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Species Distribution of Biochemical and Morphological Characters Associated with Small Pest Resistance in *Pelargonium ×hortorum*

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Abstract. **Biochemical and morphological components of 16 *Pelargonium* species and the *P. ×hortorum* interspecific complex were examined. Inflorescences and leaves of each species were analyzed for anacardic acids and the presence of glandular trichomes. Three species of the section *Ciconium*, *P. acetosum*, *P. frutetorum*, and *P. inquinans*, produced anacardic acids in association with glandular trichomes. Only *P. inquinans* and *P. frutetorum* contained ω5-anacardic acids. An evolutionary model for the origin of anacardic acids and ω5-desaturation is proposed.**

The genus *Pelargonium* consists of more than ≈250 species, currently grouped into 16 sections based on anatomy and morphology (van der Walt, 1993). Some of these species, notably those of the section *Ciconium*, are believed to have been involved in the development of the garden geranium, the interspecific *P. ×hortorum* complex (HOR). In particular, *P. inquinans* (INQ) and *P. zonale* (ZON) are considered by most authors to have contributed most of the genetic variability known to exist in HOR (Moore and Hyppio, 1982; van der Walt, 1977). Other species from the section *Ciconium* believed to have contributed to the interspecific HOR complex include *P. acetosum* (ACE), *P. frutetorum* (FRU), and *P. stenopetalum* (*P. ×Burtoniae*, BUR) (Moore and Hyppio, 1982). BUR is now generally considered to be the result of a natural hybridization between ACE and ZON. Most of these species are cross-fertile, or at least cross-fertile, with HOR. All members of the section *Ciconium* possess a diploid complement of 18 chromosomes (2N = 18) (Gauger, 1937; Knicely, 1964; Pan, 1991), although tetraploid (4N) cultivars of the domesticated HOR complex are an important segment of the commercial zonal geranium market (Badr and Horn, 1971; Knicely, 1964). These are distinguished in this report as HOR2 (diploid) and HOR4 (tetraploid).

South Africa is considered to be the center of diversity for the genus (van der Walt, 1977) with >200 of the extant species native to South Africa or the African continent. Other *Pelargonium* species have evolved in Australia and Madagascar, suggesting that the genus *Pelargonium* may have existed at least in a progenitor form while Australia and Madagascar were joined to Africa in the prehistoric supercontinent Gondwanaland (Murphy and Nance, 1992).

INQ is an upright, shrubby herbaceous plant, covered with exudate-bearing glandular trichomes and bearing pale rose to deep red-scarlet single flowers. ZON is generally described as glabrous, smooth, hairless or lightly hairy, and bearing pale pink to lavender-colored flowers (less frequently in shades of red). ZON leaves are frequently marked with dark anthocyanin-containing zones, giving rise to the botanical name, zonale, while INQ leaves are generally solid green in color without a zone. HOR shows wide variation but basically resembles INQ with its hairy leaves and typical scarlet flowers. HOR usually has a characteristic broad leaf zone, typical of ZON, providing the common name, zonal geranium.

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Table 1. Pelargonium species examined, abbreviations used, germplasm source, and presence or absence of tall glandular trichomes (TGTs) and anacardic acids (AnAs) and ω5-anacardic acids (ω5-AnAs)

