

QUANTIFICATION OF CONTACT OVIPOSITION  
STIMULANTS FOR BLACK SWALLOWTAIL  
BUTTERFLY, *Papilio polyxenes*, ON THE LEAF  
SURFACES OF WILD CARROT, *Daucus carota*

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(Received February 5, 1996; accepted August 13, 1996)

**Abstract**—Ovipositing black swallowtail butterflies, *Papilio polyxenes*, make their final host-selection decisions on the basis of compounds present on the leaf surface. Little information is available, however, on the chemistry of leaf surfaces. The purpose of this study was to develop a technique to extract and quantify the concentrations of compounds from the leaf surfaces of *Daucus carota*, one of the main host species for *P. polyxenes*, with particular reference to compounds already identified as contact oviposition stimulants, namely *trans*-chlorogenic acid (CA) and luteolin-7-*O*-(6"-*O*-malonyl)- $\beta$ -D-glucopyranoside (L7MG), as well as its degradation product luteolin-7-glucoside (L7G). Plant surfaces were extracted by dipping leaves sequentially in pairs of solvents: (1) CHCl<sub>3</sub>-MeOH, (2) near-boiling H<sub>2</sub>O, (3) CHCl<sub>3</sub>-near-boiling H<sub>2</sub>O, and (4) CH<sub>2</sub>Cl<sub>2</sub>-CH<sub>2</sub>Cl<sub>2</sub>. The resulting extracts were fractionated and analyzed using high-performance liquid chromatography. The leaf-surface concentrations of each compound were calculated using regressions relating leaf surface area to leaf weight that were obtained from measurements of field-collected carrot plants. All four methods removed the three compounds from carrot leaf surfaces, but the solvent systems differed in effectiveness. The chloroform-near-boiling water solvent system performed better than the other solvent combinations, but not significantly so. This system also extracted the highest number of polar, UV-absorbing compounds. Methylene chloride was significantly less efficient than the other methods. An additional test confirmed

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that the chloroform-near-boiling water method removed compounds from the surface alone and probably not from the apoplast or symplast. Surface concentrations of CA (up to 600 ng/cm<sup>2</sup> leaf surface) were substantially greater than those of the two flavonoid compounds. No clear seasonal trend in concentrations was evident from the limited number of sampling dates.

**Key Words**—*Papilio polyxenes*, Papilionidae, *Daucus carota*, Apiaceae, oviposition, leaf surface, contact chemoreception, HPLC, flavonoid glycosides, chlorogenic acid.

## INTRODUCTION

The initial host plants of the larval offspring of the black swallowtail butterfly, *Papilio polyxenes*, are chosen by the egg-laying female adult (Blau, 1981; Feeny et al., 1983, 1985). A gravid female locates a potential host plant on the basis of olfactory and visual cues, which may lead the female to land on the plant (Feeny et al., 1989). Once on a leaf, the female chooses whether or not to oviposit after sensing chemical cues on the leaf. Upon landing, the female drums her foretarsi on the leaf surface, bringing contact chemoreceptors located on the ventral surface of the foretarsi into contact with chemical stimuli present on the leaf surface (Feeny et al., 1983; Roessingh et al., 1991). Based on the stimuli perceived through this drumming behavior, the *P. polyxenes* female may curl her abdomen and oviposit. As in several other groups of phytophagous insects (Städler, 1986; Traynier and Hines, 1987; Linley, 1989), contact chemoreception during oviposition by butterflies appears to be limited to the leaf surface itself; no studies have yet revealed any penetration of the leaf cuticle due to drumming behavior (e.g., Städler, 1986; Bart and Williams, 1993; but see Boppré, 1978, 1983 for exception in Danaidae).

Larvae of *P. polyxenes* feed almost exclusively on plants of the family Apiaceae (Tietz, 1972). In New York, the main host plant of *P. polyxenes* is the umbellifer *Daucus carota* (Feeny et al., 1985). Commonly known as wild carrot or Queen Anne's lace, this plant is a winter annual found throughout the northeastern and northcentral United States where it has a patchy distribution and occurs in secondary successional areas (Peterson and Simon, 1986). From ethanolic extracts of carrot foliage, Feeny et al. (1988) identified two major contact stimulants for *P. polyxenes*: *trans*-chlorogenic acid (CA) and luteolin-7-*O*-(6"-*O*-malonyl)- $\beta$ -D-glucopyranoside (L7MG), a flavonoid malonate. Roessingh et al. (1991) confirmed the stimulatory characteristics of these compounds and identified some of the chemoreceptors in *P. polyxenes* that respond to these stimuli. Feeny et al. (1988) discovered that the flavonoid malonate was an unstable compound that degraded under natural conditions when the malonic acid group dissociated from the flavonoid. Luteolin-7-glucoside (L7G), the fla-

vonoid hydrolysis product, was also recovered from ethanolic extracts of carrot foliage. It was not an oviposition stimulant for *P. polyxenes*, and preliminary evidence suggested it may be an oviposition deterrent. Additional stimulant compounds, as yet unidentified, were present in the carrot fractions containing bases and polar neutrals (sugars and inositols) (P. Feeny et al., unpublished data).

