Host Recognition by the Blackmargined Aphid (Homoptera: Aphididae) on Pecan[1]

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ABSTRACT Leaf discs from pecan, *Carya illinoensis* (Wangenh.) K. Koch, pea, *Pisum sativum* L., peach, *Prunus persica* (L.) Batsch, and fig, *Fiscus benjamina* L. were presented to nymph and adult blackmargined aphids, *Monellia caryella* (Fitch) in no-choice and choice bioassays. Nymph longevity and developmental rates, and adult longevity and reproductive rates were significantly greater when aphids were placed on pecan than on pea, peach or fig. In no-choice bioassays, both nymph and adult aphids preferred to settle-on pecan, while they preferred to wander- or settle-off of pea, peach and fig. In choice bioassays, both nymph and adult aphids preferred to settle-on pecan than to settle-on pea, peach and fig. Analysis of pecan, pea, peach and fig leaf cuticular chemistry showed that n-alkane distribution patterns and the major cuticular components, specifically triterpenes and their oxidation products, differed among the four plant species. This study provides the first evidence within the pecan/aphid interactive system which suggests that the distinct foliar cuticular chemistry of pecan may have an influence on the host recognition behavior of *M. caryella*.

KEY WORDS Blackmargined aphid, *Monellia caryella,* Aphididae, pecan, host recognition, host selection, host-plant preference, leaf surface chemistry, cuticular chemistry.

Pecan, *Carya illinoensis* (Wangenh.) K. Koch, harbors a complex of three pecan aphid species: The blackmargined aphid, *Monellia caryella* (Fitch), the yellow pecan aphid, *Monelliopsis pecanis* Bissell, and the black pecan aphid, *Melanocallis caryaefoliae* (Davis). These aphid species are host specific, confined to *Carya* species within the Juglandaceae family of nut trees (primarily *C. illinoensis*), and are highly specialized as to the pecan plant tissue utilized. These aphid species, each occupying a specific niche on the leaflet, feed in the vascular system of compound leaves from the lower (abaxial) leaf surface and in veins of a particular size (Tedders 1978). Furthermore, their seasonal dynamics coincides closely with leaf phenology. It is speculated that aphid population peaks coincide with an abundance of highly nutritious young and senescent foliage in early- and late-season, respectively, while the mid-season aphid population crash coincides with the presence of nutritionally poorer mature leaves. In order to understand what mechanisms of plant origin might govern the aphid/pecan host plant specificity, and the patterns of pecan host selection

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and resource use by this complex of aphid species, initial studies were focused on the dynamics of leaflet, rachis, and nut volatiles, as well as cuticular and internal chemistry, as a function of tree developmental phenology (Smith et al., 1990a,b). Results from these studies showed pecan to be both spatially and temporally dynamic, varying chemically among the plant tissues and during the developmental stages from bud break to leaf senescence. Therefore, pecan plant chemistry could assist these aphid species in various aspects of their host selection processes, including host-finding, host-recognition and host-suitability or utilization, as well as govern, in part, their seasonal population dynamics.

Secondary plant substances have been viewed as 'flags' by which host-specific aphids recognize their host plants (Van Emden 1978). Dixon (1985) stated that these substances often act as barriers to the colonization of plants by many pathogens and herbivores, but enable aphids to recognize their host plants. This study was undertaken to test the hypothesis that pecan foliar cuticular chemistry functions in the host recognition process of *M. caryella.*

In this study, several plant species from three plant families and distantly related to pecan only at the class level (Dicotyledoneae); pea *(Pisum sativum* L.), peach *(Prunus persica* (L.) Batsch) and fig *(Fiscus benjamina* L.*)* were investigated. The objectives were: (1) to compare the developmental processes of nymph (longevity and developmental rate) and adult (longevity and reproductive rate) *M. caryella* on pecan, pea, peach and fig, under no-choice and choice (preference) conditions, as a measure of the host recognition and host suitability (utilization) processes; (2) to compare the behavioral activities of nymph and adult M. *caryella* on pecan, pea, peach and fig, under no-choice and choice (preference) conditions, as a measure of the host recognition processes; (3) to compare the foliar cuticular chemistry of pecan, pea, peach and fig as an indication of what cuticular chemistries may be involved in mediating host recognition by *M. caryella.*

Materials and Methods

Insect Rearing. A blackmargined aphid colony, developed from a single F_1 nymph of a single stem mother, was maintained in the laboratory on caged pecan plants held at 24°-27°C, under a 13-h photophase and 11-h scotophase. The pecan plants (Curtis variety), grown from seed in a greenhouse and then acclimated to colony conditions for a minimum of one week prior to use, were periodically transferred into colony cages, thereby providing a continuous supply of a high quality food source.

Leaf Material. Healthy, fully expanded leaves from greenhouse grown pecan, pea, peach and fig were harvested separately between 8.00 h and 9.00 h on the day the experiment was initiated. Leaf material utilized for bioassay was prepared in accordance with a detached-leaf method developed to study pecan aphid biology and behavior (Reilly and Tedders 1990). Following a 5 min wash with deionized water, leaf discs (ca. 3 cm x 3 cm square) were excised from whole leaves, gently washed in 1:3 chlorox:water for 2 min and then rinsed four times with sterile deionized water. The leaf discs were placed on water-agar petri plates (60 mm x 15 mm in no-choice tests and 100 mm x 15 mm in choice tests). A leaf disc of each plant species was placed in a petri plate in the no-choice test, while one leaf disc of each plant species was placed in a petri plate

in the choice tests. Preliminary experiments, using all four plant species, showed that the leaf sterilization procedure had no effect on nymph longevity and developmental rate, or any effect on adult longevity or reproductive rate. Leaves from all four species to be utilized for surface extraction were immediately placed in deionized water to maintain leaf turgor prior to surface extraction. Leaves for each plant species were processed separately so as to avoid cross-contamination.

Experimental Protocol. First instar nymph and 24 ± 12 h old adult *M. caryella* were removed from the colony. Five nymphs or one adult *M. caryella* were randomly assigned to and placed on the leaf disc in each plate in the no-choice tests. One nymph or one adult of *M. caryella* (four aphids per plate) were randomly assigned to and placed on each leaf disc in each petri plate in the choice tests. There were ten replicates for both nymphs and adults. Blank plates containing only water-agar and no leaf disc were also prepared as described. The petri dishes were maintained in an inverted position to reduce problems associated with condensation and water in the petri dish, and to allow aphids to feed in their natural orientation. The petri dishes were maintained in an environmental chamber held at 21-22°C, under a 15-h photophase and 9-h scotophase.

As a measure of host recognition (acceptance/rejection) and host suitability (suitability of the host plant to sustain aphid growth and reproduction), nymph longevity and developmental rate (number of days to develop from a first instar nymph to adult), and adult longevity and reproductive rate (number of offspring per female per day), were recorded daily. Offspring were also removed and discarded daily. In addition to these developmental processes, aphid behavioral activity (settled vs. wandering) and position (on vs. off leaf disc) were also recorded as follows: in the no-choice tests, behavioral observations were recorded three times daily (at 9.00, 12.00 and 15.00 h) for 5 min at 30 sec intervals for nymphs and for 5 min continuously for adults; in the choice tests, behavioral observations were recorded three times daily (at 9.00, 12.00 and 15.00 h) for 10 min continuously for both nymphs and adults. Data were analyzed by an analysis of variance, with mean separation performed utilizing Duncan's multiple range test.