Pelargonium Species	Accessions examined	Abbreviation	Germplasm Source[y]	Inflorescence[z]			Leaf[z]		
				TGT	AnA	ω5-AnA	TGT	AnA	ω5-AnA
Section *Ciconium*									
acetosum	1	ACE	Cornell	+	+	+ tr	0	0	0
acraeum	1	ACR	Cornell	+	0	0	0	0	0
alchemilloides	1	ALC	Cornell	0	0	0	0	0	0
×*Burtoniae*	1	BUR	Cornell	+	+	+ tr	+	0	0
caylae	1	CAY	Cornell	–	–	–	0	0	0
frutetorum	1	FRU	Cornell	+	+	+	+	0	0
×*hortorum* (2N)	16	HOR2	PSU	+	+	+	+	+	+
×*hortorum* (4N)	28	HOR4	PSU	+	+	+	+	+	+
inquinans	2	INQ	Cornell, PSU	+	+	+	+	+	+
peltatum (2N)	1	PEL2	PSU	0	0	0	0	0	0
peltatum (4N)	4	PEL4	PSU	0	0	0	0	0	0
peltatum var. lateripes	1	LAT	Cornell	0	0	0	0	0	0
tongaense	1	TON	Cornell	+	0	0	+	0	0
transvaalense	1	TRN	Cornell	+	0	0	+	0	0
zonale	1	ZON	PSU	0	0	0	0	0	0
Interspecific hybrids									
BUR x HOR2	1	BXH	Cornell	+	+	+	+	+	0
HOR4 x PEL4									
('Veronica' x 'Yale')	1	HXP	PSU	+	+	+	+	+	+
Other *Pelargonium* species									
×*domesticum*	6	DOM	PSU	0	0	0	0	0	0
echinatum	1	ECH	PSU	0	0	0	0	0	0
×*stipulatum*	1	STI	Cornell	–	–	–	+	0	0
tragacanthoides	1	TRG	Cornell	–	–	–	+	0	0

[z](+) Present, (+ tr) present but <1.5%, (0) absent, (–) tissue specimen not available.
[y]C = Cornell collection via Ernie DeMarie, PS = Penn State collection vis Richard Craig.

Recently the section *Ciconium* has been expanded to include the former section *Dibrachya* (*P. peltatum*, PEL, and *P. peltatum* var. *lateripes*) and certain species once associated with the former section *Eumorpha* (*P. multibracteatum*, *P. alchemilloides*, and *P. elongatum*) (van der Walt and Vorster, 1981a). The center of diversity on the African continent of section *Ciconium* is not clear, although the section occurs "... in a belt along the east coast from the southwestern to the extreme eastern Cape Province, and then along the escarpment through Natal, Transvaal, and northwards to the Yemen..." (van der Walt and Vorster, 1981b). The sole exception to this general distribution of section *Ciconium* is the species *P. caylae*, an extremely rare species from the island of Madagascar. All specimens of *P. caylae* known to exist outside of Madagascar are believed to be derived from the same accession (P.J. Vorster, personal communication).

Investigations of a glandular trichome-mediated small pest-resistance phenomenon in the interspecific *P.* ×*hortorum* complex have been conducted. Gerhold et al. (1984) indicated that the presence of anacardic acids in glandular trichome exudate was responsible for the pest-resistance phenomenon. Walters et al. (1989) examined the anacardic acid composition of five pest-resistant and pest-susceptible diploid HOR inbreds and concluded that the presence of a single desaturation in the omega-5 (ω5) position was characteristic of the resistant phenotype, whereas susceptible phenotypes lacked ω5-desaturation and were largely saturated in composition. Walters et al. (1989) proposed that anacardic acids were formed from fatty acid precursors and identified ω5-fatty acids in resistant HOR leaves. Hesk et al. (1991) refined the chromatographic system of Walters et al. (1989) and demonstrated that up to 20 different anacardic acids are produced by HOR inbreds. Hesk et al. (1991) identified a 23:1 w6-anacardic acid, indicating the probable presence of a 17:1 w6 fatty acid precursor. This observation led Hesk et al. (1991) to propose the existence of a Δ11 fatty acid desaturase, rather than an ω5 desaturase. Grazzini et al. (1993) identified the unusual fatty acids, which Hesk et al. had predicted in glandular trichome extracts.

In this study, we investigate the distributions of anacardic acids and tall glandular trichomes among the species of the section *Ciconium* in an attempt to determine the variation of anacardic acid composition that might exist among *Pelargonium* species and to determine a potential origin for the pest resistance phenomenon. These studies lead us to propose the use of anacardic acids as chemotaxonomic characters in distinguishing a subsection within the section *Ciconium*. Further, we propose that the components of the small pest-resistance phenomenon investigated in HOR originated in INQ.

