

Structure-activity relationships of phenolic and nonphenolic aromatic acids as oviposition stimuli for the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae)

G. G. Grant and D. Langevin

Canadian Forest Service, P.O. Box 490, Sault Ste. Marie, Ontario, Canada P6A 5M7

Abstract - In a study of carboxylic acids affecting oviposition preference of the spruce budworm, a major defoliator of conifers in North America, nonphenolic aromatic acids and phenolic acids were compared in a dual-choice bioassay to assess structure-activity relationships. Among a series of nonphenolic aromatic acids with a C₁ - C₆ alkanolic, C₃ - C₄ alkenolic or C₃ alkynolic side chain, females displayed the greatest preference for acids with a saturated C₃ - C₄ side chain (MW = 150 - 164) at dosages of 78.6 and 786 nmol/cm². This behavioral activity was consistent with the previously reported strong oviposition preference of female budworm for aliphatic acids of similar molecular weight; that is, for C₈ - C₁₀ straight chain carboxylic acids (MW = 144 - 172) and cyclohexyl carboxylic acids (MW = 142 - 170). In contrast, comparable phenolic acids at the same dosages acted as oviposition deterrents. In general, the deterrent effect of phenolic acids was greatest when the length of the acid side chain consisted of 3 carbons and the number of hydroxyl and/or methoxy groups on the aromatic ring was greater than one. Ferulic and sinapic acids exemplified this trend and were the strongest deterrents among the phenolic acids tested. Apparently, the presence or absence of an hydroxyl (or methoxy) group on the aromatic ring accounts for the difference in the observed behavioral effects between the two groups of aromatic compounds.

Key Words - phenolic acid, aromatic acid, carboxylic acid, ferulic acid, sinapic acid, *Choristoneura fumiferana*, oviposition stimulant, oviposition deterrent, structure-activity relationship

Introduction

The spruce budworm, *Choristoneura fumiferana* (Clemens), is a major defoliator of spruce and balsam fir forests in North America. It is a difficult insect to control and new pest control agents and strategies would be welcome. Phytochemicals that modify insect oviposition behavior could be useful as part of an integrated control strategy or have other practical applications. The spruce budworm also provides a useful model system to study oviposition stimuli. Recently, we found that aliphatic carboxylic acids applied to artificial substrate substantially increased oviposition preference of the spruce budworm for treated substrate (Grant et al. 2000). Peak ac-

tivity was associated with a series of straight chain C₈ – C₁₀ carboxylic acids and cyclic analogs of similar molecular size, specifically C₈ - C₁₀ cyclohexane acids (e.g. cyclohexanepropanoic acid). The structural similarity of the cyclohexane acids to simple aromatic acids such as benzoic and cinnamic acid derivatives, and to more complex phenolic acids (aromatic acids characterized by one or more hydroxyl groups on the aromatic ring), suggested that some aromatic acids might also act as oviposition stimuli for the spruce budworm. Benzoic acid, for example, is a suspected oviposition host cue for the silkworm moth, *Bombyx mori* (L.), which has antennal olfactory receptors specifically tuned to this compound (Popoff 1997). In contrast, p-coumaric acid, a phenolic acid from a nonhost plant, deters oviposition by *Etiella zinckenella* (Treitschke) (Hattori et al. 1992). Many aromatic and phenolic compounds are common constituents of plants, including conifers (Strack et al. 1989, Kraus and Spiteller 1997) that are hosts for the spruce budworm. Preliminary studies with various classes of phenolic compounds, including acids, have shown that phenolics tend to deter oviposition of the spruce budworm (Abou-Zaid et al. 2000).

The objective of this report was to expand our study of carboxylic acids as oviposition stimuli for the spruce budworm to determine how aromatic and phenolic acids affect oviposition preference, and how changes in the constituents of these acids modify this preference. The exploration of structure-activity relationships can provide useful insights into the active moiety of oviposition stimuli (Cole et al. 1989, Douglass et al. 1993, Breeden et al. 1996). This information could rationalize development of new compounds with greater stimulating or deterring effects, or with better environmental stability (Cole et al. 1989, Honda 1995).