Chemical Analysis. Leaf material utilized for surface extraction was weighed and surface area measured using a LI-3000 area meter (LI-COR, Inc., Lincoln, NE). Leaf surface extract for each plant species was obtained by dipping leaves individually for 10 sec each in 170 ml of methylene chloride (Burdick and Jackson distilled in glass grade) (Severson et al., 1984).

The cuticular extracts were reduced to 25 ml volume. A portion of the extract (pecan, 7.3 cm^2 equivalent; fig, 8.4 cm^2 equivalent; peach, 4.4 cm^2 equivalent; and pea 1.3 cm^2 equivalent) was taken to dryness under N_2 in a micro-autosampler vial, and treated with 50 ml of 1:1 bis(trimethylsily)trifluoroacetamide: dimethylformamide (BSTFA/DMF, Pierce Chemical Company), a silyation reagent, to convert alcohols and acids to their silylethers and silylesters. The vial was capped and heated for 45 min at 76°C. After cooling to room temperature, about 1 ml injections were analyzed with a HP5880 gas chromatograph equipped with splitless injector (injector temp. 250°C, purge activation time 1 min) and flame ionization detector. A 0.3 mm (i.d.) x 30 m thin film bonded

SE54 fused silica column was used with a hydrogen carrier gas flow rate of 42 cm/sec linear velocity. Column temperature was held at 100°C for 1 min, then programmed to 160°C at 10°/min, then to 300°C at 5°/min and held for 10 min at 300°C.

A 10-20 ml portion of the cuticular extract was taken to dryness, dissolved in hexane and chromatographed on a micro silicic acid column (0.5 g of 100-200 mesh Unisil silicic acid in a 5.75 in S/P dispo transfer pipet). After packing, the columns were initially washed with methylene chloride/acetone, 4:1, and reactivated overnight at 150°C. Hydrocarbons were eluted with 5 ml of hexane, followed by 5 ml of methylene chloride/acetone, 4:1, to elute the polar components. Each fraction was then taken to dryness under N_2. The hydrocarbon fraction was dissolved in iso-octane for GC analysis. The polar fraction was dissolved in methylene chloride, a portion transferred to a micro autosampler vial, taken to dryness, treated with 1:1 BSTFA:DMF and then subjected to GC analysis.

The fractions were also analyzed with a Hewlett-Packard 5985B GC/MS system modified as described by Arrendale et al. (1984). Standard hydrocarbons, fatty alcohols, fatty acids, A- and B-amyrin and sterol mixtures were used to confirm GC retention and GC/MS data and aid in the identification of cuticular components. Relative levels of leaf surface components were calculated from peak areas assuming unitary chromatographic response.

Results and Discussion

Developmental Processes. In the no-choice tests, *M. caryella* nymph and adult longevity was significantly greater when placed on pecan than when placed on pea, peach, fig or blank plates (Figures 1 and 2, respectively). *M. caryella* nymphs developed from the first instar to adult in an average of 10.5 da when placed on pecan, while no nymphs developed to adult when placed on pea, peach, fig or blank plates (Figure 1). Similarly, adult *M. caryella* reproductive rate averaged 2.2 nymphs/female/da on pecan, while no adults reproduced on pea, peach, fig or blank plates (Figure 2). In the choice tests, *M. caryella* nymph and adult longevity averaged 5.8 da and 11.0 da, respectively; reduced from their longevity of 9.3 da and 25.5 da, respectively, measured in the no-choice tests. *M. caryella* nymphs developed from the first instar to adult in an average of 9.3 da. *M. caryella* adult reproductive rate averaged 1.0 nymphs/female/da; reduced from 2.2 nymphs/female/da measured in the no-choice tests.

Behavioral Activity. *M. caryella* nymphs were observed significantly more often, and adults spent significantly more time settled-on pecan than they were observed or spent, respectively, wandering-on, settled-off or wandering-off pecan (Figures 3a and 4a, respectively). In sharp contrast, *M. caryella* nymphs were observed significantly more often, and adults spent significantly more time, settled-off and wandering-off pea, peach, and fig than they were observed or spent, respectively, settled-on or wandering-on these respective plant species (Figures 3b, c, d, and 4b, c, d, respectively). In the choice tests, *M. caryella* nymphs and adults spent significantly more time settled-on pecan than they spent settled- or wandering-on or -off pea, peach and fig, or wandering-on pecan (Figures 5 and 6). Additionally, of the 10-min observation periods where *M. caryella*

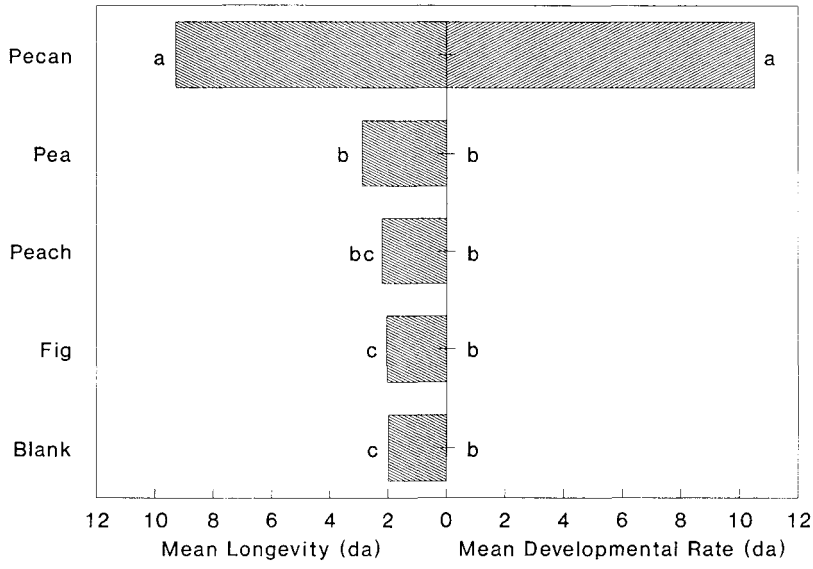


Fig. 1. *Monellia caryella* nymph longevity and developmental rate on pecan, pea, peach, and fig, and in blank plates in no-choice tests[1].
[1]Horizontal bars labelled with the same letter(s) are not significantly different at P = 0.05.

nymphs and adults settled-on pecan, they spent, respectively, 99.3% and 97.7% of their time engaged in this behavior. Furthermore, *M. caryella* nymphs and adults wandered-on pecan less than one percent of the time (0.16% and 0.42%, respectively) and of the 10-min observation periods in which they did so, they engaged in this behavior only 16.7% (1.67 min) and 15.8% (1.58 min) of the time, respectively.