Materials and Methods

Individual plants of *Pelargonium* species were obtained from two sources (Table 1). In each case, we attempted to utilize accessions originally obtained from South African sources by Cornell Univ. and Pennsylvania State Univ. Cuttings were taken and rooted under mist. Plants were grown in a warm greenhouse with supplemental lighting (total daylength maintained at 16 to 18 h) and under a routine greenhouse fertilizer regime (200 ppm N weekly, supplied from Peters 15–16–17 Peat-lite Special). Irrigation and fertilization were done manually, using a hose with a

Table 2. Relative percentage anacardic acid composition of those species found to produce anacardic acids. Only major components are included in this table; therefore, totals may not sum to 100%.

		Major anacardic acid components (%)								
Species	Cultivar or inbred	22:1 ω5	24:1 ω5	23:1 ω6	22:0	23:0 anteiso[z]	24:0	24:1 ω9	24:2 ω6,9	UI[y]
ACE		1.3	0.0	0.7	7	2.7	3.1	50.4	31.6	115.6
BUR		0.8	0.0	0.9	9.1	5.7	3.2	29.9	43.4	118.4
FRU		25.9	14.4	2.4	13.8	4.7	5.8	10.6	17.1	87.5
HOR2	71-17-7 (mite-resistant)	38.9	41.7	4.8	4.9	2.0	1.4	0.0	0.3	86.0
HOR2	71-10-1 (mite-susceptible)	0.0	0.0	0.0	26.8	7.5	25.3	7.3	15.7	38.7
HOR2	F_1 (71-17-7 x 71-10-1)	45.9	21.3	6.3	5.1	7.7	3.6	0.0	1.9	77.3
HOR4	'Veronica'	34.4	32.5	2.9	5.2	4.5	4.8	2.4	14.9	102.0
INQ		33.2	45.8	2.1	2.6	2.7	1.9	0.5	4.2	90.0
HXP	F_1 ('Veronica' x 'Yale')	44.2	22.8	7.9	6.5	8.9	2.1	1.9	7.8	92.4
BXH		28.4	34.3	2.0	8.0	4.6	3.3	1.0	12.6	90.9

[z]The 16-carbon chain possesses a methyl branch at the ω3 position.
[y]UI = unsaturation index.

breaker nozzle and directing the stream of water to the base of the plant to minimize plant-to-plant transfer of anacardic acids. Plants were grown in 4-liter nursery pots filled with a loose potting mix (ProMix BX amended with coarse horticultural perlite, 3:1, v/v).

We attempted to screen a wide range of *Pelargonium* species, and utilized *Pelargonium* collections maintained at Pennsylvania State Univ. by. Richard Craig and at Cornell Univ. by Ernest DeMarie. The parents and primary interspecific hybrid (Laughner, 1986) between a tetraploid HOR4 and *P. peltatum* (PEL4) were included, as well as a selection of *P. ×domesticum* (DOM) breeding lines from the Penn State *Pelargonium* genetics program.

Chemical analyses. The analyses reported here used the method of Hesk et al. (1991) in which anacardic acids are extracted from the plant tissue in dichloromethane (DCM). The methylated anacardic acids were then separated with silica gel thin-layer chromatography (TLC) and visualized on the TLC plates with ultraviolet (UV). They were then eluted from the adsorbent and analyzed by isocratic reversed phase high-performance liquid chromatography (HPLC) using a 25-cm × 4.6-mm 5-μ LC8 DB column (Supelco, Bellefonte, Pa.) at a flow rate of 0.9 ml·min^{-1}. and UV detection at 212 nm. The dimethylated anacardic acid residues were dissolved in the HPLC mobile phase (490 isopropanol : 147 acetonitrile : 343 0.01 M acetic acid, by volume), before analysis.

It had been previously determined that inflorescences were a rich source of tall glandular trichomes and anacardic acids (Yerger et al., 1992). Tissue specimens consisted of whole inflorescences harvested when approximately one-third to one-half of the florets had opened. Open florets were removed by hand, and ≈15 to 20 g of the remaining inflorescence (composed of sepal, pedicel, peduncle, and unopened floret tissue) was extracted by dipping the plant tissue in DCM for 10 to 15 min. Fully expanded leaf specimens were extracted similarly.

Cross-contamination of plants within a greenhouse bench due to close physical proximity, handling, or vigorous overhead watering is always a possibility. In an attempt to avoid plant-to-plant contamination, we selected young tissue from the center of each plant and routinely took tissue samples using everted polyethylene bags. Plant and leaf samples thus collected were exposed only to the inside surface of the polyethylene bag during harvesting.

