

'Floral' scent production by *Puccinia* rust fungi that mimic flowers

ROBERT A. RAGUSO*‡ and BARBARA A. ROY†

*Department of Biology, University of Michigan, Ann Arbor, MI 48109–1048, USA, †Geobotanisches Institut, Swiss Federal Institute of Technology (ETH), Zürichbergstrasse 38, CH-8044 Zürich, Switzerland

Abstract

Crucifers (Brassicaceae) in 11 genera are often infected by rust fungi in the *Puccinia monoica* complex. Infection causes a 'pseudoflower' to form that is important for attracting insect visitors that sexually outcross the fungus. 'Pollinator' attraction is accomplished through visual floral mimicry, the presence of a nectar reward and floral fragrances. Here we used gas chromatography and mass spectrometry to identify and quantify fragrance production by these rust fungi on several *Arabidopsis* hosts, and by co-occurring true flowers that share insect visitors. Fungal pseudoflowers produced distinctive floral fragrances composed primarily of aromatic alcohols, aldehydes and esters. Pseudoflower fragrances were chemically similar to noctuid-moth-pollinated flowers, such as *Cestrum nocturnum* and *Abelonia grandiflora*, but were very different from host flowers, host vegetation and the flowers of coblooming, nonhost angiosperms. There was variation in the quantity and composition of fragrance profiles from different fungal species as well as within and among hosts. The evolution of scent chemistry is relatively conservative in these fungi and can be most parsimoniously explained in three steps by combining chemical data with a previously determined rDNA ITS sequence-based phylogeny. Pseudoflower scent does not appear to represent a simple modification of host floral or vegetative emissions, nor does it mimic the scent of coblooming flowers. Instead, we suspect that the unique fragrances, beyond their function as pollinator attractants, may be important in reducing gamete loss by reinforcing constancy among foraging insects.

Keywords: gas chromatography, headspace volatiles, ITS region, mass spectrometry, phylogenetic reconstruction, pollination

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Introduction

Rust fungi in the *Puccinia monoica* complex (Pucciniaceae, Uredinales, Basidiomycetes) infect 11 species of Brassicaceae throughout the northern hemisphere, modifying host leaf morphology to produce fungal 'pseudoflowers' that aid in the sexual reproduction of the fungus through the movements of foraging insects (Roy 1993a; 1994a, b). Rosettes of infected leaves are encrusted by bright yellow spermatogonia (the outcrossing fungal structures that bear spermatia and receptive hyphae) and visually resemble yellow coblooming true flowers. Not only

do *Puccinia* pseudoflowers look like flowers, they also produce a sweet, pungent fragrance and exude a fructose-rich solution that is consumed by numerous insects, including andrenid and halictid bees, ants, nymphalid butterflies, anthomyiid, muscid and sarcophagid flies (Roy 1993a; 1996; Roy & Raguso 1997). Thus, pseudoflowers produce 'floral' advertisements and rewards and may be considered generalized floral mimics (Roy 1993a, 1994a, 1996).

We previously demonstrated that both visual and olfactory cues attract bees and flies to fungal pseudoflowers in field settings (Roy & Raguso 1997). Halictid bees (*Dialictus* spp.) were attracted to yellow paper artificial flowers and to lures charged with complete and fractionated pseudoflower fragrance, but the combination of yellow colour and *Puccinia* scent was required to elicit normal levels of bee approaches and landings. In contrast,

Correspondence: R. A. Raguso. ‡Present address for correspondence: Center for Insect Science and ARL Div. of Neurobiology, 611 Gould-Simpson Building, University of Arizona, Tucson AZ 85721, USA. Fax: +1-520-621-8385; E-mail: rar@neurobio.arizona.edu

anthomyiid flies were not attracted to yellow colour alone, nor to individual scent compounds, but only to the complete blend of pseudoflower volatiles (Roy & Raguso 1997). Thus, fragrance production appears to be an important component of insect attraction and sexual reproduction in *Puccinia monoica* and related rust fungi.