Chemical Analysis. The cuticular extract components were fatty acids, fatty alcohols, hydrocarbons, sterols and triterpenoid alcohols and acids (Figure 7a, b, c and d; identifications in Table 1). Major components identified in the cuticular extract of pecan leaves (Figure 7a) were hexadecanoic acid (peak 4), n-hentriacontane (peak 20), β-amyrin (peak 28), α-amyrin (peak 29), an uncharacterized triterpenol (M.W. 426, peak 30) and 24-methylenecycloartenol (peak 32). Other lower level triterpenes identified were hydroxy-amyrins (peaks 33 and 34), oleanolic acid (peak 36) and ursolic acid (peak 37). The major components identified in the cuticular extract of fig leaves (Figure 7b) were n-nonacosane and triterpenols. The most abundant components of fig leaves were lanosterol (peak 31) and three triterpenols also present in the pecan extract, β-amyrin (peak 28), α-amyrin (peak 29) and the unidentified terpene alcohol (peak 30). The major fatty alcohols identified in peach leaves were 1-hexacosanol (peak 17), 1-octacosanol (peak 21), 1-triacontanol (peak 26), 1-dotriacontanol (peak 35) and 1-tetratriacontanol (peak 38), and are in agreement with those characterized by Baker et al. (1979). The cuticular triterpenes identified

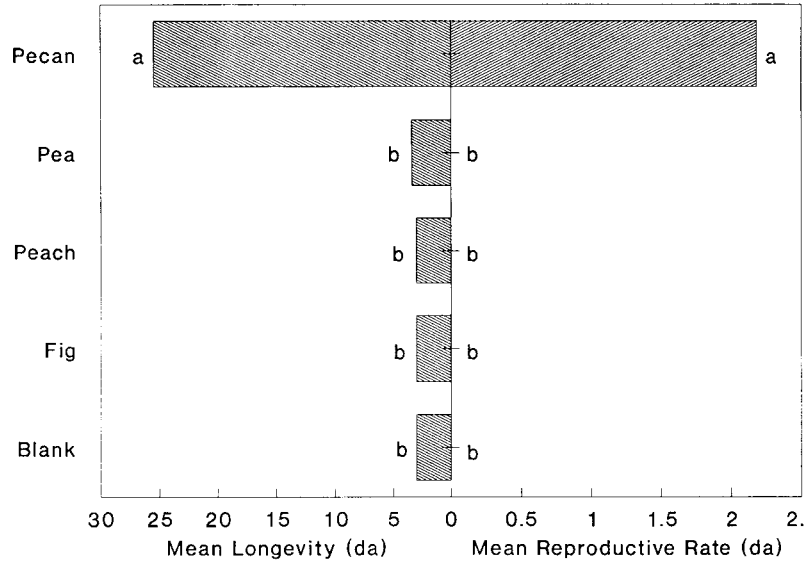
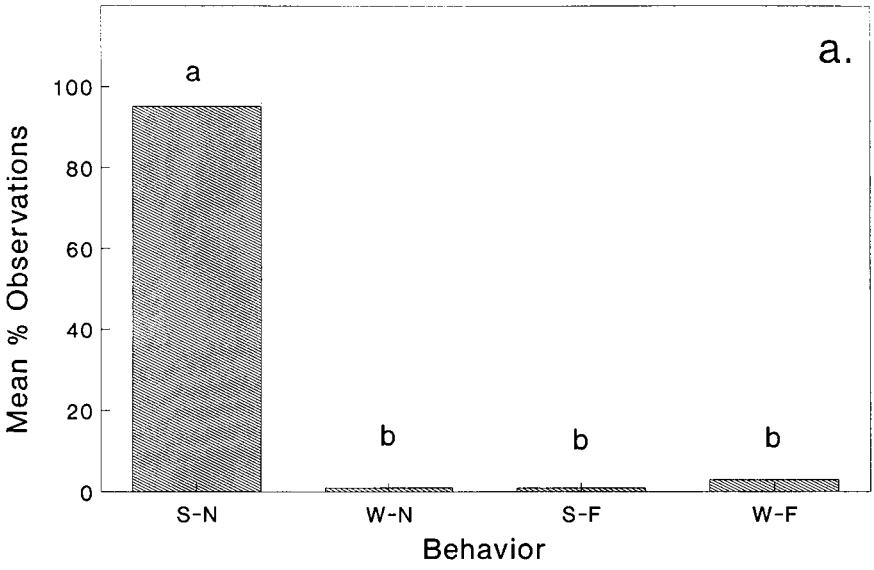


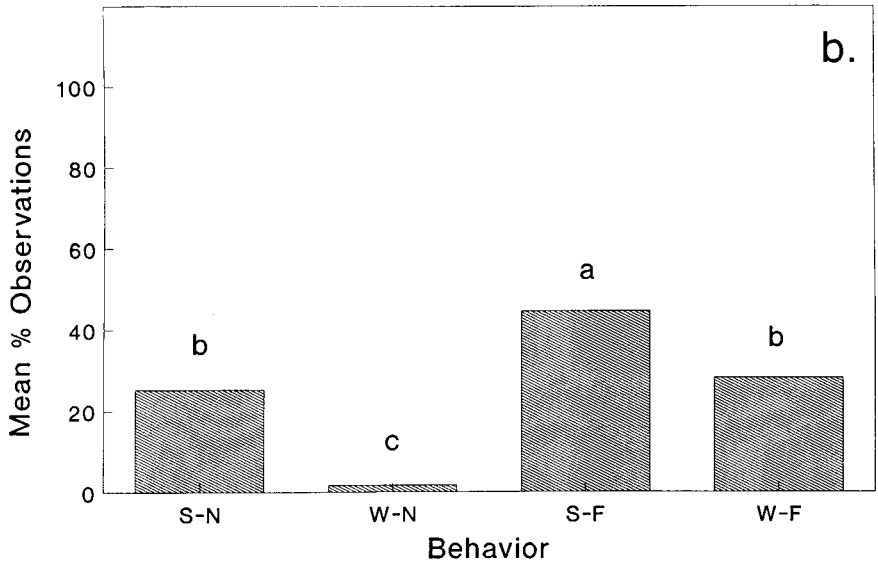
Fig. 2. *Monellia caryella* adult longevity and reproductive rate on pecan, pea, peach, and fig, and in blank plates in no-choice tests[1].
[1]Horizontal bars labelled with the same letter(s) are not significantly different at P = 0.05.

were oleanolic acid and ursolic acid and their monohydroxy derivatives. In agreement with Marcy and Barber (1970) and Kolattukudy (1969), the major classes of compounds identified in the cuticular extract of pea leaves were hyrdrocarbons and fatty alcohols. The hydrocarbon fraction from silicic acid chromatography yielded composition data given in Table 2. The pecan and peach hydrocarbon fraction contained C_{25} to C_{35} normal chain hydrocarbons with n-hentriacontane (C_{31}) being the most abundant. The fig hydrocarbon isolates contained C_{23} to C_{35} homologs with n-nonacosane (C_{29}) being the major component. The principle component of the pea hydrocarbon fraction (C_{25} - C_{34}) was n-hentriacontane

Comparison of Behavior. Most aphid species are autoecious, living on one or a few species of a particular genus of plants (Eastop 1973). Although host-alternating or heteroecious species are classified as polyphagous, it is noteworthy that most of them live only on one species of plant at a time, thereby they are sequentially monophagous. Thus, most aphids show a very high degree of host specificity (Dixon 1988), with truly polyphagous species (e.g., *Myzus persicae)* being rare.

