsilon_{ijkl}$ where β_k is the random effect of the k^{th} block. The model used for thrips damage was $y_{ijtl} = \mu + g_i + e_j + b_t + \epsilon_{ijtl}$ where μ is the overall mean, g_i is the fixed effect of the i^{th} onion accession, e_j is the random effect of the j^{th} evaluator, b_t is the random effect of the t^{th} block, and ϵ_{ijtl} are residuals. Evaluator by block interaction was not significant and therefore not included in the mixed model. Pearson's correlations between proportions of individual waxes using estimated accession means and mean estimates of thrips damage were calculated based on these models.

Results

EPICUTICULAR WAX PROFILES. Eight waxes were detected on foliage of the onion accessions (Tables 2 and 3): 2-methyloctacosane (Met), H16, hexacosanol-1 (Hex), heptacosane (Hepc), heptadecane (Hepd), octacosanol-1 (Oct1), octadecane (Octd), and triacontanol-1 (Tri1), in agreement with Damon et al. (2014). H16 was the most prevalent wax on the foliage of all accessions except glossy B9885 (Tables 2 and 3) and ranged from 21% to 71% of the total epicuticular wax (Table 4). A visually assessed waxy phenotype was most obvious when H16 comprised more than two-thirds of total wax amounts, consistent with Gülz et al. (1992) who proposed that wax crystals build up on the leaf surface when one lipid class predominates. However in some cases waxy and semiglossy accessions had the same amounts of total wax on foliage (e.g., as waxy 288903 and semiglossy 546101 in Tables 2 and 3), and similar percentages of H16 to total wax (waxy 264648 and semiglossy 546201 in Table 4).



Fig. 1. Visually scored phenotypes of onion foliage from left to right: 1 = glossy, 3 = semiglossy, and 5 = waxy.

Table 2. Mean peak areas adjusted to internal standard as measured by gas chromatography for eight epicuticular waxes, total wax amount and visually assessed foliar phenotypes of 14 onion accessions grown in completely randomized design in coldframe 2016 and greenhouse 2017. Accessions sorted from highest to lowest amount of hentriacontanone-16 (H16).

Accession	Phen ^z	Waxes ^y											Total						
		H16	Oct1	Tri1	Met	Hex	Octd	Hepd	Hepc										
546096	WX	3.48	a	0.49	a	0.19	cde	0.06	a	0.14	a	0.05	ab	0.16	ab	0.18	a	4.75	a
22452AA	WX	2.39	b	0.32	abc	0.14	de	0.14	a	0.02	a	0.07	a	0.22	a	0.23	a	3.53	ab
264648	WX	2.29	b	0.28	abc	0.19	cde	0.03	a	0.04	a	0.03	bcde	0.15	bc	0.15	a	3.16	bc
DH2107	WX	2.27	b	0.34	abc	0.20	bcde	0.04	a	0.09	a	0.02	cde	0.09	cde	0.11	a	3.16	bc
546106	WX	2.00	bc	0.33	abc	0.17	cde	0.09	a	0.04	a	0.04	bc	0.10	cde	0.27	a	3.04	bc
288903	WX	1.94	bcd	0.29	abc	0.14	de	0.05	a	0.04	a	0.05	ab	0.11	bcde	0.10	a	2.72	bcd
22452AI	WX	1.72	bcd	0.24	bc	0.12	e	0.14	a	0.06	a	0.05	ab	0.13	bcd	0.31	a	2.77	bcd
546201	SG	1.72	bcd	0.33	abc	0.38	a	0.02	a	0.00	a	0.00	e	0.06	de	0.02	a	2.53	bcd
546192	SG	1.72	bcde	0.50	a	0.32	abc	0.04	a	0.00	a	0.02	de	0.09	cde	0.06	a	2.75	bcd
546101	SG	1.62	bcde	0.41	ab	0.33	ab	0.15	a	0.10	a	0.03	bcde	0.13	bcd	0.40	a	3.17	bc
B5351C	SG	1.23	cde	0.15	c	0.09	e	0.08	a	0.01	a	0.03	bcde	0.15	abc	0.18	a	1.92	cde
264320-2	SG	1.13	de	0.42	ab	0.27	abcd	0.13	a	0.05	a	0.03	bcde	0.11	bcde	0.21	a	2.35	bcd
264320-1	SG	0.75	ef	0.32	abc	0.18	cde	0.10	a	0.05	a	0.02	de	0.08	de	0.15	a	1.65	de
9885C	GL	0.25	f	0.32	abc	0.14	de	0.06	a	0.01	a	0.04	bcd	0.05	e	0.10	a	0.97	e

^zVisually classified phenotypes of glossy (GL), semiglossy (SG), or waxy (WX) foliage.