Here we identify the fragrance compounds produced by fungal pseudoflowers on *Arabis* and compare them with: (i) the vegetative and floral volatiles of their uninfected host *Arabis* plants; and (ii) the floral volatiles of coblooming plants in Colorado, USA, with which *Puccinia* pseudoflowers share insect visitors. In addition, we analysed variation in scent production by pseudoflowers in a phylogenetic context, searching for intra- and interspecific differences in fragrance chemistry that are correlated with host species. Specifically, we asked: do flower-mimicking crucifer rust fungi emit amplified levels of volatiles already produced by host vegetation and flowers, or are their fragrance compounds unique to infected tissues? Are pseudoflower fragrances similar to those of some coblooming flowers, or are they chemically distinct? How has fragrance production evolved in this floral mimicry system?

Materials and methods

Plant and fungal materials were collected at numerous localities, including Almont, Cement Creek and Taylor River (2500–2700 m; see Roy 1996) near the Rocky Mt. Biological Laboratory in Gunnison County, Colorado, USA. We enclosed cut *Arabis* leaves, flowers, and tissues infected with *Puccinia* rust fungus within 0.5 L polyacetate bags (Reynolds, Inc.). Due to the small masses and relative weakness of fragrance in these samples, tissues from many individuals were combined so that each trial had a minimum of ≈ 0.5 g of plant/fungal tissue. In addition, we collected and identified floral scent compounds from *Anemone (Pulsatilla) patens* and *Ranunculus inamoenus* (Ranunculaceae), *Phlox bryoides* (Polemoniaceae), *Pseudocymopterus montanus* (Apiaceae) and *Thlaspi montanus* (Brassicaceae) plants that share flowering phenology, habitat and insect visitors with *Puccinia* pseudoflowers. Headspace volatiles accumulated in the bags and were swept over cylindrical glass cartridges packed with 100 mg of Porapak Q sorbent (80–100 mesh) by a Manostat, Inc. peristaltic vacuum pump at a flow rate of 250 mL/min for 8 h. Trapped volatiles were eluted from cartridges with 3 mL of HPLC-grade hexane, concentrated to a 50 μ L volume with gaseous N₂, and supplemented with toluene (5 μ L of a 0.3% v/v solution in hexane) as an internal standard.

Fragrance compounds were analysed by injecting 1 μ L aliquots of processed samples into a Shimadzu GC17A gas chromatograph, using a Shimadzu QP5000 mass spectrometer as a detector. Constituent peaks were separated

on a nonpolar SE-54 glass capillary column (30 m long, 0.32 mm internal diameter (ID), 1 μ m film thickness) using the following temperature program: inject at 50 °C and hold for 2 min, then increase at 10 °C/min to 275 °C, hold for 3.5 min). Tentative identifications of scent compounds were provided by on-line mass spectral libraries (Wiley and NIST, > 125 000 spectra) and confirmed, when possible, through comparison with retention times and mass spectra of synthetic standards (Sigma Inc. and Aldrich Inc.). Integrated peak areas were quantified by comparison with the internal standard (toluene) and with calibration curves based on serial dilutions of other standards.

Results

Volatiles from uninfected *Arabis* vegetative tissues

We identified numerous fatty-acid-derived volatiles, a few aromatics and one sesquiterpene from the vegetation of six *Arabis* species (Table 1). The vegetative headspace of these plants was dominated by the 'green-leaf volatiles' (GLVs), a class of C₆–C₈ aliphatic compounds that are ubiquitous in vegetative tissues, especially when cut or wounded, and are derived from linolenic acid through the catabolic lipoxygenase pathway (Visser & Avé 1978, Tollsten & Bergström 1988; Mookherjee *et al.* 1989; Buttery & Ling 1993; Dickens *et al.* 1993). In addition, we identified isopropyl isothiocyanate, a glucosinolate derivative that is characteristic of crucifer foliage (Fenwick *et al.* 1983; Tollsten & Bergström 1988; Evans & Allen-Williams 1992), from the vegetative headspace of *A. crandallii* and *A. holboellii*. Within *A. holboellii*, rosette leaves differed from bolting leaves in overall volatile emissions per fresh mass and in the presence of the aromatic compounds benzaldehyde and benzyl alcohol (Table 1). Bolting leaves were used for all other *Arabis* samples.