It can be expected that there will be a strong parallelism between the evolutionary development towards a monophagous way of living and the dependance on specialized signal systems between host plant and insect (Patterson 1973).





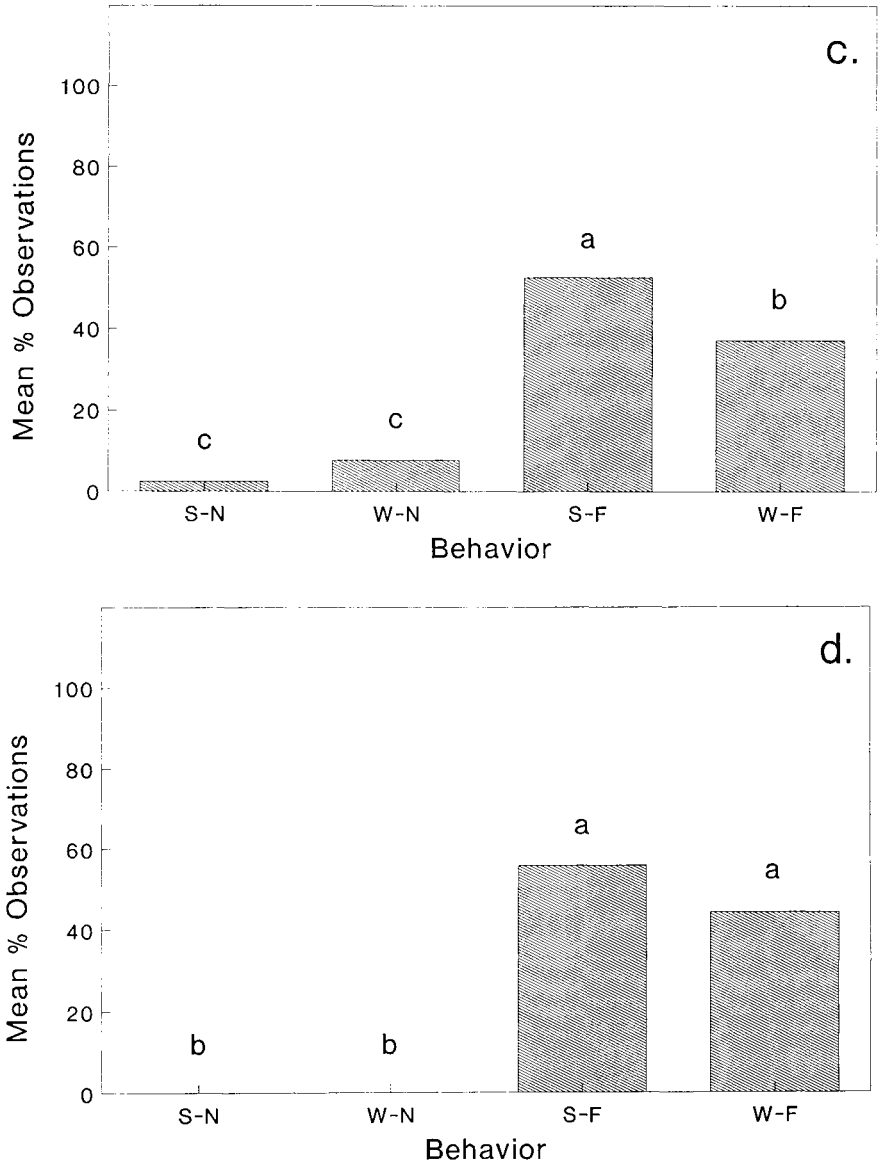
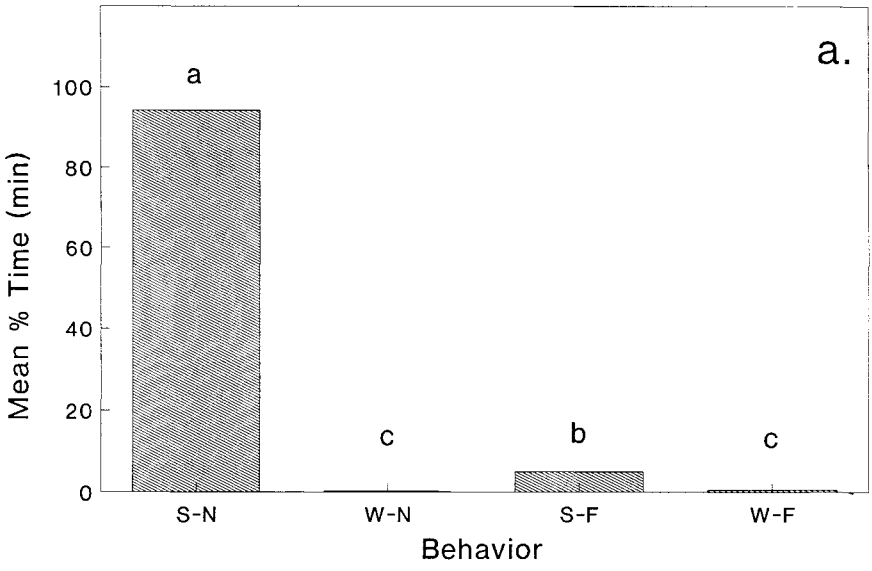


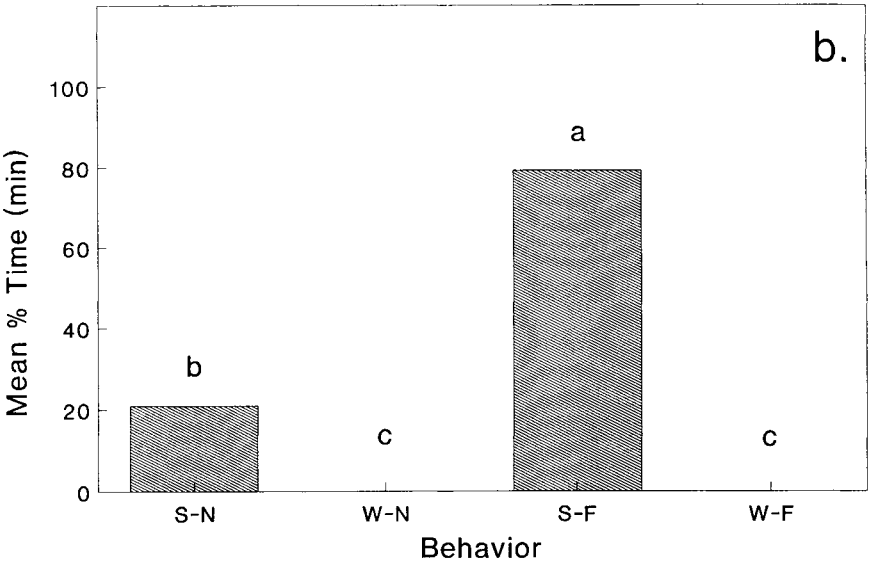
Fig. 3. *Monellia caryella* nymph behavior on (a) pecan, (b) pea, (c) peach, and (d) fig in no-choice tests[1,2,3].