Methods and Materials

Insects were obtained from a long-established laboratory colony at the Canadian Forest Service, Sault Ste. Marie, ON, Canada. Larvae were reared on artificial diet but females were provided host foliage for oviposition. Candidate compounds were evaluated in a dual-choice oviposition bioassay (Grant and Langevin 1994, 1995). Mating pairs of spruce budworm were placed singly in small screen cages (9 cm diam.) with a top and bottom made from plastic Petri dishes lined with filter paper (Whatman No. 1), which served as the oviposition substrate. The top and bottom dishes were separated by a 4.5 cm high aluminum screen spacer. Bioassays were conducted in a well ventilated room maintained at 23-25EC, 50-60% RH, and on a 16:8 (L:D) h cycle.

Test chemicals were obtained from Sigma-Aldrich Canada (Oakville, ON). Candidate nonphenolic aromatic acids included six compounds with an alkanolic side chain of one carbon (benzoic acid) to six carbons (6-phenylhexanoic acid), two compounds with an alkenolic side chain (3-phenyl-*trans*-2-propenoic acid [= cinnamic acid] and 4-phenyl-*trans*-3-butenoic acid), and one compound with an alkynoic

side chain (3-phenyl-2-propionic acid). Thirteen candidate phenolic acids with aliphatic side chains of 1 - 3 carbons or a 3-carbon alkenoic side chain (i.e. cinnamic acid derivatives) and with 1- 3 hydroxy and 0 - 2 methoxy groups on the aromatic ring were tested. The choice of test compounds was limited by commercial availability.

Test compounds were dissolved in 95% or absolute alcohol to produce concentrations of 10 mM and 100 mM (Grant et al. 2000). In addition, five of the phenolic acids with significant oviposition activity at the 10 mM level were tested further at a 1 mM concentration. A 0.25 ml aliquot of a test solution was pipetted evenly over one-half (31.8 cm²) of the filter paper substrate (visibly divided by a light pencil line), resulting in dosages of 7.86, 78.6 and 786 nmol/cm², respectively, for the 1,10 and 100 mM concentrations. The 78.6 nmol/cm² dosage was deemed to be a physiologically realistic stimulus level (Zhao et al. 1998, Grant et al. 2000).

A 0.25 ml aliquot of the solvent used to dissolve the test compound was applied to the other half of the filter paper to serve as the control. Both the top and bottom filter papers in 25 arenas were treated for an experiment, which was replicated at least once.