Past work on the identification of the oviposition stimulants for *P. polyxenes* from *D. carota* has been based on whole-leaf extracts. Since stimulant isolation and chemical characterization require large quantities of plant material, whole-leaf extracts are necessary in order to obtain sufficient amounts of the compounds of interest. It has been assumed, but never proved, that the stimulant compounds are present on the leaf surface (Feeny et al., 1983).

Although Richmond and Martin (1959) reported the extraction of phenolic compounds from apple leaves by 10-sec dips in diethyl ether, they noted some leaching of compounds from the leaf interior, and it remains unclear how much material originated from the leaf surface. There has been much subsequent discussion as to the likelihood that flavonoid glycosides and other polar compounds can occur within the nonpolar waxy matrix of the leaf surface (Harborne, 1973; Hunt and Baker, 1980; Wagner, 1982; Robinson, 1991). Recent studies, reviewed below, have demonstrated clearly that several classes of polar compounds are indeed present on leaf surfaces, although the mechanisms of transport and storage remain unclear.

Oviposition stimulants for insects have typically been extracted from leaf surfaces by dipping leaves for several seconds in various solvents. The suitability of different solvents for particular extractions depends critically on the polarity of the stimulant compounds. Most chemical explorations of the leaf surface have used methylene chloride to extract the surface compounds (Städler and Buser, 1984; Proksch et al., 1985, 1986; Bohm and Constant, 1990; Bohm and Chan, 1992; Bohm, 1993; Reid and Bohm, 1993). This organic solvent of medium polarity has been used with success to extract sesquiterpenoids and other relatively nonpolar compounds (Städler and Roessingh, 1991), but it may have been insufficient to remove polar compounds present in the epicuticular waxes or the cuticle proper. Cold water was used to extract more polar compounds such as fructose and other water-soluble carbohydrates from leaf surfaces of *Zea mays* (Derridj et al., 1987; Fiala et al., 1990). Neither cold water nor methanol alone were effective in extracting stimulants for *Delia radicum* or *Pieris rapae*; however, extraction of glucosinolates and other stimulants for these insects required either hot water or methanol preceded by a chloroform or methylene chloride dip (Städler and Roessingh, 1991; Renwick, et al., 1992; van Loon et al., 1992; Baur et al., 1996). Hot water was also used by Zobel and Brown (1988) to extract furanocoumarins from the leaves of *Ruta graveolens*. Städler and Roes-

singh (1991) concluded that stimulants must generally be embedded quite deeply in the waxy leaf cuticle so that they can be extracted only when the waxy layer is dissolved (chloroform/methylene chloride) or melted (hot water).

In early work on oviposition stimulants for *P. polyxenes*, Feeny et al. (1983) found that stimulant activity could not be extracted from the surfaces of carrot leaves by individual organic solvents or by cold water. In fact, the leaves retained their stimulant activity even after immersion in 2 N HCl for 30 min at 40°C; only as temperatures were increased above 60°C, presumably causing softening and disruption of the waxes, did the leaves lose their ability to stimulate oviposition. Since the major stimulants, CA and L7MG, are both easily hydrolyzed by dilute HCl, this was evidence that these polar stimulants are indeed embedded in the surface wax, as concluded by Städler and Roessingh (1991).

The purpose of this study was threefold: (1) to confirm the presence of contact oviposition stimulants for *P. polyxenes* on the leaf surfaces of *D. carota*, (2) to develop the most efficient extraction technique for the removal of these stimulants from the surface of *D. carota* leaves, and (3) to quantify the concentrations of these chemicals on the leaf surface.

#### METHODS AND MATERIALS

**Surface Area Regressions.** Foliage of carrot, *Daucus carota*, was taken fresh from the field in the vicinity of Ithaca, New York, in spring 1990 and transported to the laboratory on ice. Additional carrot foliage was taken from greenhouse cultures at Cornell University. All petioles were cut just below the point of attachment of the first leaflets, and the fresh weight and length of each leaf was recorded. All parts of each leaf were then mounted on paper sprayed with Scotch brand Spray Mount Artist's Adhesive (3M). This paper was covered with clear plastic and photocopied to make a permanent record of leaf surface area before wilting began.

The photocopied images of leaves were scanned using an Apple flatbed scanner. On all photocopied sheets, a 0.5-square-inch standard was included for calibration. The pixels of the scanned images were then counted using a pixel-counting program provided by M. Lapidus. The calculated surface area of each leaf was doubled to account for both the upper and lower leaf surfaces. Least-squares regressions were calculated for leaf surface area versus leaf weight and for leaf surface area versus leaf length.