[1]Vertical bars labelled with the same letter(s) are not significantly different at P = 0.05.

[2]Behavior: S-N=settled-on disc; W-N=wandering-on disc; S-F = settled-off disc; W-F = wandering-off disc.

[3]Total observations: (a) pecan = 1,025; (b) pea = 163; (c) peach = 64; (d) fig = 84.





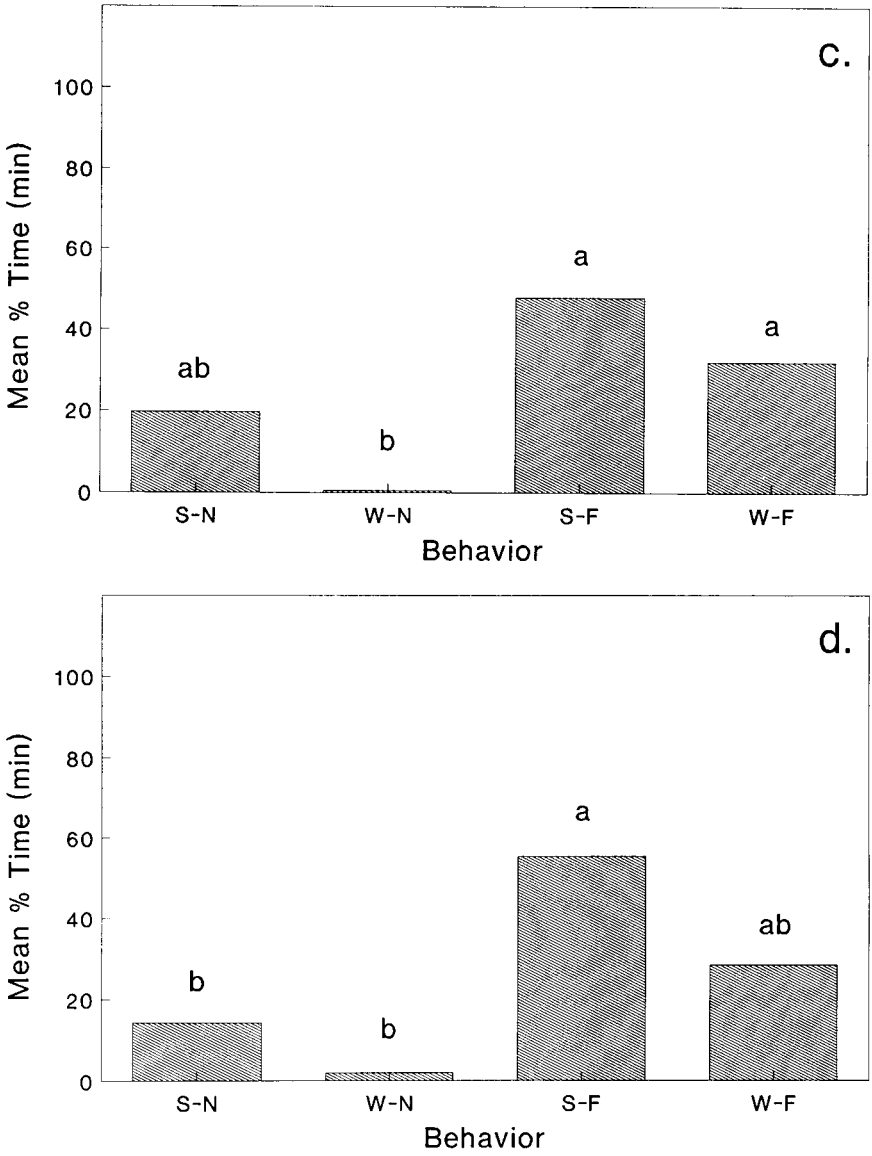


Fig. 4. *Monellia caryella* adult behavior on (a) pecan, (b) pea, (c) peach, and (d) fig in no-choice tests[1,2,3].

[1]Vertical bars labelled with the same letter(s) are not significantly different at P = 0.05.

[2]Behavior: S-N=settled-on disc; W-N=wandering-on disc; S-F = settled-off disc; W-F = wandering-off disc.

[3]Total observations: (a) pecan = 1,655 min; (b) pea = 120 min; (c) peach = 60 min; (d) fig = 70 min.

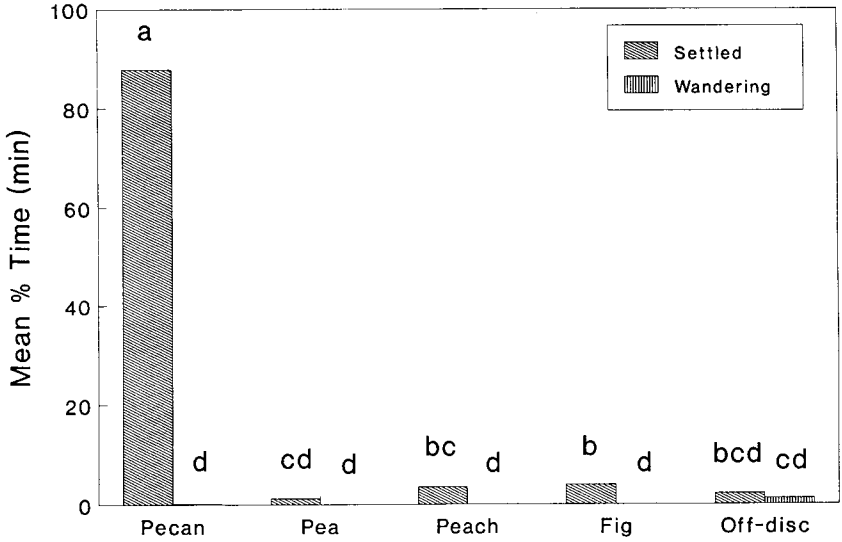


Fig. 5. *Monellia caryella* nymph behavior in choice tests[1,2].
[1]Vertical bars labelled with the same letter(s) are not significantly different at P = 0.05.
[2]N = 312 10-min observation periods (3,120 min total).