^yWaxes are hentriacontanone-16 (H16), octacosanol-1 (Oct1), and triacontanol-1 (Tri1), 2-methyloctacosane (Met), hexacosanol-1 (Hex), octadecane (Octd), heptadecane (Hepd), and heptacosane (Hepc). Means followed by the same letter are not significantly different using Tukey's honestly significant differences test at $P = 0.05$.

Table 3. Mean peak areas adjusted to internal standard as measured by gas chromatography for eight epicuticular waxes, total wax amount and visual foliar phenotypes on the foliage of 13 onion accessions grown in a randomized complete block design at two locations on the UW Horticulture Research Farm in 2017. Accessions sorted from highest to lowest amount of hentriacontanone-16 (H16).

Accession	Phen ^z	Waxes ^y															
		H16	Oct1	Tri1	Met	Hex	Octd	Hepd	Hepc	Total							
546096	WX	2.24 a	0.67 a	0.28 ab	0.02 c	0.04 abc	0.06 abc	0.20 a	0.07 abc	3.58 a							
288903	WX	2.07 ab	0.39 bcd	0.14 cde	0.03 bc	0.03 abcd	0.07 ab	0.15 ab	0.05 bc	2.93 ab							
DH2107	WX	1.85 ab	0.30 cdef	0.27 abc	0.02 c	0.02 cd	0.02 ef	0.12 bc	0.06 abc	2.66 ab							
22452AI	WX	1.85 ab	0.35 cde	0.12 de	0.07 a	0.04 abc	0.07 a	0.19 a	0.08 ab	2.77 ab							
264648	WX	1.72 abc	0.47 bc	0.22 abcd	0.01 c	0.04 abcd	0.04 bcd	0.16 ab	0.05 bc	2.71 ab							
546106	WX	1.67 abc	0.47 bc	0.26 abc	0.02 c	0.03 bcd	0.04 cd	0.09 bcd	0.06 abc	2.64 ab							
264320-2	SG	1.36 abcd	0.40 bcd	0.23 abcd	0.03 bc	0.05 a	0.02 ef	0.12 bc	0.09 ab	2.30 bcd							
22452AA	WX	1.30 bcd	0.39 bcd	0.15 bcde	0.06 ab	0.04 abc	0.09 a	0.17 ab	0.07 abc	2.27 bcde							
546101	SG	1.30 bcd	0.52 ab	0.31 a	0.02 c	0.05 ab	0.04 cd	0.14 abc	0.07 abc	2.45 abc							
546192	SG	0.88 cde	0.23 def	0.11 de	0.03 bc	0.02 cd	0.03 def	0.09 bcd	0.08 ab	1.47 cdef							
B5351C	SG	0.70 de	0.17 ef	0.08 e	0.03 bc	0.01 d	0.03 de	0.15 abc	0.11 a	1.28 def							
264320-1	SG	0.66 de	0.14 f	0.09 e	0.03 bc	0.03 cd	0.01 f	0.08 cd	0.08 ab	1.12 ef							
9885C	GL	0.08 e	0.33 cde	0.18 bcde	0.03 bc	0.04 abc	0.04 cd	0.04 d	0.02 c	0.76 f							

^zVisually classified phenotypes of glossy (GL), semiglossy (SG), or waxy (WX) foliage.

^yWaxes are hentriacontanone-16 (H16), octacontanol-1 (Oct1), triacontanol-1 (Tri1), 2-methyloctacosane (Met), hexacosanol-1 (Hex), octadecane (Octd), heptadecane (Hepd), and heptacosane (Hepc). Means followed by the same letter are not significantly different using Tukey's honestly significant differences test at $P = 0.05$.

Table 4. Visual assessed foliar phenotypes, mean feeding damage by onion thrips, and percentages of individual epicuticular waxes for onion accessions as measured by gas chromatography across evaluations. Accessions sorted from highest to lowest feeding damage.