**Plant Material for Surface Extracts.** Four collections of *D. carota* foliage were taken fresh from the field in the vicinity of Ithaca, New York, from June 1994 to October 1994. The cut petioles of individual leaves were placed in Aquapiks (Cleveland Plant and Flower Co., Wholesale Florists, Binghamton,

New York 13905) and transported to the laboratory on ice. The leaves within a single collection were assigned randomly to four treatment groups. Within each treatment group the fresh weight of the leaves, including petioles, was obtained prior to surface extraction. Each treatment group contained between 10.0 and 13.0 g of leaves. After surface extraction, the petioles were cut from the leaves below the point of attachment of the first leaflets and weighed separately. The petiolar weight was subtracted from the total leaf weight to obtain the laminar weight.

**Solvent Extraction Systems.** Four solvent systems (treatments) were used to extract chemicals from the surfaces of carrot leaves. For system 1 (CHCl<sub>3</sub>-MeOH), a freshly cut leaf in this treatment group was dipped in 200 ml of CHCl<sub>3</sub> for 10 sec and held above the solvent to drip for 10 sec. The leaf was then dipped in 200 ml MeOH for 7 sec and allowed to drip for 10 sec. All leaves in the group were dipped individually in the same aliquot of solvent. For system 2 (near-boiling H<sub>2</sub>O), leaves were dipped individually in 300–350 ml of near-boiling H<sub>2</sub>O (HPLC grade, temperature at least 94°C) for 2 sec and were allowed to drip for 10 sec. For system 3 (CHCl<sub>3</sub>-near-boiling H<sub>2</sub>O), each leaf was first dipped for 10 sec in 200 ml of CHCl<sub>3</sub> and allowed to drip for 10 sec. The leaf was then dipped for 1 sec in 300 ml of near-boiling H<sub>2</sub>O and allowed to drip for 10 sec. For system 4 (CH<sub>2</sub>Cl<sub>2</sub>-CH<sub>2</sub>Cl<sub>2</sub>), leaves were dipped individually in 300 ml of CH<sub>2</sub>Cl<sub>2</sub> for 30 sec and allowed to drip for 10 sec. This was repeated in a second volume of CH<sub>2</sub>Cl<sub>2</sub>. Since the CH<sub>2</sub>Cl<sub>2</sub> evaporated rapidly, solvent volume was kept constant throughout the extraction by addition of more solvent. All solvent fractions were stored in the freezer until preparation for HPLC analysis.

**Solvent Timing Study.** Two collections of *D. carota* foliage were taken fresh from the field in the vicinity of Ithaca, New York, in July 1995. The cut petioles of individual leaves were placed in Aquapiks and transported to the laboratory on ice. The leaves from both collections were combined and assigned randomly to six treatment groups. Within each treatment group the fresh weight of the leaves, including petioles, was obtained prior to surface extraction. After surface extraction, the petioles were cut from the leaves below the point of attachment of the first leaflets and weighed separately. The petiolar weight was subtracted from the total leaf weight to obtain the laminar weight. The leaves from each treatment were extracted with solvent system 3 (CHCl<sub>3</sub>-near-boiling H<sub>2</sub>O). In these extractions, the dipping time in CHCl<sub>3</sub> was kept constant at 10 sec, but each treatment group received successively longer dipping times in the near-boiling H<sub>2</sub>O (with times of 1, 2, 3, 4, 5, and 7 sec, respectively). All solvent fractions were stored in the freezer until preparation for HPLC analysis.

**Preparation of Surface Extracts for HPLC Analysis.** The organic fractions were concentrated in vacuo at 40°C to a volume of 20–40 ml and then extracted three times with equal volumes of H<sub>2</sub>O (HPLC grade). The resulting aqueous

layer was kept, and the original organic layer discarded. The aqueous surface washes were concentrated in vacuo at 40°C to a volume of 20–40 ml and then extracted three times with equal volumes of CHCl<sub>3</sub>. The original aqueous layer was kept, and the CHCl<sub>3</sub> wash discarded. In a solvent system containing both an organic and aqueous wash, the final aqueous layers after extraction for both washes were combined. All final extracts were concentrated in vacuo at 40°C to a final volume of 1 ml.

**HPLC Methodology.** Analyses of surface extracts were carried out on a Waters multisolvent delivery system (M6000 pump, U6K injector) and monitored between 200 and 600 nm (Waters 991 photodiode array (PDA) detector). The analytical reverse-phase C-18 column (Phenomenex IB-sil, 4.6 × 250 mm, 5 μm with 4.6 × 30 mm C-18 guard column) was eluted at a flow rate of 1.0 ml/min using a stepped gradient (Table 1). Calibration and quantification of the PDA data were done by analysis of eight standard solutions containing CA, L7G, and L7MG (500, 250, 150, 60, 30, 15, 7.5, and 4.875 μg of each compound per ml, respectively, giving from 5000 to 48.750 ng of each compound per injection). Calculation of calibration curves and quantification of the known compounds in the surface extracts was performed by Millennium 2010 Chromatography Manager software (Millipore, Inc.).