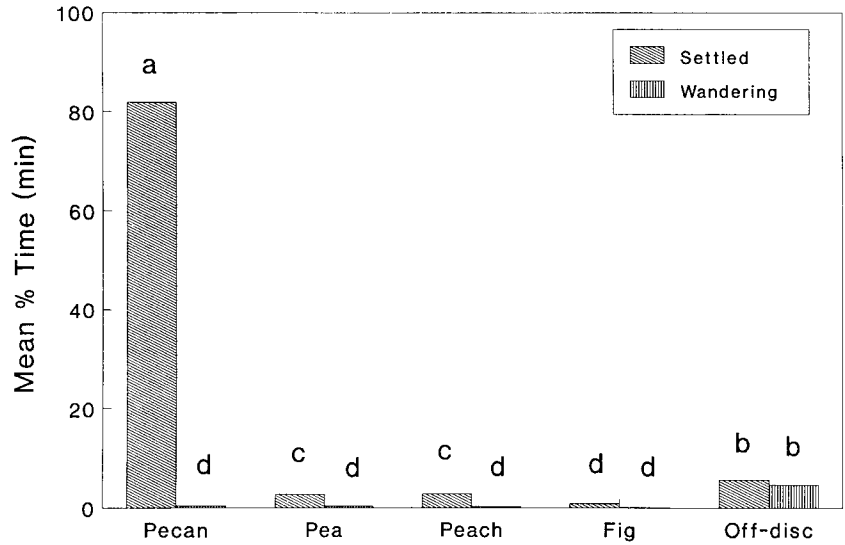
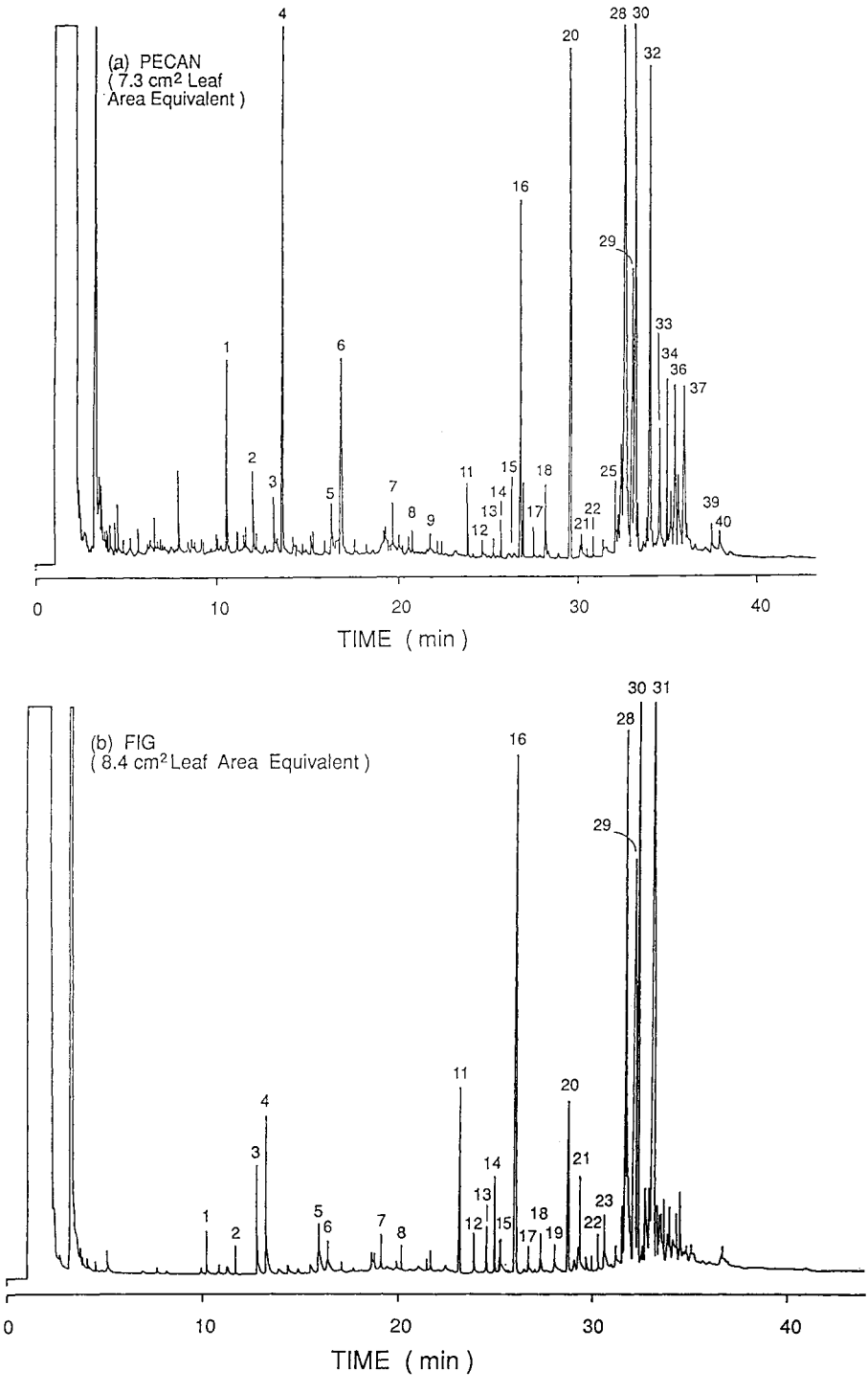


Fig. 6. *Monellia caryella* adult behavior in choice tests[1,2].
[1]Vertical bars labelled with the same letter(s) are not significantly different at P = 0.05.
[2]N = 717 10 - min observation periods (7,170 min total).