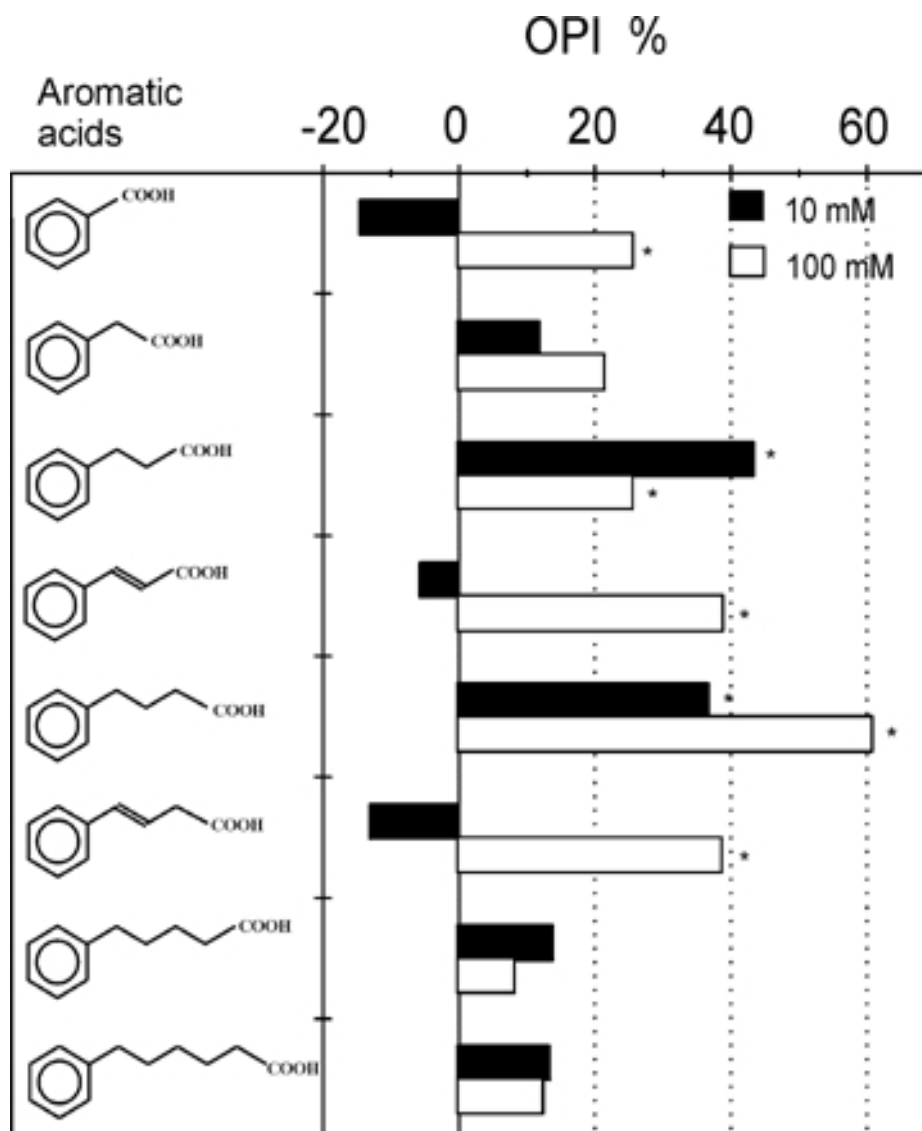
Oviposition preference, as indicated by the number of egg masses on treated and control substrates after 2 days, was quantified by means of an oviposition preference index (OPI) = (treated - control) x 100 / (treated + control). Mean OPI values ranged from -100% to +100%; statistically significant positive values indicated preference while significant negative values indicated nonpreference (deterrence). Results of replicated experiments were pooled if statistically homogeneous (i.e. $P > 0.05$), as indicated by the Mann-Whitney test for 2 replicates or the Kruskal-Wallis test for more than 2 replicates (Zar 1984). Statistical significance ($P \leq 0.05$) of OPIs was assessed with the Wilcoxon matched-pairs, signed-rank test (Zar 1984).

Results

Female spruce budworm oviposited on substrate treated with several of the nonphenolic aromatic acids. At 78.6 nmol/cm², preference was limited to three compounds: 3-phenylpropionic acid and 4-phenylbutanoic acid with saturated side chains of 3 and 4 carbons respectively (Fig. 1), and 3-phenyl-2-propionic acid (not shown in Fig.1) with a triple bond in the C₃ side chain (OPI = 35.1%, $p < 0.001$). In contrast to their saturated analogues, the two aromatic acids with a double bond in the C₃ side chain (cinnamic acid and 4-phenyl-*trans*-3-butenoic acid) were not active at this dosage. However, at the higher dosage (786 nmol/cm²), these unsaturated aromatic acids had a significant effect on oviposition along with their saturated analogs (Fig. 1). The activity of 3-phenyl-2-propionic acid increased at this higher dosage (OPI = 68.1%, $p < 0.001$).