Unsaturation index (UI). For the purposes of comparison, we developed an unsaturation index (UI), which permitted the comparison of overall anacardic acid composition based on the number of double bonds in the alkyl side chain of the molecule. This index is similar in concept to that used in lipid chemistry (Mumma et al., 1971). The UI is calculated by multiplying the percentage of each individual component times the number of double bonds in the alkyl side chain, then summing the resultant values. As a formula: $UI = \sum (f \times n)$, where f = percent composition of an individual anacardic acid fraction and n = number of double bonds in the side chain.

Microscopic observation of plant surface. We observed trichomes on the surfaces of specimen plants of *Pelargonium* species with a standard binocular microscope at 25× magnification and used side-lighting to highlight any trichomes present. A visual comparison was consistently made to the resistant HOR2 inbred 71-17-7. Plants were observed periodically during the course of a year.

Results and Discussion

Anacardic acid content of Pelargonium *species.* Of the *Pelargonium* species currently considered to be distinct, only three species (INQ, ACE, FRU) had any detectable anacardic acids (Table 1). These three species belong to the section *Ciconium*. BUR (*P. ×burtonii* = *P. stenopetalum*, a hybrid complex believed to result from the hybridization of ACE and ZON); HOR2 (diploid *P. ×hortorum*); HOR4 (tetraploid *P. ×hortorum*); BXH (the primary hybrid between BUR and an unknown selection of HOR2); and HXP (the primary hybrid between HOR4 'Veronica' and PEL4 'Yale') also contained anacardic acids.

It is relevant to note that one of the species (ZON) implicated as having a primary role in the development of HOR was completely lacking in anacardic acids. The species PEL2, formerly assigned to section *Dibrachya*, and its presumed close relative, *P. tongaense* (TON), both lacked anacardic acids as did *P. acraeum* (ACR), which is often proposed to be the progenitor of both INQ and ZON (van der Walt and Vorster, 1981a). None of the PEL4 or DOM cultivars or DOM breeding lines surveyed contained detectable anacardic acids.

These results suggest that the biosynthetic capacity to produce anacardic acids in tall glandular trichomes only exists in a small number of members of the section *Ciconium*. Anacardic acid biosynthesis may have evolved in INQ, or in a progenitor species from