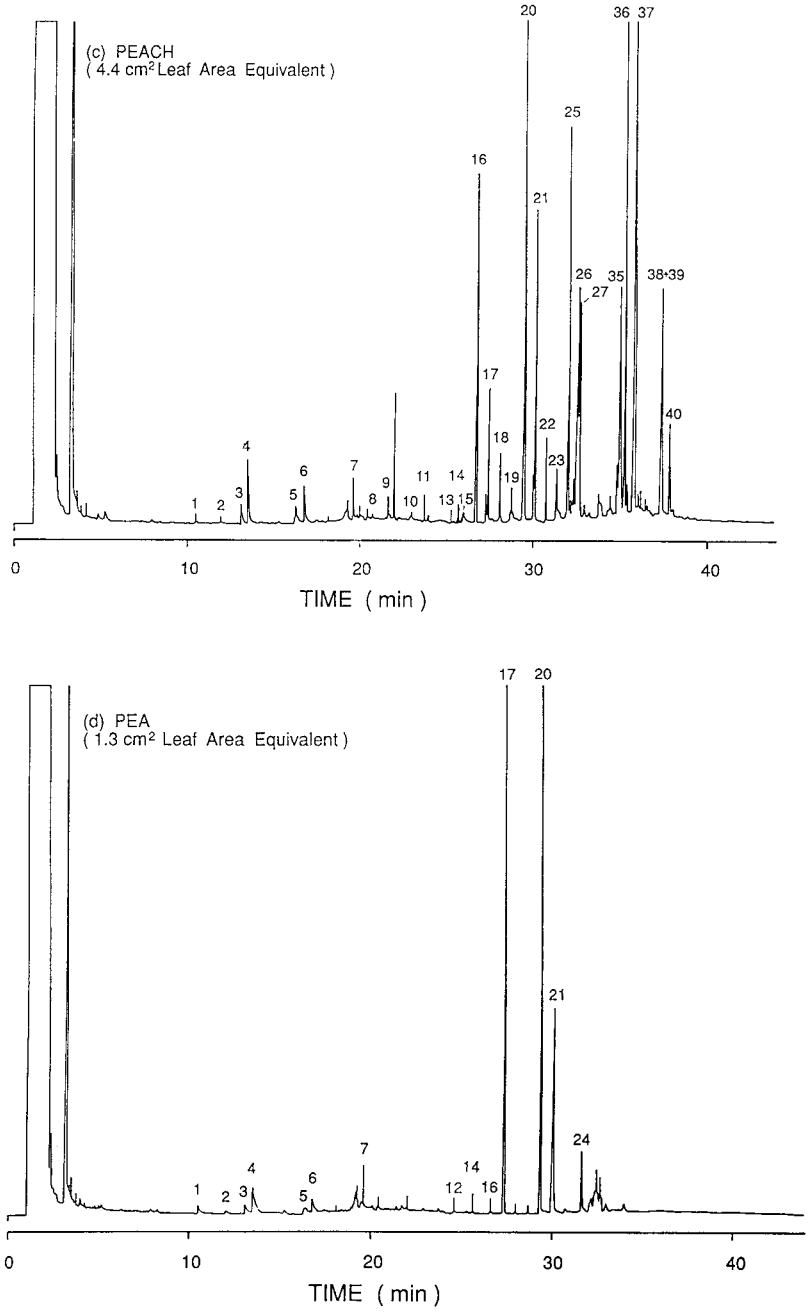


Fig. 7. Capillary gas chromatogram of the leaf surface extracts: (a) pecan; (b) fig; (c) peach; (d) pea. See Table 1 for peak identification.

Table 1. Cuticular Components Extracted From Pecan, Fig, Pea and Peach Foliage.

Compound	Peak #	Relative Distribution (%)* Pecan	Fig	Pea	Peach
1-Tetradecanoic Acid	1†	6	2	< 1	< 1
1-Pentadecanoic Acid	2†	3	1	< 1	< 1
cis-9-Hexadecenoic Acid	3†	2	6	< 1	2
1-Hexadecanoic Acid	4†	23	9	5	5
cis-9-Octadecenoic Acid	5†	3	5	< 1	3
1-Octadecanoic Acid	6†	7	4	3	4
1-Eicosanoic Acid	7†	2	2	7	3
n-Pentacosane	8†	< 1	2	–	< 1
1-Docosanol	9†	1	–	–	8
1-Docosanoic Acid	10†	–£	–	–	< 1
n-Heptacosane	11†	3	11	–	2
1-Tetracosanol	12†	< 1	3	2	–
n-Octacosane	13†	1	4	–	< 1
Squalene	14†	2	6	3	1
1-Tetracosanoic Acid	15†	< 1	6	–	< 1
n-Nonacosane	16†	15	38	2	23
1-Hexacosanol	17†	1	3	71	8
n-Triacontane	18†	4	3	–	5
1-Hexacosanoic Acid	19†	–	3	–	3
n-Hentriacontane	20†	24	13	100	74
1-Octacosanol	21†	< 1	9	36	21
n-Dotriacontane	22†	< 1	4	–	5
1-Octacosanoic Acid	23†	–	11	–	5
16-Hentriacontanol	24†	–	–	13	–
n-Tritriacontane	25†	5	–	–	29
1-Triacontanol	26†	–	–	–	22
β-Sitosterol	27†	–	–	–	16
β-Amyrin	28†	100	52	–	–
α-Amyrin	29†	15	35	–	–
Triterpenol	30‡	83	58	–	–
Lanosterol	31†	–	100	–	–
24-Methylenecycloartenol	32§	24	–	–	–
Hydroxy-amyrin (erythrodiol)	33¶	6	–	–	–
Hydroxy-amyrin	34¶	8	–	–	–
1-Dotriacontanol	35†	–	–	–	13
Oleanolic Acid	36¶	10	–	–	52
Ursolic Acid	37¶	10	–	–	100
1-Tetratriacontanol	38†	–	–	–	22
Hydroxy-oleanolic Acid	39¶	2	–	–	–
Hydroxy-ursolic Acid	40¶	1	–	–	9

* Relative distribution = (area of GC peak X/area of most abundant component) x 100; based on unitary chromatographic response.

† GC retention and GC/MS data identical to authentic standard.

‡ M.W. 426; trimethylsilyl ether M/Z 498 (m+), 483 (m-15), 408 (m-HOTMS), 369(m-129). Possible 3-hydroxy-5-ene triterpene. (Diekman and Djerassi, 1967).

§ GC retention and GC/MS data consistent with that reported by Severson et al., 1978.

¶ GC retention and GC/MS data of the trimethylsilyl ethers and/or esters consistent with literature (Burnouf-Radosevich et al., 1965).

£ Absent or below detection limits.

Table 2. Relative Distribution of Cuticular Hydrocarbons of Pecan, Peach, Fig and Pea.

Hydrocarbon	Relative Distribution* Pecan†,‡	Peach‡	Fig‡	Pea‡
n-23	–§	–	1.7	–
n-24	–	–	2.0	–
n-25	6.8	1.0	4.1	2.8
n-26	1.2	0.9	4.0	0.6
n-27	15.2	3.2	32.2	2.9
n-28	6.0	2.2	9.1	0.5
n-29	65.6	33.9	100.0	3.3
n-30	12.0	6.1	7.8	0.6
n-31	100.0	100.0	32.3	100.0
n-32	6.3	7.2	2.5	1.5
n-33	14.8	73.2	5.2	2.7
n-34	2.7	1.0	1.0	0.8
n-35	1.5	1.7	4.0	–

* Relative Distribution = (area of GC peak hydrocarbon X/area of GC peak for most abundant hydrocarbon) x 100; based upon unitary chromatographic response.
† Identified by GC/MS.
‡ Identified by GC retention times.
§ Absent or below detection limits.

As one such system, secondary plant substances are important in determining the host range of aphid species. While acting to protect plants against polyphagous aphid species and pathogens, these devices may also act as barriers to the extension of the host range in host-specific aphid species. Van Emden (1978), however, argued that it may be more appropriate to regard plant secondary substances as flags for insect orientation rather than as plant barriers to insect colonization. These same substances also function as phagostimulants, feeding deterrents or repellents (Wensler 1962; Kogen 1977; Van Emden 1978; Dixon 1985).

The successive phases of the host plant selection process of aphids have been described in detail by Dixon (1985) and Klingauf (1987). The major steps include: (1) uncontrolled directional flight; (2) host finding (controlled landing once within the boundary layer of relatively still air around vegetation); (3) host recognition (testing of the plant surface and the outer plant tissues); (4) penetration, and (5) testing of the phloem. Final host selection may be dependent on several factors, including environmental factors such as gravity, light, temperature, relative humidity, barometric pressure and wind; and host plant factors such as the host's shape, color, odor, structure of the surface and texture of the tissues; chemical composition of the surface, the outer and the inner tissues, as well as the host plant spacing and neighboring plants (Klingauf 1987). Besides secondary plant substances, hairs, physical form of the wax, a thick cuticle, rough or smooth surfaces, amino acids, nutrients, and pesticide treatments are considered to be significant for walking and probing behavior. The focus of the research reported here has been the potential role of foliar cuticular chemistry in the host recognition phase of *M. caryella.*

Host recognition by aphids usually consists of a characteristic behavior of walking and probing. During probing, usually the cuticle only or at most the epidermis of a leaf is explored by the labium and the stylets. The term probing (also called 'trial probes') is used to describe short-distance and brief penetrations that are insufficient for feeding but may be used for host recognition and finding of nutrient tissue (Pollard 1973). Probes last from a few seconds up to a few minutes, averaging ca. 1 min. Aphids may also tap the leaf surface with their rostrum or slide the apex over the surface prior to the probing activity (Ibbotson and Kennedy 1959). Positive gustatory stimuli usually induce an increase in frequency and duration of the probes.