**Sources of Standard Compounds.** CA was purchased from Sigma. L7G was purchased from Indofine Chemical Company. The L7MG standard was purified from fresh *D. carota* foliage using a modification of the procedure described by Feeny et al. (1988). Foliage of carrot was taken fresh from the field in the vicinity of Ithaca, New York, in May 1994. The cut petioles of individual leaves were placed in Aquapiks and were transported to the laboratory in coolers containing ice packs. Leaves, 40 g fresh weight at a time, were

TABLE 1. HPLC ELUTION PROGRAM FOR CHEMICAL ANALYSES OF *Daucus carota* SURFACE EXTRACTS ON ANALYTICAL REVERSE-PHASE C-18 COLUMN<sup>a</sup>

Time (min)	Proportion of 1% aq HOAc	Proportion of MeOH	Curve
0.0	100	0	
7.5	65	35	6
25.0	55	45	9
50.0	55	45	6
55.0	0	100	6
65.0	0	100	6

<sup>a</sup>Curve specifies the rate of change of eluent composition over time (6 produces a linear gradient curve; 9 represents a concave gradient curve).

immediately blended in boiling 95% EtOH for 5–10 min and the extract filtered. After removal of the EtOH by evaporation in vacuo at 40°C, the resulting aqueous suspension was centrifuged and then extracted sequentially (each three times) with equal volumes of hexane, diethyl ether (Et<sub>2</sub>O), CHCl<sub>3</sub>, and ethyl-acetate (EtOAc). The post-EtOAc aqueous fraction was concentrated in vacuo at 40°C and adjusted with H<sub>2</sub>O to a concentration of 20 gle/ml (1 gle = amount of material extracted from 1 g fresh weight of carrot leaves).

Preparative fractionation of the post-EtOAc aqueous fraction was carried out on an open column (4.1 cm ID) packed with 100.0 g C-18 prep LC packing (Bakerbond, 40-μm particle size). Five milliliters (100 gle) of the post-EtOAc aqueous fraction was applied to the column and eluted successively with 1000 ml 1% acetic acid (HOAc) in H<sub>2</sub>O, 1000 ml 20% MeOH in 1% aq. HOAc, 1200 ml 40% MeOH in 1% aq. HOAc, and 500 ml pure MeOH. The brown band that was eluted from the column at the beginning of the pure MeOH fraction was concentrated in vacuo at 40°C and was further fractionated on a second open column (1.9 cm ID × 25.0 cm) packed with C-18 prep LC packing. The column was successively eluted with 275 ml 30% MeOH in 1% aq. HOAc (fraction 1), 460 ml 20% MeOH in 1% aq. HOAc (fraction 2), 230 ml 40% MeOH in 1% aq. HOAc (fraction 3), and 100 ml pure MeOH. The L7MG was collected by repeated HPLC of fraction 3 on a semi-prep C-18 column (Phenomenex IB-sil, 10 × 250 mm, 5 μm). Its identity was confirmed by matching its retention time and UV spectra with the time and spectra of previously isolated luteolin-7-*O*-(6"-*O*-malonyl)-β-D-glucoside (Feeny et al., 1988).

**Data Analysis.** Statistical comparisons between the regression lines of the surface area correlations for field and greenhouse leaves were carried out using the *F* test for difference between two regression coefficients (Sokal and Rohlf, 1981). Statistical comparisons among the four solvent methods were carried out using the Quade test (Conover, 1980).

## RESULTS

**Leaf Surface Areas.** Although surface area varied linearly with both leaf length and leaf weight, weight was a better predictor of surface area, explaining 98% of the surface area variation in field carrot leaves (Figure 1) and 97% of the variation in greenhouse leaves (Figure 2). The variation of surface area with leaf weight differed significantly between field carrot leaves and greenhouse carrot leaves, as greenhouse leaves had more surface area per gram of leaf ( $P < 0.001$ , *F* test). The variation of surface area with leaf length also differed significantly between the two groups ( $P < 0.01$ , *F* test).

**Solvent System Comparison.** CA, L7G, and L7MG were present in surface extracts obtained using each solvent system, but the systems differed in their