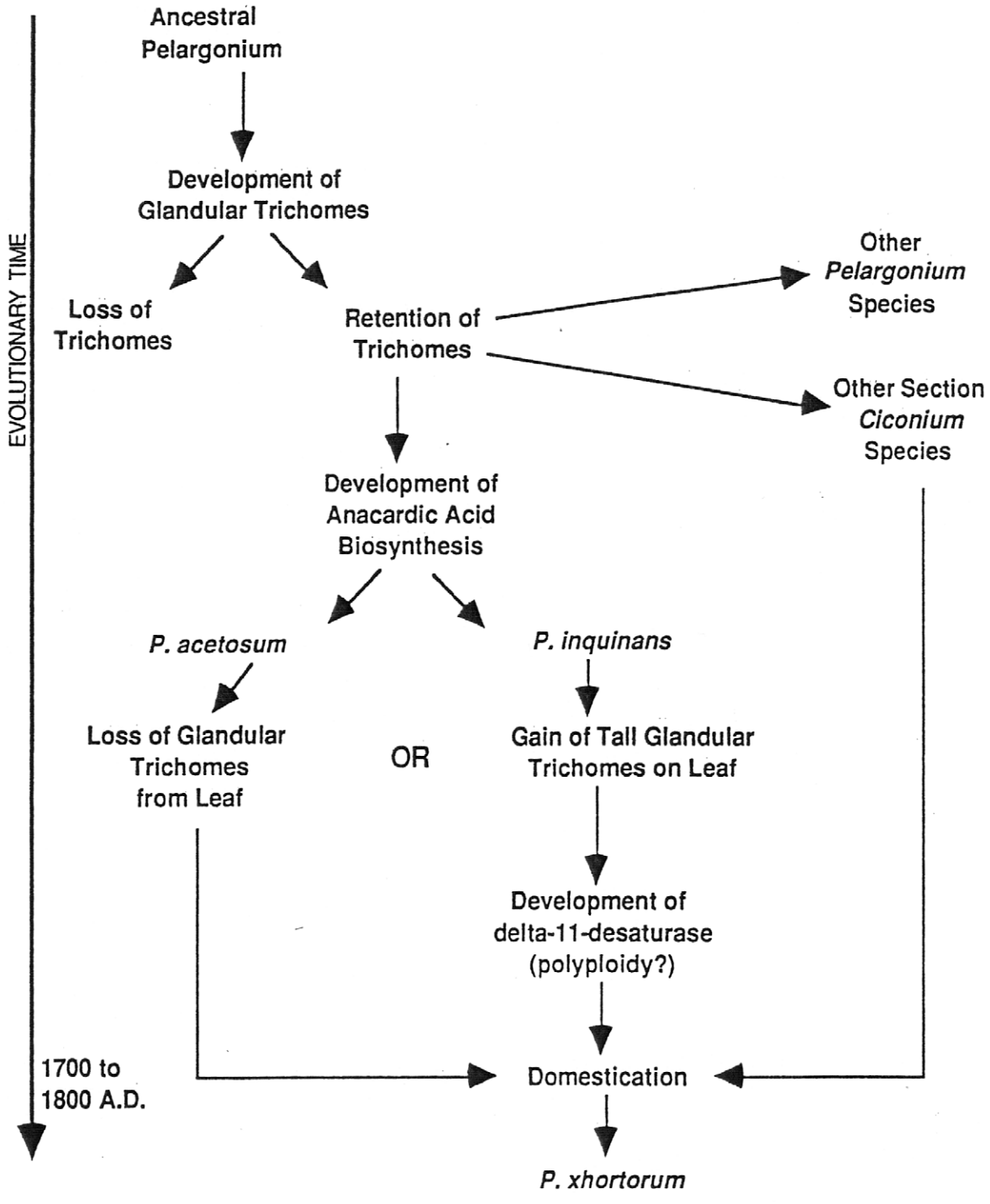

Fig. 1. Proposed evolutionary scheme of *Pelargonium* section *Ciconium* based on anacardic acid composition.

which INQ, ACE, and FRU evolved. It is interesting to note that ACR is proposed (van der Walt and Vorster, 1981b) as a possible progenitor species to both INQ and ZON, yet ACR and ZON lacked anacardic acids in our studies. It is also important to note that we sampled a limited number of accessions for each of the species used in these studies. Both ZON and PEL are reported to have nonglabrous (and presumably tall glandular trichome-bearing) types in their native distributions (van der Walt, 1977). However, the ZON and PEL accessions observed in these studies were glabrous, and neither produced detectable amounts of anacardic acids. We believe that the accessions in our collection are indeed representative of the species type. Individual accession descriptions closely match those described in the literature. Furthermore, field observations in South Africa (R.O. Mumma, personal observation) of ACE, INQ, and ZON indicated that the accessions of the species used for this report are indeed representative.

It should also be noted that FRU is highly polymorphic and hybridizes freely with INQ and HOR2. The FRU accession analyzed displayed many characteristics of INQ and HOR2, including the presence of ω5-anacardic acids. It is possible that the FRU accession examined was not representative of the species; however, it was the only specimen available.

Tissue-specific anacardic acid distribution. We observed that in some species (ACE, BUR, FRU, and BXH), anacardic acids

were present only in the extracts made from inflorescences (Table 1). From leaf extracts of these species and species hybrids, no anacardic acids were observed. In other species of the section *Ciconium* (namely INQ and HOR), anacardic acids were present in leaf and inflorescence extracts.

In species producing anacardic acids, the tissue-specific anacardic acid distribution almost completely parallels the distribution of tall glandular trichomes. In INQ and HOR, species in which tall glandular trichomes are found on leaf and inflorescence surfaces, anacardic acids are also found in those tissues. In ACE and BUR, which are glabrous and lack tall glandular trichomes on the leaves, there are no anacardic acids from leaf extracts. However, from ACE and BUR inflorescences, on which there are an abundance of tall glandular trichomes, there is also an abundance of anacardic acids.