On host plants, the sequences of probes and walks result in walking time becoming shorter and probing time longer (Klingauf 1975). The significantly high percentage of time *M. caryella* spent settled-on pecan in both the no-choice and choice tests (Figures 3a, 4a, 5 and 6) clearly provides evidence that *M. caryella* recognized pecan as a potential host plant, and in fact prefers pecan to pea, peach and fig.

In contrast, aphids often behave differently on host and non-host plants (Klingauf 1976, Gibson and Rice 1989). A stay on a non-host plant increases locomotary activity, whereas a stay on a host plant reduces it (Kennedy 1965, 1966). On non-hosts the duration of successive probes decreases and the walking time increases, while the stays on the leaves are mostly shorter. Aphids often abandon non-hosts soon after a short probe (Klingauf 1970, 1975). The low percentage of time *M. caryella* spent settled-on pea, peach and fig, indicates that they fail to recognize these three plant species as host plants (Figures 3b, c and d, 4b, c and d, 5 and 6).

Prevention of penetration is a major method of plants to defend themselves against aphid attack. Young instars of *Aphis fabae, Acyrthosiphon pisum* and *Megoura viciae* penetrate cultivated *Vicia* species, while they fail to penetrate the cuticle barriers on several perennial *Vicia* species (Birch 1984). The fact that pea, peach and fig serve as host plants for five, ten and four aphid species, respectively, suggests that their plant defenses, either physical or chemical in nature, do not form an impenetrable barrier to all aphid species (Blackman and Eastop 1984). Moreover, *M. caryella is* a relatively large aphid species. Therefore, it is unlikely that pea, peach and fig possess a physical cuticular barrier to penetration by *M. caryella.*

These behavioral results are supported by the differential longevity, developmental rate and reproductive rate of *M. caryella* on pecan versus pea, peach and fig. The statistically equivalent longevity, developmental rate and reproductive rate of *M. caryella,* when provided pea, peach, fig or no plant leaf, either implies that *M. caryella* did not feed on these three plant species, or that, if *M. caryella* did feed, the three plant species and the water-agar plates were equally unsuitable as food sources.

Comparison of Chemistry. Klingauf et al. (1971) investigated the effects of different surface extracts of *V. faba* on host selection behavior of A. *pisum.* His criterion for attractiveness was the duration of the first probe; where long-time-probes were valued positive and short-time-probes negative. He found that short-time water-extracts from uninjured plants and extracts obtained by aeration of uninjured plants were highly attractive. Furthermore, of the three fractions isolated (n-alkanes, acetates of n-alcohols and n-alcohols), only the n-alkane fraction had

a significant positive effect. One of three tested n-alkane-standards, n-dotriacontane, was attractive to the aphid. He concluded that wax components of plant surfaces can play a part during the first phase of host selection by *A. pisum.*

Klingauf et al. (1978) later reported that the qualitative and quantitative composition of plant alkanes from leaf waxes were more important than individual compounds in influencing the probing behavior of *A. pisum* on V. *faba.* The n-alkane pattern of V. *faba,* mainly n-C_{27}, n-C_{29}, n-C_{31}, and n-C_{33}, favored probing behavior in contrast to the major n-alkane, n-C_{29} of the non-host *Brassica.* The distribution pattern of n-alkanes in pecan are different from pea, peach and fig (Table 2), providing possible evidence that the distribution pattern of hydrocarbons in the cuticular layer of pecan leaves could enable *M. caryella* to recognize or distinguish pecan as an acceptable host plant.

Significant differences in other major cuticular components in pecan, pea, peach and fig were also found (Figure 7; Table 1). Pecan, peach and fig produced triterpene constituents which differed qualitatively and quantitatively. Cuticular extract of pecan and fig contained four major triterpenols: three of the four being identical [α- and β-amyrin and an unidentified triterpenol (Peak 30); Figure 7a and b], and one of the four being different (pecan produced 24-methylenecycloartenol, while fig produced lanosterol). In contrast, major triterpenes in peach were oleanolic and ursolic acids, which are higher oxidation products of β- and α-amryin, respectively, and a sterol, β-sitosterol. All of the above terpenes and sterols have similar condensed A, B, C and D rings (24-methylenecycloartenol, lanosterol, β-sitosterol) or A, B, C, D, and E rings (α- and β-amyrin, oleanolic and ursolic acids). If aphids use triterpenols to differentiate between pecan and other plants then: (1) they can likely detect and utilize 24-methylenecycloartenol as the key host recognition compound; (2) they can likely detect either differences between side chain chemistries in 24-methylenecycloartenol and lanosterol or differences in relative distribution of the other triterpenols, in order to differentiate pecan and fig; (3) they can likely detect differences between α- and β-amyrin and their higher oxidation products, oleanolic and ursolic acids, in order to differentiate pecan and peach; (4) they can reject pea due to its lack of triterpenols. These data, coupled with previous literature, indicate that aphids possess a sophisticated sensory apparatus capable of differentiating small structural and compositional differences in plant cuticular chemistries. Therefore, the distinct cuticular chemistries of pecan may provide the major 'flags' for host recognition by *M. caryella,* and thereby promote probing, while the distinct cuticular chemistries of fig, peach and pea may provide a barrier to host recognition and/or insect colonization by *M. caryella.*

In conclusion, perhaps the most crucial area of aphid biology, which demands study from the basic as well as applied points of view, is the effect that chemicals from the surface and wax layers of plants have on the probing and feeding behavior of aphids on host and non-hosts, on plants in different stages of development, as well as on susceptible and resistant varieties (Mittler 1988). The study reported herein, provides evidence that: (1) *M. caryella* recognized pecan as a potential host plant, while it failed to recognize pea, peach and fig as potential host plants; (2) the distribution pattern of n-alkanes of pecan differed from pea, peach and fig; (3) the triterpenes of pecan differed qualitatively and/or

quantitatively from those of peach and pea; and (4) while pecan and fig have several triterpenols in common, fig (as well as pea and peach) lacks 24-methylenecycloartenol. Therefore, this study provides the first evidence within the pecan/aphid interactive system which suggests that the distinct foliar chemistry of pecan may have an influence on the host recognition behavior of *M. caryella.*

Although the linkages between aphid behavior and cuticular chemistry reported herein are inconclusive, the differential chemical analysis of the cuticular chemistry of pecan and the three non-host plant species should enhance our ability to identify the key compound(s) which mediate host plant recognition by *M. caryella.* Therefore, these studies have set the stage for more definitive bioassays of host plant recognition by *M. caryella,* which have focused current research efforts on disguising these non-host plants as pecan host plants via the application of whole and fractionated pecan foliar cuticular extracts to their foliar surfaces. This same principle has been utilized and reported by Wensler (1962), Tarn and Adams (1982) and Mansour et al. (1982).

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