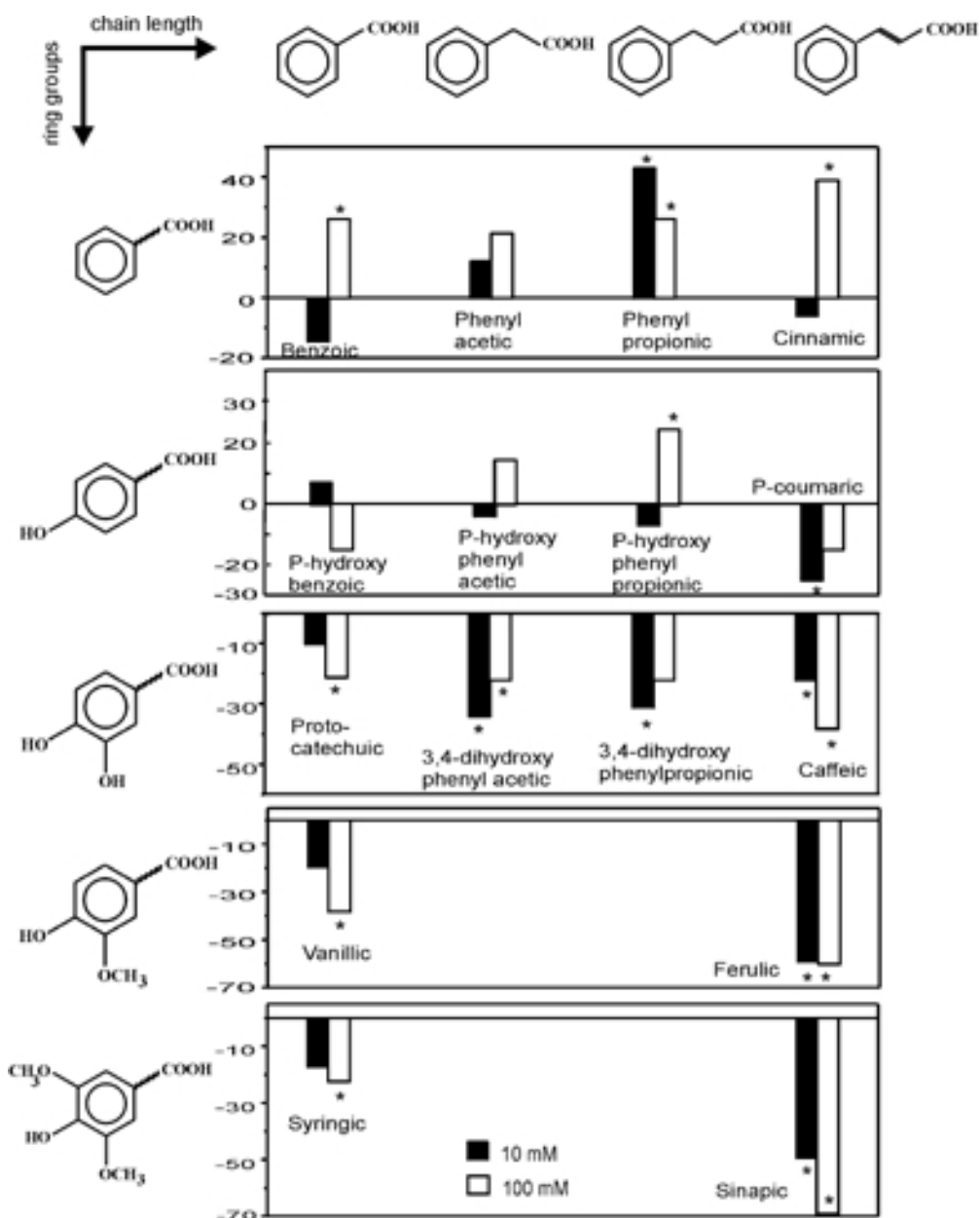
In contrast to the above aromatic acids, the phenolic acids tended to deter oviposition by the spruce budworm. Of the 13 phenolic acids tested, five were

Figure 1 Effect of nonphenolic aromatic acids at two concentrations on oviposition preference of the spruce budworm as indicated by the oviposition preference index (OPI %). Aromatic acids illustrated from top of figure to bottom are respectively, benzoic acid, 2-phenylacetic acid, 3-phenylpropanoic acid, 3-phenyl-*trans*-2-propenoic acid, 4-phenylbutanoic acid, 4-phenyl-*trans*-3-butenoic acid, 5-phenylpentanoic acid, and 6-phenylhexanoic acid. An asterisk indicates a significant OPI value ($P \leq 0.05$, Wilcoxon matched-pairs, sign rank test).



significantly deterrent at 78.6 nmol/cm^2 and eight were deterrent at the 10-fold higher dosage (Fig. 2), including gallic acid, 3,4,5-trihydroxybenzoic acid (not shown in Fig. 2), which generated OPI values of -31.4% ($p < 0.01$) and -20.2% ($p < 0.05$) at the low and high dosages, respectively. In general, the deterrent effect of phenolic acids was greatest when the acid side chain consisted of three carbons (horizontal comparison of compounds in Fig. 2) and the number of functional groups on the

Figure 2 Structure-activity relationship between phenolic and nonphenolic aromatic acids and oviposition by spruce budworm. Each panel represents a group of aromatic acids with the same hydroxyl and/or methoxy substituents on the aromatic ring. Within each panel, the length of the acid side chain on the aromatic ring increases from C₁ to C₃, with unsaturation present in the last C₃ compound. The Y-axis for each panel is the oviposition preference index (OPI %). An asterisk over a column indicates a significant OPI value (P # 0.05, Wilcoxon matched-pairs, sign rank test). The first panel is modified from Fig. 1.



aromatic ring was greater than one (vertical comparisons, Fig. 2). Sinapic and ferulic acids, as the most deterrent compounds tested, exemplified this trend. Unsaturation in

the C₃ side chain of the phenolic acids did not appear to affect behavioral activity as occurred with two of the nonphenolic aromatic acids mentioned above.