FRU, however, is an exception. In the accession examined of this species, tall glandular trichomes were observed on both leaf surfaces and on the petiole. However, no anacardic acids were found in these tissues. The only visibly noticeable trichome exudate in FRU tissues was observed on inflorescence tall glandular trichomes and those tissues contained anacardic acids. A similar situation occurred with the complex interspecific hybrid BXH. Tall glandular trichomes were observed on the leaf surface but no anacardic acids were detectable in leaf extracts.

Glandular trichomes the approximate height of the tall glandular trichomes of the reference HOR2 inbred (71-17-7) were observed on the surface of other members of the section *Ciconium*, regardless of the presence of anacardic acids (Table 1). Some of these trichomes secreted an obvious exudate; however, at the detection limits of our analyses, these exudates did not contain anacardic acids. ZON pedicels were observed to be densely covered with glandular trichomes that were the height of the short glandular trichomes observed on resistant and susceptible HOR2 inbreds. However, in contrast to the short glandular trichomes of HOR2 inbreds, these ZON pedicel short glandular trichomes exuded an abundant clear, nonadhesive, fluid exudate. Two-spotted spider mites (*Tetranychus urticae* Koch) were observed to move freely among the ZON pedicel glandular trichomes without noticeable reaction to the ZON exudate, even when the exudate appeared to completely cover the mite. This response to exudate contact is dramatically different from the response to HOR tall glandular trichome exudate.

Anacardic acid composition of Pelargonium *species.* Anacardic acid composition differed among species (Table 2). The presence of ω5-unsaturated anacardic acid as a primary component characterized INQ and resistant HOR2 and any hybrid containing HOR in its parentage. ACE and BUR contained abundant anacardic acids, but only trace amounts of 22:1 ω5-anacardic acid and no 24:1 ω5-anacardic acid. FRU contained ω5-anacardic acid but not in the abundance of INQ and HOR. FRU appeared to have an anacardic acid composition intermediate between INQ and ACE, containing substantial amounts of ω5-anacardic acids (25.9%) but also the elevated levels of the ω6,9- and ω9-anacardic acids (17.1% and 10.6%, respectively) characteristic of ACE, BUR and many commercial diploid and tetraploid *P.* ×*hortorum* cultivars (Grazzini et al., 1995).

The anacardic acid composition of BXH contained ω5-anacardic acids (28.4%), like resistant HOR2, and ω6,9-anacardic acid (12.6%), like susceptible HOR2, ACE and BUR. In contrast to ACE and BUR, however, BXH contained only trace levels of the ω9-anacardic acid (1.0%) found in abundance in ACE and BUR.

Resistant HOR2 71-17-7 and INQ contained almost no 24:1 ω9-anacardic acid in contrast to ACE, which contained >50% of its total anacardic acid content as the 24:1 ω9-component. This anacardic acid is produced from the elongation of an oleic acid (C18:1 ω9) precursor. In spite of the lack of oleic acid incorporation into anacardic acids, resistant HOR2 contains significant amounts of oleic acid in all tissues examined, including glandular trichomes (Grazzini, 1993).

None of the species observed contained the high amounts of saturated anacardic acids characteristic of the mite-susceptible HOR inbreds examined by Walters et al. (1989). Of the species examined, FRU contained 13.8% 22:0 saturated anacardic acids, but this amount was little more than half of the amount found in susceptible HOR extracts (26.8%). Similarly, no other anacardic acid-containing species produced >6% 24:0 saturated anacardic acid, yet susceptible HOR inbreds contained >25%.

UIs were calculated for the anacardic acid-containing species and compared to the HOR2 inbreds and the F_1 hybrid between them (Table 2). The UI values ranged from 87.5 for INQ to 118.4 for BUR. It is interesting to note that the highest UI values (and therefore the highest relative amount of unsaturation) observed were for BUR and its putative parental species, ACE (UI = 115.6). ACE and BUR are succulent *Pelargonium* species, with glandular trichomes only on the inflorescences. The high UI values observed would suggest that ACE and BUR have very fluid trichome exudates at moderately warm temperatures.