Accession	Phen ^z	Feeding damage (1–9 scale) ^y			Composition of wax (%) ^x							
		Mean	SE		H16	Oct1	Tri1	Met	Hex	Octd	Hepd	Hepc
DH2107	WX	7.6	0.40	a	71.5	10.7	6.3	1.3	3.0	0.6	3.0	3.6
546106	WX	7.5	0.71	a	65.6	10.9	5.6	3.0	1.3	1.4	3.3	8.9
22452AI	WX	7.3	0.40	a	61.2	8.7	4.2	5.4	2.4	1.8	4.6	11.7
288903	WX	7.2	0.40	a	71.3	10.6	5.0	1.8	1.6	1.7	4.0	4.0
546096	WX	6.8	0.40	a	73.0	10.2	4.0	1.3	3.1	1.0	3.4	4.0
22452AA	WX	6.6	0.40	a	67.4	9.0	3.8	3.9	0.6	1.9	6.2	7.2
264648	WX	5.9	0.40	ab	72.3	8.7	5.9	1.0	1.5	0.9	4.7	5.0
546201	SG	4.2	0.40	bc	61.5	11.9	13.1	2.3	2.2	0.1	2.9	6.0
546101	SG	3.5	0.40	cd	50.1	12.6	10.2	5.1	3.4	1.0	4.2	13.4
546192	SG	3.3	0.40	cd	56.8	17.1	10.1	2.7	2.0	0.5	3.8	7.0
9885C	GL	3.2	0.40	cd	24.1	32.5	14.1	6.7	1.7	3.7	5.3	11.9
264320-2	SG	2.8	0.40	cd	47.5	17.9	11.2	5.7	2.3	1.2	4.7	9.4
B5351C	SG	2.3	0.40	cd	58.3	7.2	3.7	5.1	2.0	1.6	8.1	14.0
264320-1	SG	1.6	0.40	d	44.9	19.4	10.9	6.3	3.1	0.9	4.9	9.5
Correlation with feeding damage					0.73**	-0.52	-0.65*	-0.63*	-0.25	-0.04	-0.44	-0.56

^zVisually classified phenotypes of glossy (GL), semiglossy (SG), or waxy (WX) foliage (Table 1).

^yScores for feeding damage by onion thrips were from four evaluators using scale from 1 = no damage to 9 = severe damage. Means followed by the same letter are not significantly different using Tukey's honest significant difference test at $P < 0.05$.

^xWaxes are hentriacontanone-16 (H16), octacontanol-1 (Oct1), triacontanol-1 (Tri1), 2-methyloctacosane (Met), hexacosanol-1 (Hex), octadecane (Octd), heptadecane (Hepd), and heptacosane (Hepc).

*, **Significant at $P < 0.05$ or 0.01, respectively.

Amounts of the fatty alcohol Oct1 ranged from 10% to 36% of the total wax load; glossy B9885 had the largest proportion of Oct1 at 36%, and 264320-1 and 264320-2 had relatively large proportions at $\approx 18\%$ each (Table 4). Amounts of the fatty alcohol Tri1 were lower than Oct1 and ranged from 5% to 15% of the total wax load (Table 4). The proportion of the alkane Hepc was relatively high in accessions B5351, 264320-1, and 546101 (Table 4). The other waxes represented relatively minor components (<10%) of the total wax profile (Tables 2–4). We detected an average of 20% less total wax on accessions grown in the Arl17A and Arl17B vs. CF16 and GH17 evaluations

(Tables 2 and 3), indicating that visual foliar phenotypes may be more distinct under field conditions due to lower amounts of total wax.

From analyses of the CF16 and GH17 evaluations, accession was highly significant ($P < 0.001$) for the three most prevalent waxes (H16, Oct1, and Tri1) and explained 41.1%, 14.7%, and 6.9% of the variation, respectively. Evaluation (CF16 vs. GH17) was significantly different for H16 ($P = 0.025$) and Tri1 ($P < 0.01$); however, evaluation explained only 0.7%, 4.0%, and 0.1% of the total variation for H16, Oct1, and Tri1, respectively. The accession-by-evaluation interaction was significant for H16

Table 5. Pearson correlation coefficients among amounts of hentricontanone-16 (H16), octacosanol-1 (Oct1), and triacontanol-1 (Tri1) on foliage of onion accessions grown in coldframe in 2016 (CF16), greenhouse in 2017 (GH17), and at two locations on the University of Wisconsin Horticulture Research Farm in 2017 (Arl17A and Arl17B). All correlations were significant at $P = 0.05$

	H16			Oct1			Tri1		
	GH17	Arl17A	Arl17B	GH17	Arl17A	Arl17B	GH17	Arl17A	Arl17B
CF16	0.89	0.81	0.76	0.34	0.34	0.34	0.39	0.45	0.68
GH17		0.79	0.73		0.50	0.53		0.55	0.46
Arl17A			0.8			0.97			0.89