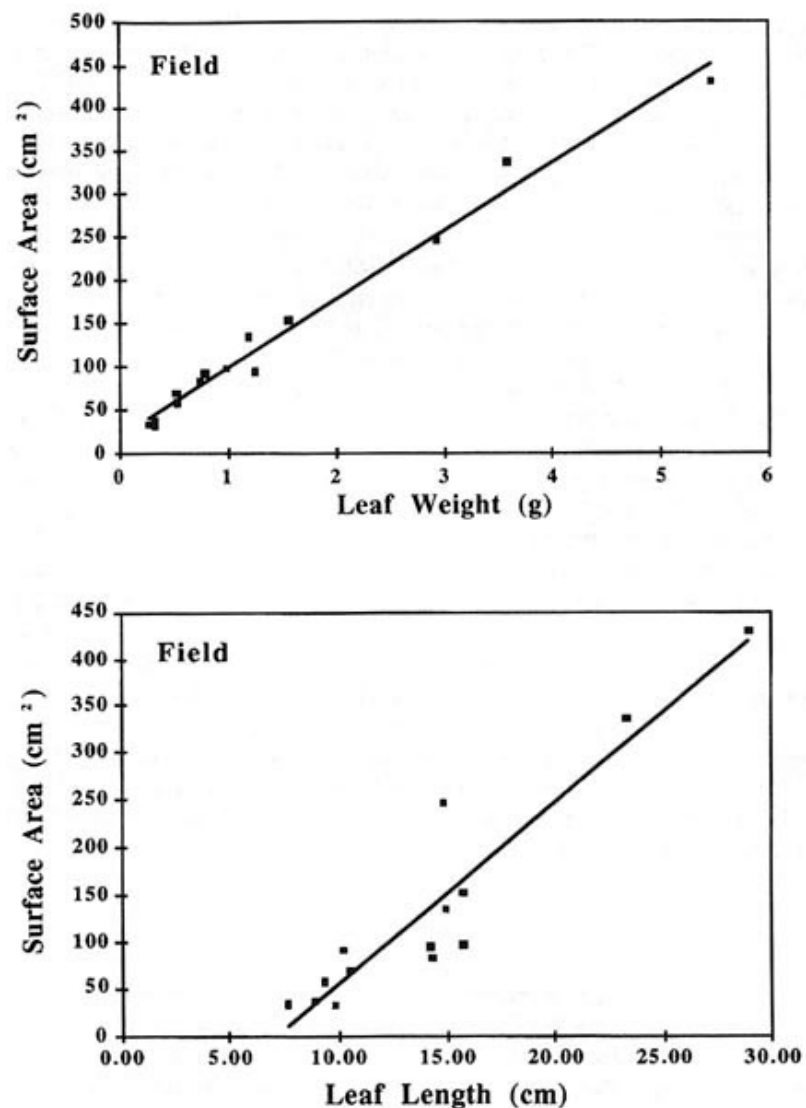


FIG. 1. Relationship of leaf surface area to leaf weight and leaf length, respectively, of field-collected *Daucus carota*. For leaf weight:  $y = 78.731x + 18.615$  ( $r^2 = 0.9827$ ). For leaf length:  $y = 19.231x - 139.92$  ( $r^2 = 0.8803$ ).

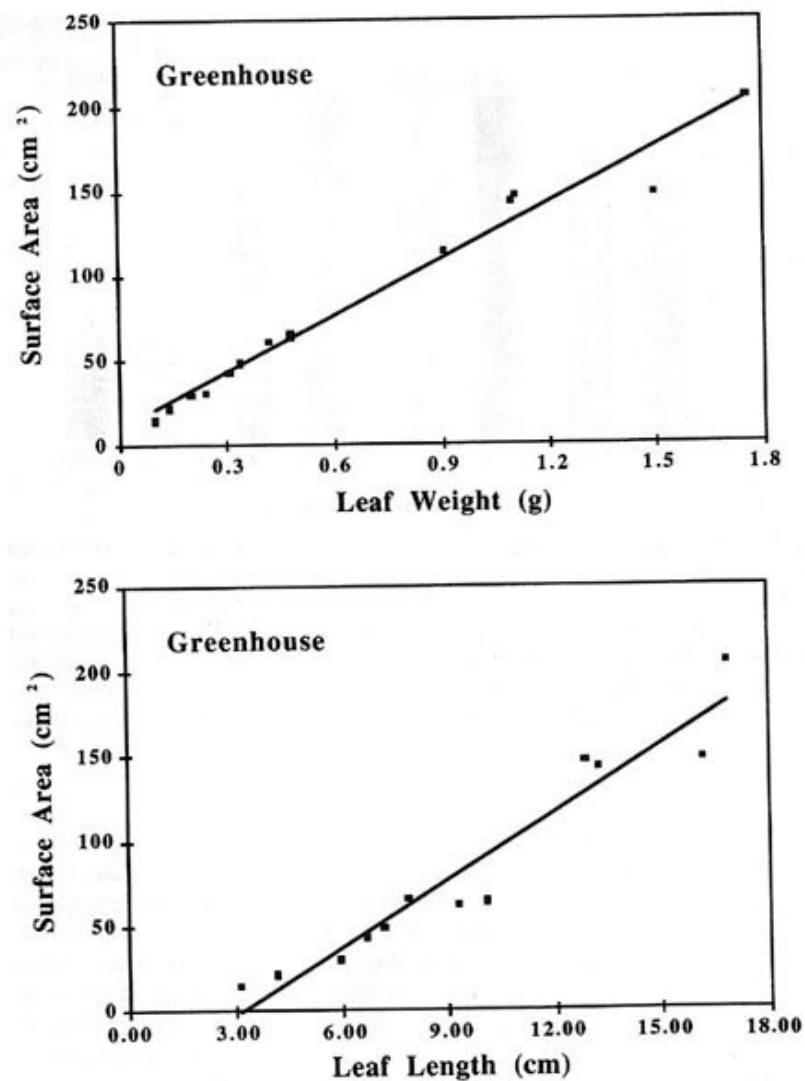


FIG. 2. The relationship of leaf surface area to leaf weight and leaf length, respectively, of greenhouse-grown *Daucus carota*. For leaf weight:  $y = 109.37x + 9.7964$  ( $r^2 = 0.9725$ ). For leaf length:  $y = 13.192x - 42.924$  ( $r^2 = 0.9263$ ).