The UI for the susceptible HOR2 inbred, 71-10-1, is very low (UI = 38.7) in comparison to the resistant 71-17-7 (UI = 86). The UI decreases to UI = 71 in the hybrid of these two lines. This change reflects the increased content of saturated anacardic acids present in the F_1, an observation that is not readily apparent because of the dramatic heterotic increase in 22:1 ω5 content.

The origins of the components of small pest resistance in Pelargonium. The ω5-anacardic acid characteristic of the small pest-resistant phenotype in our genetic studies appears to have its origin in INQ. We observed this characteristic anacardic acid almost exclusively in INQ and HOR (diploid and tetraploid). Of the other anacardic acid-synthesizing species, only tall glandular trichome exudate from FRU inflorescences contained any appreciable ω5-anacardic acid. This exudate composition may be representative of FRU as a species, or it may indicate that the particular FRU accession analyzed is an interspecific hybrid with INQ and HOR. Other INQ-like characters (upright habit, flower color, tall glandular trichomes) lead us to speculate that the FRU accession examined is an interspecific hybrid, and the relationship of FRU to the other members of section *Ciconium* will need to be examined further.

The species ACE and BUR appear to be closely related. BUR is proposed by some taxonomists to be a natural hybrid between ACE and ZON (van der Walt and Vorster, 1981a). From the anacardic acid composition, and the tall glandular trichome distributions, we conclude that ACE and BUR appear to be closely related and that BUR may be a derivative of ACE. This is also supported by the presence in BUR and ZON (but not ACE) of strong UV-absorbing bands on the TLC plate used to isolate monomethylated anacardic acids. The identity of the strong UV-absorbing bands is not known.

The presence of tall glandular trichomes on the adaxial and abaxial leaf surfaces also appears to be characteristic of INQ. In none of the other *Ciconium* species observed were there tall glandular trichomes on leaf surfaces and inflorescence surfaces. Thus, it appears that the presence of tall glandular trichomes on the leaf surface is an INQ characteristic that was transferred to HOR in a dominant fashion, since tall glandular trichomes have been observed on all HOR accessions examined (>250 different cultivars and inbreds) (Grazzini et al., 1995).

It is interesting to note the differences (Table 2) in relative composition among species, and in species hybrids. In INQ and HOR, there is almost no 24:1 ω9 anacardic acid. In ACE and BUR, this component is abundant. In the hybrid BXH, the 24:1 ω9-anacardic acid accounts for only one percent of the total. This may indicate that INQ and HOR lack the ability to incorporate 18:1 ω9-fatty acids into 24:1 ω9-anacardic acids, or lack the capacity to transport ω9-fatty acids into an appropriate compartment for incorporation into anacardic acids. This result may also simply indicate that the two desaturation systems are competing for substrate in the same localized pool of lipid precursors and that the ω5-desaturation system has a much higher affinity for the saturated fatty acid substrate. The interspecific transfer of this phenomenon from HOR or INQ appears to be dominant or partially dominant, since the interspecific hybrid BXH resembles HOR and INQ in that it produces very low amounts of 24:1 ω9-anacardic acids.

In any case, the interspecific HOR complex can be used as a practical source of tall glandular trichome anacardic acid-mediated small pest resistance. These mechanisms can be transferred within the section *Ciconium* via interspecific hybridization. Tracking the movement of this resistance mechanism should be as simple as visually observing tall glandular trichomes with a visible and fluid orange exudate.

Furthermore, there appears to be significant variation in anacardic acid composition within the *Pelargonium* species surveyed. This suggests that these species may be important as sources of economically important lipid biosynthesis genes, novel allelochemicals, and the biosynthetic genes producing those allelochemicals.

These studies suggest that the section *Ciconium* can be further subdivided into anacardic acid producers (INQ, ACE, FRU) and nonanacardic acid producers (all of the remaining species). We propose to further examine the biochemical and molecular relationships in the section *Ciconium*.