Volatiles from uninfected *Arabis* flowers

Volatile emissions from floral tissues of *A. demissa*, *A. drummondii* and *A. holboellii* were barely detectable, and included the sesquiterpene caryophyllene, aliphatic GLVs (probably from vegetative tissues) and trace amounts of the aromatic 2-phenyl ethanol and the nitrogen-bearing indole (Table 1). Most of these compounds have been previously identified from the vegetative and floral headspace of other crucifers, including *Brassica napus*, *B. campestris*, *B. juncea*, *B. nigra* (Tollsten & Bergström 1988; Evans & Allen-Williams 1992, Jakobsen *et al.* 1994), *Hesperis matronalis* (Nielsen *et al.* 1995) and *Sinapis alba* (Tollsten & Bergström 1988).

Volatiles from *Puccinia* fungal pseudoflowers

Arabis vegetative tissues infected with *Puccinia* rust fungus produced numerous structurally related aromatic or

Table 1 Volatile production from uninfected *Arabis* plants

Chemical class compound	<i>A. holboellii</i>			<i>A. demissa</i>		<i>A. crandallii</i>	<i>A. lignifera</i>	<i>A. drummondii</i>		<i>A. gunnisoniana</i>
	veg*	veg†	floral	veg	floral	veg	veg	veg	floral	veg
Aromatics										
Benzaldehyde	19.28%‡								ND	
Benzyl alcohol	24.19									
Indole		<1.0			26.51	<1.0	<1.0			
2-phenylethanol					<1.0					
Terpenoids										
Caryophyllene			50.00							
Fatty acid derivatives										
1-hexanol		11.07				13.60				
<i>n</i> -hexanal		13.47		20.00		29.30				
2-ethyl hexanol§		1.63								
Hexyl acetate		2.20								
(Z)-3-hexen-1-ol										20.6
(Z)-3-hexenal	9.24	59.00		80.00		43.80	29.33	34.55		
(Z)-3-hexenyl acetate	29.65	8.79			57.14	8.30	36.48	33.20		79.4
(Z)-3-hexenyl butyrate								6.09		
(Z)-3-hexenyl	4.16	3.83	50.00		16.65	2.36	34.19	12.61		
3-methyl butyrate										
2-methyl butyrate								12.52		
Diethyl ethanone§								1.00		
Isopropyl iso-thiocyanate octadecanal§	8.49				<1.0	2.65				
Total fresh mass (g)	4.23	1.21	0.50	0.59	1.13	1.94	3.56	4.06	0.70	7.24
Emissions ng/g fresh mass/8 h	0.27	8.36	1.55	6.45	6.65	2.88	0.05	0.22	ND	0.30

*Leaves taken from bolting tissues.

†Leaves taken from basal rosette.

‡Per cent of total volatile emissions.

§Indicates tentative identification due to mass spectrum library search. All other identifications verified by mass spectrum and retention time through coinjection of standards.

benzenoid compounds, such as benzaldehyde, phenylacetaldehyde and various phenylethyl esters (Tables 2 and 3), in addition to aliphatic GLVs that are emitted by uninfected *Arabis* foliage (Table 1, Fig. 1). Phenylacetaldehyde and 2-phenyl ethanol were present in all *Puccinia* samples and, to our human noses, were the dominant 'character-bearing notes' of the distinctive pseudoflower fragrance. One aromatic ester, benzyl acetate, was only detected in the headspace of some (but not all) *A. crandallii* plants infected with *P. consimilis* (Table 2, 3). Interestingly, benzyl acetate was not detected when *P. consimilis* used *A. lignifera* as a host. In addition, we identified what appears to be an α -oxidation series of phenylethyl esters (Fig. 1; see Croteau & Karp 1991), ranging in size from phenylethyl senecioate and phenylethyl isovalerate (C_{13}) to phenylethyl acetate (C_{10}). Scent profiles did not vary greatly between fungal species or between host races within fungal species (Tables 2 and 3), except in cases

where poor scent production was probably an artefact of small, senescent tissue samples (*P. monoica* on *A. drummondii* and *P. thlaspeos* on *A. demissa*). Two additional aromatic esters, methyl benzoate and amyl salicylate, were detected in most samples in 1994 and were absent in 1996, but were not present as contaminants in ambient controls. Phenylacetaldehyde, 2-phenylethanol, and benzaldehyde (along with indole) were previously identified from the headspace of another rust fungus, *P. punctiformis*, which uses *Cirsium arvense* thistles as a sexual stage host but does not modify host vegetation to produce pseudoflowers (Connick & French 1991).