There was no consistent difference in the effect of the phenolic acids at the 78.6 and 786 nmol/cm² dosages (Fig. 2). However, of the five phenolic acids bioassayed at a lower dosage (7.8 nmol/cm²), only ferulic acid remained deterrent (Table 1). In contrast, the effect of gallic acid was reversed and females showed a significant preference for substrate treated at this dosage (Table 1). This result may illustrate the reversal in behavioral response sometimes observed in bioassays covering a range of dosages (Torto et al. 1991).

Table 1 Effect of selected phenolic acids at 7.8 nmol/cm² on oviposition of spruce budworm

Compound	OPI(%) ^a	P ^b
gallicacid	31.4	0.001
syringicacid	9.4	0.52
caffeicacid	-5.2	0.69
ferulicacid	-28.6	0.03
sinapicacid	-16.9	0.20

^aOviposition preference index

^bWilcoxon matched-pairs, sign rank test

Discussion

The results suggest that spruce budworm preference for nonphenolic aromatic acids is dependent on the length of the acid side chain and dosage. The most stimulating aromatic acids had C₃ – C₄ acid side chains. They also closely matched the size (9 - 10 carbons, MW = 146 - 164) of the most stimulating straight chain and cyclohexane aliphatic acids (C₈ - C₁₀, MW = 142 - 172) reported in Grant et al. (2000). At the highest dosage (786 nmol/cm²), these aromatic acids remained stimulating in contrast to the C₈ - C₁₀ aliphatic acids, which became repellent or behaviorally inactive at this higher dosage (Grant et al. 2000). The lower volatility of the aromatic acids in contrast to the corresponding aliphatic acids probably accounts for this difference. Lower volatility would keep airborne concentrations from reaching too a high a level and thus becoming repellent.

The introduction of a double bond (*trans*) into the acid side chain of two stimulating saturated aromatic acids (i.e. 3-phenylpropanoic acid and 4-phenylbutanoic acid) apparently reduced their oviposition activity, as the unsaturated analogues were inactive at 78.6 nmol/cm² (although they were active at the higher dosage). The reason for this inactivity relative to the saturated analogues at this dosage is not

clear. The result could be an anomaly, as 3-phenyl-2-propionic acid with a triple bond in the side chain was an effective stimulus at the same dosage. However, Douglass et al. (1993) found that the introduction of a double bond into the acid side chain of analogs of β -bergamotenoic acid, a host-produced oviposition stimulus for *H. zea*, significantly reduced oviposition preference of this moth relative to the corresponding saturated analogues.

With respect to the phenolic acids, the results show that they tend to deter oviposition by the spruce budworm. Although the series of compounds tested was limited, it appears that the most deterrent phenolic acids have a $C_6 + C_3$ structural skeleton similar to the most stimulating aromatic acids ($C_6 + C_3$ and $C_6 + C_4$). Compounds with this structure, particularly phenylpropanoid ($C_6 + C_3$) derivatives, often have broad stimulating or deterring effects on the behavior of other adult insects (Metcalf 1987, Cowels et al. 1990, Hattori et al. 1992, Dudareva and Pichersky 2000). The difference in the respective behavioral effects of the simple aromatic acids versus phenolic acids for the spruce budworm is apparently due to the presence or absence of a hydroxyl (and/or methoxy) group on the aromatic ring, which would affect both chemoreception and volatility. As the phenolic acids have little or no volatility, they presumably act through contact chemoreceptors and thus function as deterrents rather than repellents. However, mechanical or visual effects of the phenolics cannot be ruled out (Tabashnik 1985).