Possible evolutionary origin of mechanism. An examination of the *Pelargonium* species distribution of anacardic acids (Tables 1 and 2) indicates that anacardic acid production is limited to a very small number of species. Anacardic acids were only found in ACE, FRU, and INQ of the sixteen wild species examined. HOR has INQ as a presumed ancestor. Similarly, the ω5-*cis* double bond that is characteristic of the anacardic acids found in the glandular trichome exudate of the resistant phenotype in inbred lines of the HOR complex is limited to INQ. This evidence suggests that the ω5-desaturation mechanism originated in INQ or its ancestor.

The presence of glandular trichomes on various plant surfaces seems to be common throughout the genus *Pelargonium*. However, the leaf trichome-specific production of anacardic acids appears to only occur in INQ. Glandular trichomes of similar size do not produce anacardic acid-rich exudate in other species.

Unique genes appear to be present in INQ that provide the three components of the pest-resistance mechanism: anacardic acids, ω5-fatty acids (the precursors of ω5-anacardic acids), and tall glandular trichomes. INQ and ACE produce anacardic acids, indicating that anacardic acid biosynthesis in *Pelargonium* may have evolved in the immediate progenitor to these two extant species. ACR is occasionally referenced as a possible progenitor; however, based on the absence of anacardic acids in any tissue of ACR examined, this may not be likely unless ACR lost the potential to produce anacardic acids after ACE and INQ diverged.

The unique presence of exudate-producing glandular trichomes on the leaves of INQ and its corresponding absence in ACE may indicate either the retention of foliar glandular trichomes in INQ from an ancestral species or a loss of such trichomes in the more succulent and more xerophytic ACE. Tall glandular trichomes and the parallel anacardic acid production in ACE only occurs in floral tissues, and more specifically, only on peduncles, pedicels and sepals, at least in the plant biotypes that we examined. It is interesting to note that the UI (the number of double bonds per mole of the hydrocarbon chain of the anacardic acid) of ACE is among the highest observed in any species or cultivar, in spite of the fact that ACE is xerophytic. An exudate with a high UI indicates a very fluid exudate, and therefore one that is not likely to be particularly effective as a sticky trap. Such an observation leads one to speculate on the action of such a highly fluid exudate. Certainly, in a dry and xerophytic environment, the chance occurrence of exudate removal by falling rain (and subsequent pest susceptibility) is minimal. Perhaps a fluid exudate facilitates adhesion of the exudate to the exterior of a pest, and subsequent spreading of the exudate over the surface of the pest, enhancing penetration of any bioactive components in the exudate through the epidermis of the pest. Grazzini et al. (1991) reported that both crude trichome exudate and purified anacardic acids inhibited the activities of lipoxygenase and prostaglandin endoperoxide synthase, enzymes known to be involved in insect reproduction.

It is possible to consider the presence of tall glandular trichomes and anacardic acids as dominant markers of natural hybridization. Reports of hirsute PEL and ZON exist (van der Walt, 1977). From our limited observations, we would anticipate the presence of anacardic acids in these hirsute types and suggest that both of these related characters have introgressed from outcrossing to INQ.

The relatively widespread distribution of glandular trichomes of at least two distinct height classes across much of the genus *Pelargonium* suggests that this character evolved early in the development of the genus and was retained by many of the developing species. Alternatively, the very narrow species distribution of anacardic acid biosynthesis suggests that this pathway may have evolved late in the development of the genus and after the ancestor of ACE and INQ had already begun to diverge from the remainder of the genus.

The development of the Δ11(ω5)-fatty acid desaturase appears to be a very recent evolutionary event, since it would seem to provide such a strong selective advantage to any individual in which it was present, and since genetic transmission of the character is strongly dominant. Since this trait is only present in INQ and has not yet been incorporated into other species hybrids, it must have developed relatively recently. If the Δ11(ω5)-desaturase is a modification of a preexisting desaturase system (and we believe that it is), a likely time for this event to have happened would have been subsequent to the occurrence of polyploidy in the basic genus. Gibby and Westfold (1983) suggest that the section *Ciconium*, which has a haploid complement of nine, may be derived from allotetraploidy between species of 2n = 8 and 2n = 10. Such an event would provide gene duplication for one of the basic desaturases, the duplicated gene could have then developed an altered substrate specificity and resulted in the development of a unique desaturase. One possible model for the evolution and retention of these characters through speciation and the eventual domestication that resulted in HOR is presented in Fig. 1.

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