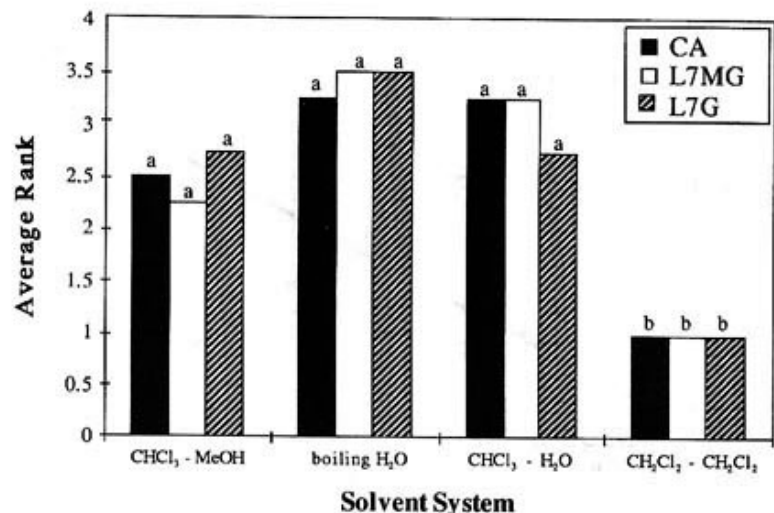


FIG. 3. Comparison of the efficiency of four solvent systems for extracting chlorogenic acid (CA), luteolin-7-*O*-(6"-*O*-malonyl)- $\beta$ -D-glucoside (L7MG), and luteolin-7- $\beta$ -D-glucoside (L7G) from leaf surfaces of *Daucus carota*. Efficiency was based on the amounts of a compound detected in surface extracts obtained by each solvent system. The method that removed the highest quantity of a compound received a rank of 4; the method that removed the lowest quantity received a rank of 1. The score given is the average of four replicate samples. Bars with different letters are statistically different ( $P < 0.05$ , Quade test).

efficiency of extraction (Figure 3). System 4 (CH<sub>2</sub>Cl<sub>2</sub>-CH<sub>2</sub>Cl<sub>2</sub>) was significantly less efficient in extracting the polar compounds from the leaf surfaces ( $\alpha < 0.05$ , Quade test). In fact, no detectable amounts of L7G were found in one of the four methylene chloride samples, and L7MG was not detectable in three of the four methylene chloride samples. System 2 (near-boiling H<sub>2</sub>O) and system 3 (CHCl<sub>3</sub>-near-boiling H<sub>2</sub>O) tended to extract higher quantities of the polar compounds from the carrot leaf surfaces than did system 1 (CHCl<sub>3</sub>-MeOH). The superiority of the aqueous methods was not significant statistically, however, probably due to the low number of replicates in the study.

The four solvent systems also varied in the overall number of polar, UV-absorbing compounds extracted from the carrot leaf surfaces and revealed in HPLC traces. System 3 (CHCl<sub>3</sub>-near-boiling H<sub>2</sub>O) extracted 34 compounds on average from the leaf surfaces. Near-boiling water (system 2) removed 32 compounds on average, and the chloroform-methanol system (system 1) extracted

26 compounds. The methylene chloride solvent system (system 4) extracted an average of only 15 compounds from carrot leaf surfaces.

**Compound Quantification.** CA was present at much higher concentrations, ranging from 19.2 to 596.2 ng/cm<sup>2</sup>, in the CHCl<sub>3</sub>-near-boiling H<sub>2</sub>O surface extracts than were the flavonoid glycosides (Figure 4). This same pattern was also found in the surface extracts in the other three solvent systems. L7G and L7MG were present in extracts at much lower concentrations, ranging from 0.8 to 26.8 ng/cm<sup>2</sup> and 0.9 to 8.2 ng/cm<sup>2</sup>, respectively (Figure 4).

There was also seasonal variation in the concentrations of the compounds on the leaf surface (Figure 4). Leaves from the fall rosette foliage had lower concentrations of CA on their surfaces than did the foliage of adult leaves (those from the July and August collections). Although there were also seasonal differences in the concentrations of the flavonoid compounds, no apparent trend was evident from the limited number of sampling dates.

**Solvent Timing Study.** In the chloroform-near-boiling water solvent system, the amount of time the leaves were dipped in the water affected the amounts of compounds extracted from the leaves (Figure 5), with the quantity extracted from leaves increasing sharply when dipped in water for more than 2 sec.

## DISCUSSION

There is debate in the literature as to the possibility of flavonoid glycosides being located on the leaf surface. Harborne (1973) and Wagner (1982) present evidence to show that flavonoid glycosides are confined almost entirely to plant cell vacuoles. However, Hunt and Baker (1980) found naringenin-7-glucoside in the cuticle of tomato fruits and concluded that "flavonoid glycosides occur extensively in the outer epidermis of leaves and fruits but their precise location within this layer has not been clearly established" (p. 1418). Recent studies comparing leaf surface and interior flavonoid composition found flavonoid glycosides in the leaf interior, and only the less polar flavonoid aglycones on the leaf surface (Proksch et al., 1985, 1986; Bohm and Constant, 1990; Bohm and Chan, 1992; Bohm, 1993; Reid and Bohm, 1993). Robinson (1991) described the consensus of research in this area when he stated, "it has been suggested as a general rule that the [flavonoid] glycosides are found within cells" (p. 192).