Phenolic compounds are important oviposition stimuli for other Lepidoptera. Chlorogenic acid (a phenylpropanoid derivative) and several flavonoids are oviposition host stimulants for various butterfly species (Honda 1995, Haribal and Renwick 1996, Carter et al. 1998) and for *Helicoverpa armigera* (Hübner), a moth species (Simmonds 1998). On the other hand, phenolics derived from nonhost sources have proved to be oviposition deterrents for a number of moth species, including *Plutella xylostella* (L.) (Tabashnik 1985), *Heliothis virescens* (F.) (Ramaswamy et al. 1992), *E. zinckenella* (Hattori et al. 1992) and *Sitotroga cerealella* (Olivier) (Ge and Weston 1995).

Structure-activity relationships of carboxylic acids as oviposition stimuli have been studied in a few other insects. Oviposition preference of female *H. zea* for β -bergamotenoic acid, a host stimulant, appears to be due primarily to the carboxylic function but, as in the spruce budworm, oviposition activity is modified substantially by the length of the side chain to which the carboxyl function is attached and by the presence or absence of a double bond in the side chain (Douglass et al. 1993, Breden et al. 1996). In the case of the cabbage rootworm fly, *Delia radicum* (L.), the situation is much simpler. Sinapic acid is a naturally occurring oviposition deterrent for this fly, produced in the frass of a moth caterpillar feeding on the same plants (Jones et al. 1988). The deterrent activity of sinapic acid was dependent solely on the carboxylic acid function. Neither the phenolic ring nor other structural features conferred or affected deterrent activity (Cole et al. 1989). It remains to be seen how a change at the carboxylic function would affect the spruce budworm response to the

behaviorally active compounds reported here.

Few studies have examined how adult Lepidoptera detect nonvolatile phenolic compounds. Electrophysiological studies with a *Papilio* butterfly species have shown that tarsal contact chemoreceptors of females respond to host-derived phenolics (*trans*-chlorogenic acid and a flavonoid) (Roessingh et al. 1991). Hence phenolic acids can be expected to stimulate contact chemoreceptors on the tarsi or ovipositor of the spruce budworm, or possibly chemoreceptors on the proboscis (Rivet and Albert 1990). The deterrent effect of phenolic acids on the spruce budworm may reflect an ability of its sensory receptors to detect potentially noxious substances through a deterrent neuron (Schoonhoven 1991). We have shown previously that quinolizidine alkaloids have strong deterrent effects on ovipositing spruce budworm (Zhao et al. 1998) and the action of these deterrents may have a similar chemosensory pathway. The relationships between the chemical structure of carboxylic acids and their behavioral activity will provide useful comparisons for electrophysiological studies of the contact chemoreceptors of the spruce budworm stimulated with these compounds, and would help confirm the sensory mode of action of these compounds. Those results could also lead to a better understanding of the chemical constituents that stimulate or deter oviposition behavior.

Acknowledgement

We thank D. Lombardo, M. Seccariccia, D. Poitras and M. Roboek for excellent technical assistance, and colleagues B. Helson and L. Gringorten for helpful review comments.

References

- Abou-Zaid MM, Grant GG, Helson BV, Beninger CW, de Groot P (2000) Phenolics from deciduous leaves and coniferous needles as sources of novel control agents for lepidopteran forest pests. pp 398-416, In Shahidi S, Ho C (eds.) *Phytochemicals and Pharmaceuticals*, AOCS Press, Champaign, Ill.
- Breeden DC, Young TE, Coates RM, Juvik JA (1996) Identification and bioassay of kairomones for *Helicoverpa zea*. *J. chem. Ecol* 22: 513-539
- Carter M, Sachdev-Gupta K, Feeney P (1998) Tyramine from the leaves of wild parsnip: a stimulant and synergist for oviposition by the black swallowtail butterfly. *Physiol Entomol* 23: 303-312
- Cole RA, Jones TH, Finch S (1989) Deterrent effect of carboxylic acid on cabbage root fly oviposition. *Ann appl Biol* 115: 39-44
- Cowles RS, Miller JR, Hollingworth RM, Abdel-Aal MT, Szurdoki F, Bauer K, Matolcsy G (1990) Cinnamyl derivatives and monoterpenoids as nonspecific ovipositional deterrents of the onion fly. *J chem Ecol* 16: 2401-2428
- Dudareva N, Pichersky E (2000) Biochemical and molecular genetic aspects of floral scents. *Plant*