Volatiles from coblooming alpine flowers

The floral fragrances of *Anemone patens*, *Phlox bryoides*, *Pseudocymopterus montanus* and *Thlaspi montanus* were dominated by monoterpenoid compounds, especially

Table 2 Volatiles emitted by *Puccinia* rust fungi associated with *Arabis* host plants: 1994

Chemical class compounds	<i>P. monoica</i> on <i>A. holboellii</i>	<i>P. consimilus</i> on <i>A. crandallii</i>	<i>P. consimilus</i> on <i>A. demissa</i>	<i>P. thlaspeos</i> on <i>A. gunnisoniana</i>	<i>P. thlaspeos</i> on <i>A. holboellii</i>
Aromatics					
Benzaldehyde	3.4%*	2.1	1.2	1.3	<1.0
Phenylacetaldehyde	61.6	47.5	30.6	35.9	<1.0
2-phenylethanol	11.9	3.2	2.7	24.6	13.2
Methyl benzoate	1.5	<1.0	2.0	1.7	
Benzyl acetate		60.5			
Phenethyl acetate	11.3	29.0		16.4	<1.0
Phenethyl propionate	<1.0	1.0	<1.0	1.1	
Phenethyl isobutyrate	<1.0	<1.0	<1.0	<1.0	
Phenethyl isovalerate†	5.2	12.7	1.2	9.6	<1.0
Phenethyl senecioate†	<1.0	<1.0	<1.0	1.8	
Amyl salicylate	1.0	<1.0	<1.0	<1.0	
Fatty acid derivatives					
Isopropyl isothiocyanate					20.7
2-methyl hexanoic acid†	2.2	1.0	1.1	<1.0	
Hexyl acetate		<1.0		<1.0	
(Z)-3-hexen-1-ol					28.8
(Z)-3-hexenyl acetate		<1.0		<1.0	37.2
Total fresh mass (g)	4.57	5.58	6.54	3.39	0.50
Scent ng/g fresh mass/8 h	6.71	12.97	4.45	37.90	10.73

*Per cent of total volatile emissions

†Indicates tentative identification due to mass spectrum library search. All other identifications verified by mass spectrum and retention time through coinjection of standards.

myrcene, (Z)- and (E)- β -ocimene, α - and β -pinene and, in *Phlox*, oxygenated monoterpenoids such as 1,8 cineole, linalool and verbenone (Table 4, Fig. 1). Ocimene was previously reported as the major floral volatile of the related European plant, *Anemone* (= *Pulsatilla*) *vulgaris* (Nilsson 1980), and monoterpene blends such as that identified from *Pseudocymopterus montanus* are characteristic of related umbellifers, such as *Angelica* spp. (Tollsten *et al.* 1994). Despite several attempts, we did not detect volatiles from the yellow flowers of *Ranunculus inamoenus*, which *Puccinia* pseudoflowers resemble most closely in size, shape and colour (Roy 1993a, 1994a). Floral headspace analyses of the related *Ranunculus acris* in Öland, Sweden revealed a scent profile dominated by (E)- β -ocimene and other monoterpenes, 14 sesquiterpenes and about 1% 2-phenylethanol (Bergström *et al.* 1995).

Scent production and phylogenetic trends in the *P. monoica* complex

Previous phylogenetic analyses based on DNA sequence variation in the ITS-1 and ITS-2 regions had revealed that both *P. monoica* and *P. thlaspeos* are polyphyletic 'species' (Roy *et al.* 1998). According to the phylogenetic hypothesis presented in Fig. 2, races of *P. monoica* using *A. drummondii* and *A. holboellii* as aecial hosts (Group II) bear a

sister-group relationship to another clade (Group I) in which races of *P. thlaspeos* and *P. monoica* sharing *A. demissa* as a host are more closely related to each other than to conspecific races that use different *Arabis* hosts. *P. consimilus* appears to have evolved from within Group I, and is most similar to rusts using *A. demissa* as an aecial host.