Although the nonpolar cuticle provides a barrier between the plant leaf interior and the leaf surface, movement of polar compounds through the cuticle has been shown in several plant species. Tukey (1970) documents the diversity of chemicals found in plant leachates, including sugars, sugar alcohols, organic acids, and phenolics. The pesticide literature provides many examples of polar components of applied pesticides moving from the leaf surface into the leaf

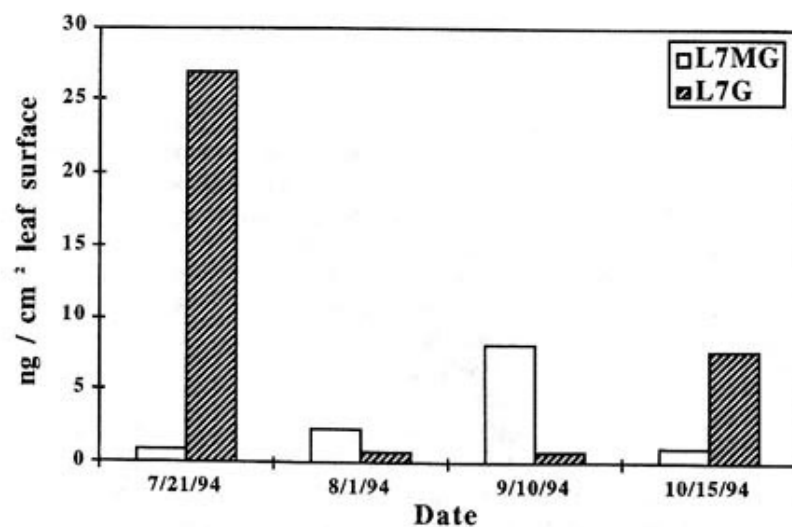
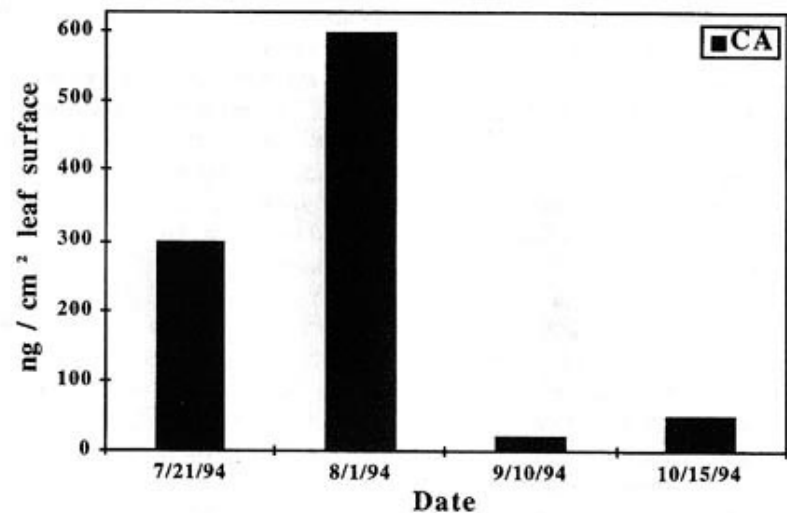


FIG. 4. Quantities of chlorogenic acid (CA), luteolin-7-*O*-(6''-*O*-malonyl)- $\beta$ -D-glucoside (L7MG), and luteolin-7- $\beta$ -D-glucoside (L7G) removed from leaf surfaces of *Daucus carota* using the chloroform-near-boiling water method.