- Phys. 122: 627-633
- Douglass SK, Juvik JA, Pyun H-J, Coates RM (1993) Structure-activity relationships for analogs of (+)-(E)-*endo*- β -bergamoten-12-oic acid, an oviposition stimulant of *Helicoverpa zea*. J chem Ecol 19 11-27
- Ge X, Weston PA (1995) Ovipositional and feeding deterrent from Chinese prickly ash against Angoumois grain moth (Lepidoptera: Gelechiidae). J econ Entomol 88 1771-1775
- Grant GG, Langevin D (1994) Oviposition responses of four *Choristoneura* (Lepidoptera: Tortricidae) species to chemical and physical stimuli associated with host and nonhost foliage. Environ Entomol 23 447-456
- Grant GG, Langevin D (1995) Oviposition deterrence, stimulation, and effect on clutch size of *Choristoneura* (Lepidoptera: Tortricidae) species by extract fractions of host and nonhost foliage. Environ Entomol 24: 1656-1663
- Grant GG, Zhao B, Langevin D (2000) Oviposition response of the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae) to aliphatic carboxylic acids. Environ Entomol 29:164-170
- Haribal M, Renwick JAA (1996) Oviposition stimulants for the monarch butterfly: flavonol glycosides from *Asclepias curassavica*. Phytochemistry 41: 139-144
- Hattori M, Sakagami Y, Marumo S (1992) Oviposition deterrents for the limabean pod borer, *Etiella zinckenella* (Treitschke) (Lepidoptera; Pyralidae) from *Populus nigra* L. c.v. Italica leaves. Appl Entomol Zool 27: 195-204
- Honda K (1995) Chemical basis of differential oviposition by lepidopterous insects. Arch Insect Biochem Physiol 30: 1-23
- Jones TH, Cole RA, Finch S (1988) A cabbage root fly oviposition deterrent in the frass of the garden pebble moth caterpillars. Entomol. exp. appl 49: 277-282
- Kraus C, Spitteller G (1997) Comparison of phenolic compounds from galls and shoots of *Picea glauca*. Phytochemistry 44: 59-67
- Metcalf RL (1987) Plant volatiles as insect attractants. Crit Rev Plant Sci 5: 251-301 Popoff B (1997) Olfactory responses recorded from sensilla coeloconica of the silkworm *Bombyx mori*. Physiol Entomol 22: 239-248
- Ramaswamy SB, Cohen NE, Hanson FE (1992) Deterrence of feeding and oviposition responses of adult *Heliothis virescens* by some compounds bitter-tasting to humans. Entomol exp appl 65: 81-93
- Rivet M-P, Albert PJ (1990) Oviposition behavior in the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). J Insect Behav 3: 395-400
- Roessingh P, Städler E, Schoni R, Feeny P (1991) Tarsal contact chemoreceptors of the black swallowtail butterfly *Papilio polyxenes*: responses to phytochemicals from host and nonhost plants. Physiol Entomol 16: 485-495
- Simmonds, MSJ (1998) Chemoecology: the legacy left by Tony Swain. Phytochemistry 49: 1183-1190
- Schoonhoven LM (1991) The sense of distaste in plant-feeding insects - A reflection on its evolution. Phytoparasitica 19: 3-
- Strack D, Heilemann J, Wray V, Dirks H (1989) Structures and accumulation patterns of soluble and insoluble phenolics from Norway spruce needles. Phytochem 28: 2071-2078
- Tabashnik BE (1985) Deterrence of diamondback moth (Lepidoptera; Plutellidae) oviposition by plant compounds. Environ Entomol 14: 575-587
- Torto B, Hassanali A, Saxena KN, Nokoe S (1991) Feeding responses of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) larvae to sorghum plant phenolics and their analogs. J chem Ecol 17: 67-78

Zhao B, Grant GG, Langevin D, MacDonald L (1998) Deterring and inhibiting effects of quinolizidine alkaloids on the spruce budworm (Lepidoptera: Tortricidae) oviposition. *Environ Entomol* 27: 984-992

Zar JH (1984) *Biostatistical analysis*, 2nd ed. Prentice-Hall, Englewood Cliffs, N.J.