($P = 0.009$) and Tri1 ($P < 0.011$) and contributed more to the total variation at 6.8%, 7.4%, and 10.6% for H16, Oct1, and Tri1, respectively. For the Arl17A and Arl17B evaluations, accession was highly significant ($P < 0.001$) for H16, Oct1, and Tri1. Evaluation was significant for H16 and Tri1 (both $P < 0.001$), accession-by-evaluation interaction was significant for H16 and Tri1 (both $P < 0.011$), and blocks were not significant for H16, Oct1, and Tri1. For H16, accessions explained the largest proportion of the variation at 33.9%, followed evaluation at 21.4% and accession-by-evaluation at 4.7%. For Oct1, the proportion of variance explained by accession was 57.5%; the evaluation and accession-by-evaluation interaction were essentially zero. For Tri1, accession contributed the largest proportion at 28.7%, followed by the evaluation at 15.7% and accession-by-evaluation at 6.8% of the total phenotypic variance.

ONION THRIPS DAMAGE. In the 2016 field evaluation, visually scored feeding damage by onion thrips was significantly different ($P < 0.001$) among accessions, and the glossy and semiglossy selections showed significantly lower damage compared with the waxy onion (Table 4). Feeding damage was significantly ($P < 0.05$) correlated with total amounts of H16 at 0.69 and 0.68 from evaluations CF16/GH17 and Arl17A/Arl17B, respectively. Amounts of Oct1 and Tri1 were not significantly correlated with feeding damage at the 0.05 level in CF16/GH17, and only Oct1 for plants grown in the Arl17A/Arl17B evaluations was significantly ($P < 0.05$) correlated with less feeding damage by onion thrips. The percentages of H16, Met, and Tri1 to total wax amounts were significantly correlated with onion thrips damage at 0.73, -0.63, and -0.65, respectively (Table 4), indicating that lower amounts of H16 and Met and higher amounts of the other waxes were correlated with less feeding damage. No significant differences were observed for damage scores in the 2017 evaluation due to low numbers of onion thrips.

PHENOTYPIC CORRELATIONS AND VARIANCE COMPONENTS. Pearson correlations among evaluations (CF16, GH17, Arl17A, and Arl17B) were all significant ($P < 0.05$) for the three main waxes and were consistent with those reported in a segregating family by Damon and Havey (2014). Correlations for amounts of H16 ranged from 0.89 to 0.73 (Table 5). Correlations for amounts of Oct1 ranged from 0.97 to 0.34 and for Tri1 between 0.89 and 0.39 (Table 5). The lower correlations for Oct1 and Tri1 across evaluations could be due to greater environmental effect on amounts of these fatty alcohols, or greater variation in their measurements because of lower amounts.

Discussion

We measured types and amounts of epicuticular waxes on the foliage of glossy, semiglossy, and waxy onions and

confirmed that glossy and semiglossy phenotypes suffer significantly less feeding damage by onion thrips compared with waxy onion (Table 4), agreeing with previous researchers (Cramer et al., 2014; Damon et al., 2014; Diaz-Montano et al., 2010; Jones et al., 1935; Maughan and MacLeod, 1936) but disagreeing with Njau et al. (2017). Glossy and semiglossy

accessions had lower amounts of H16 relative to the other wax components; however, these phenotypes may have larger amounts of other waxes such as fatty alcohols or alkanes (Tables 2 and 3). Amounts of total epicuticular wax varied among accessions within the same visual phenotypes, and semiglossy accessions were identified with the same total epicuticular wax as waxy plants (Tables 2 and 3). Selections from PIs 546192 and 546201 had smaller proportions of H16 on foliage and increased amounts of other waxes such as the alkane Hpc and fatty alcohols Oct1 and Tri1 (Table 4). The selection from PI 546192 possessed as much total wax as some waxy phenotypes and showed lower feeding damage by onion thrips, agreeing with observations by Cramer et al. (2014). In subsequent research, Cramer et al. (2017) concluded that lighter leaf color and/or a reduced amount of epicuticular wax did not always result in the fewest number of onion thrips per plant; however, Cramer et al. (2017) relied on visual classification of foliage phenotypes and did not quantitatively measure amounts and types of leaf waxes. Our research demonstrates that the composition of epicuticular waxes is important for reduced damage by onion thrips. Selection of onion for lower amounts of H16 and higher amounts of other waxes, such as alkanes and fatty alcohols, should produce phenotypes that suffer less feeding damage by onion thrips. These onions would still be damaged by onion thrips; however, they may require fewer insecticide applications for adequate control (Nault and Huseth, 2016) and potentially delay or avoid the development of insecticide resistance often associated with frequent spraying.

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