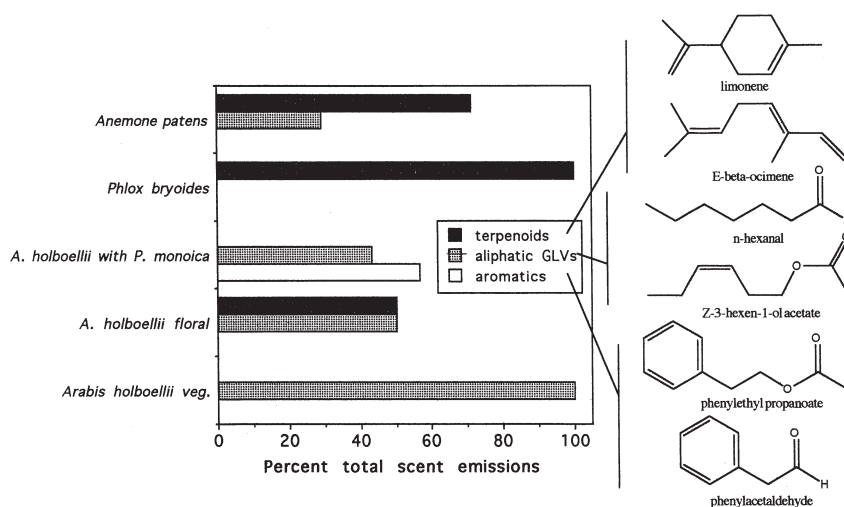
The evolution of scent chemistry is relatively conservative among these fungi, and is most parsimoniously explained in three steps. First, the emission of benzaldehyde, phenylacetaldehyde and 2-phenylethanol occurs in the basal taxon *P. punctiformis*, which lacks pseudoflowers but requires insect-mediated sexual reproduction (Connick & French 1991). These three aromatic compounds, derived from phenylalanine through the shikimic acid pathway, are common to all taxa that we have examined within the more derived *P. monoica* complex, in which both pseudoflowers and insect-mediated sexual reproduction occur. Second, the phenylethyl esters are shared among all members of the *P. monoica* complex, but were not reported to be present in *P. punctiformis* by Connick & French (1991). They must have arisen as components of pseudoflower fragrance after the historical split between the ancestors of *P. punctiformis* and the *P. monoica* complex (Fig. 2). Third, benzyl acetate is emitted exclusively by the derived taxon *P. consimilis*, is present

Table 3 Volatiles emitted by *Puccinia* rust fungi associated with *Arabis* host plants: 1996

Chemical class	<i>P. monoica</i> on <i>A. holboellii</i>	<i>P. monoica</i> on <i>A. drummondii</i>	<i>P. consimilis</i> on <i>A. crandallii</i>	<i>P. consimilis</i> on <i>A. lignifera</i>	<i>P. thlaspeos</i> on <i>A. demissa</i>	<i>P. thlaspeos</i> on <i>A. gunnisoniana</i>
Aromatics						
Benzaldehyde	7.6%*		6.4	6.8	5.6	1.1
Benzyl alcohol		3.6				
Phenylacetaldehyde	20.6	<1.0	7.2	1.9	7.5	2.1
2-phenylethanol	20.2	1.4	16.2	12.4	20.0	15.2
Benzyl acetate				<1.0		
Phenethyl acetate	<1.0		<1.0	7.9	<1.0	3.4
Phenethyl propionate	<1.0			<1.0		
Phenethyl isobutyrate	<1.0		3.1	1.2	5.6	<1.0
Phenethyl isovalerate†	8.6		25.4	8.7	11.2	3.3
Phenethyl senecioate†	<1.0		5.0	<1.0	5.6	<1.0
Fatty acid derivatives						
2-methyl butanoate		5.4		25.9		
<i>n</i> -hexanal				5.7		20.3
Hexyl acetate					2.2	
2-ethyl hexenol					1.1	
(<i>Z</i>)-3-hexenal	8.3	61.9	9.6	3.8	24.0	12.9
(<i>Z</i>)-3-hexenyl acetate	23.8	20.0	20.9	15.8	14.9	34.5
(<i>Z</i>)-3-hexenyl butanoate†	3.4	3.3	6.2		5.6	5.0
(<i>Z</i>)-3-hexenyl-3-methyl butanoate	7.7	4.5		8.7	15.2	6.9
Fresh mass (g)	3.66	1.10	2.30	2.18	1.26	1.72
ng/g fresh mass/8 h	2.09	4.54	2.29	5.06	4.25	6.69
					0.58	2.31

*Per cent of total volatile emissions.