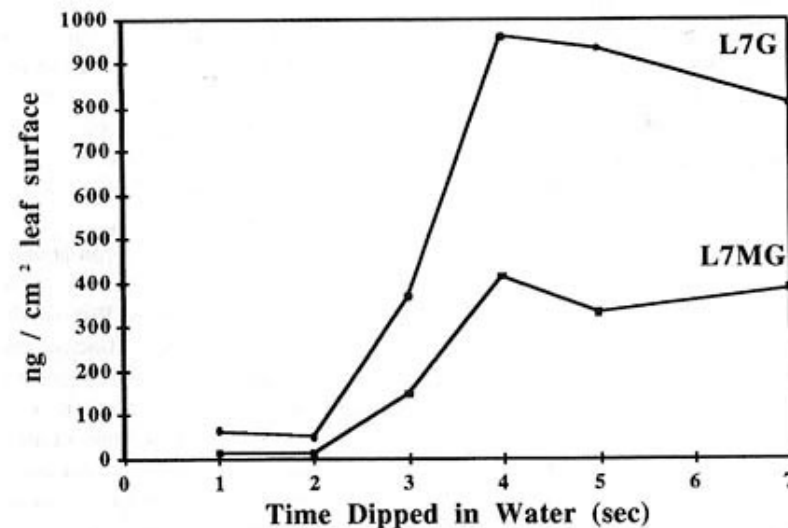
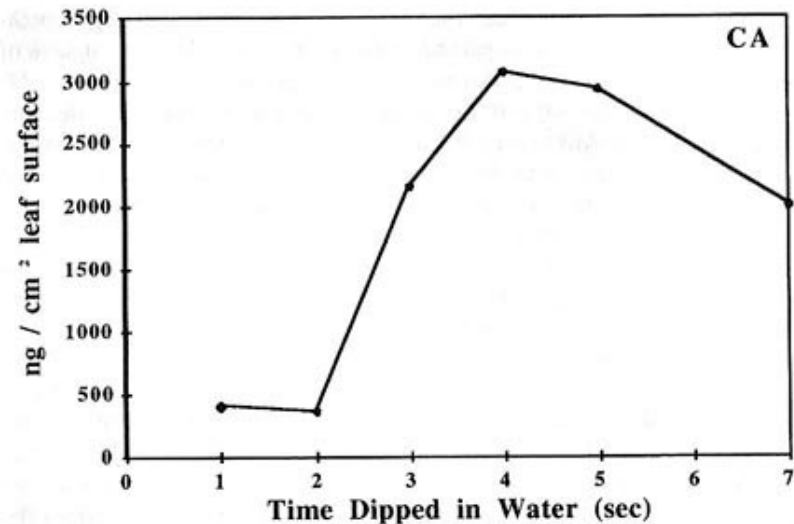


FIG. 5. Quantities of chlorogenic acid (CA), luteolin-7-*O*-(6''-*O*-malonyl)- $\beta$ -D-glucoside (L7MG), and luteolin-7- $\beta$ -D-glucoside (L7G) removed from *Daucus carota* leaf surfaces using chloroform and near-boiling water, with successively longer dipping times in water.

interior (see Merrall, 1981; Price, 1982 for references). Merrall (1981) hypothesized that an aqueous or polar pathway existed to explain the facile uptake of hydrophilic and ionic materials that were incompatible with lipids. Price (1982) also reviewed literature support for possible hydrophilic pathways extending from the aqueous apoplast to the cuticle surface but concluded that the evidence in favor of such a theory was rather indirect. More recent work has documented the presence of cuticular pores and transcuticular canals in 63 taxa among 32 families (Miller, 1985, 1986). Derridj et al. (1996) described the kinetics of soluble carbohydrate movement from leaf tissues through the cuticle to the leaf surface. From these studies it can be concluded that the cuticle is not a complete barrier to polar compounds, although the actual mechanism for the passage of polar compounds through the cuticle is unknown.

This current work confirms the presence of the flavonoid glycosides luteolin-7- $\beta$ -D-glucoside and luteolin-7-O-(6"-O-malonyl)- $\beta$ -D-glucoside in leaf surface extracts of *Daucus carota*. The solvent timing study with the chloroform-near-boiling water solvent combination suggests that brief extractions in the water (1-2 sec) remove compounds from the surface alone and not from the apoplast or symplast. It is reasonable to believe that these extracts reflect the presence of the compounds on the leaf surface itself.

Many of the past studies conducted on leaf surfaces used methylene chloride as the solvent for surface extraction (Proksch et al., 1985, 1986; Bohm and Constant, 1990; Bohm and Chan, 1992; Bohm, 1993; Reid and Bohm, 1993). Since methylene chloride significantly underperforms as an extraction solvent in comparison to more polar solvents, and often fails to extract the flavonoid glycosides to any detectable extent, it does not appear to be a suitable solvent for extracting polar compounds from the leaf surface of wild carrot.

Since extraction methods can vary greatly in the amounts of compounds extracted from the leaf surface, it is important to find the method best suited for the compounds of interest to the investigator (see also Städler and Roessingh, 1991; van Loon and van Meer, 1991; Renwick et al., 1992). For extracting the leaf surfaces of *D. carota*, a solvent combination of chloroform and near-boiling water performs somewhat better than other solvents and solvent combinations tested for extraction of polar compounds. It is also a versatile solvent combination that can extract nonpolar compounds in addition to polar compounds. With brief dipping times in water, the method appears to remove compounds from the leaf surface without sampling from the leaf interior to any appreciable extent.

Since swallowtail butterflies make their final oviposition decisions based on leaf surface chemistry, it is important to study chemical stimulants present on the leaf surface itself. Little is yet known concerning the relationship between chemical profiles of leaf surfaces, as perceived by egg-laying females, and the

chemical content of leaf interiors, which are consumed by the feeding larvae. The work described here provides a possible avenue for investigating such relationships.

*Acknowledgments*—We thank Erich Städler and an anonymous reviewer for their helpful comments on this manuscript. The research was supported by a Grant-In-Aid from the Cornell Chapter of Sigma Xi to J. S. B. and by NSF research grants IBN-9119674 and IBN-9420319 to P. F.

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