†Indicates tentative identification due to mass spectrum library search. All other identifications verified by mass spectrum and retention time through coinjection of standards.

**Fig. 1** Summary of qualitative differences in volatile composition, as measured using headspace trapping and GC-MS analysis, between uninfected *Arabis holboellii* tissues (mostly fatty-acid-derived products), tissues infected with *Puccinia monoica* (dominated by aromatic compounds), and coblooming plants (rich in terpenoid volatiles). Chemical structures of representative compounds from each biochemical class are provided. (Complete data are given in Tables 1–4.)

only when *A. crandallii* is used as a host, and varies widely in relative emission rates (Tables 2 and 3, Fig. 2). This observed variation may represent a population-level polymorphism or may simply be a consequence of the unusually high variation in spore stage expression that characterizes the demicyclic *P. consimilis* (Roy *et al.* 1998).

Discussion

Pseudoflower fragrance, insect attraction and floral mimicry

Diverse groups of fungi interact with 'pollinators' and other insects, both to effect spore dispersal (Smith 1956;

Table 4 Volatile compounds detected in coblooming plants

Class Compound	<i>Pseudocymopterus</i> <i>montanus</i>	<i>Anemone patens</i>		<i>Phlox bryoides</i>		<i>Ranunculus</i> <i>inamoenus</i>		<i>Thlaspi</i> <i>montanus</i>		
	1993 floral	1994 floral	1996 floral	veg.	1994 floral	veg.	1996 floral	1996 floral	1994 floral	veg.
Terpenoids										
unknown (C ₉ H ₁₆) (mw 135, b. pk. 69)					8.2	12.2	Scent not detected			
α pinene					22.4	7.2			3.5	
β pinene	5.0%*					4.3				
Sabinene	2.7					10.0				
Myrcene	36.9				5.5					
δ-carene	3.0									
Limonene		2.8	3.8		5.8				15.0	18.7
(Z)-β-ocimene	42.7	3.0	<1.0							
(E)-β-ocimene	6.5	94.2	66.5							
α terpinolene					3.2					
Verbenone					6.6					
Camphor					8.4					
1,8 cineole					22.7	25.8				
Linalool					2.6				7.5	10.1
Linalool oxide (furanoid)					3.3					
Linalool oxid (pyranoid)									43.9	42.4
Nerol									16.4	
Aromatics										
Cymene	3.2								Scent not detected	
Benzaldehyde									5.3	7.0
Benzyl alcohol				21.1						
1-propenyl benzenet				8.2						
Propadienyl benzenet				17.6						
Fatty acid derivatives										
(Z)-3-hexen-1-ol						10.4				6.0
(Z)-3-hexenyl acetate						13.0				<1.0
(Z)-3-hexenyl 3-methyl butyrate				36.3						
4-methyl nonanol†			5.1							
Dodecanet			24.1							
γ valerolactone†				16.8						
Valeric acid									8.2	
Fresh mass (g)	4.50	25.44	10.19	7.62	10.49	9.12	0.60	0.33	6.87	5.37
Emissions, ng/g fresh mass/8 h	7.52	0.40	1.48	0.33	1.47	0.91	Not detected		1.48	1.60

*Percent of total volatile emissions.

†Indicates tentative identification due to mass spectrum library search. All other identifications verified by mass spectrum and retention time through co-injection of standards.

Alexander & Antonovics 1988; Webber & Gibbs 1989; Alexander 1990; Jennersten & Kwak 1991; Lin & Phelan 1992; Young 1993; Roy 1994b; Shykoff & Bucheli 1995) and to mediate the exchange of mating-type gametes (Craigie 1931, Bultman & White 1988; Connick & French 1991; Roy 1993a). We have shown that *Puccinia* pseudoflowers use fragrance to attract bees and flies that pro-

vide an essential 'pollination' service during the sexual cycle of these fungi (Roy & Raguso 1997). Our surveys of scent production by *Puccinia* pseudoflowers, their *Arabis* hosts and coblooming true flowers demonstrate that pseudoflower fragrance is chemically distinct and consists entirely of aromatic alcohols, aldehydes and esters (Fig. 1, Tables 2 and 3).

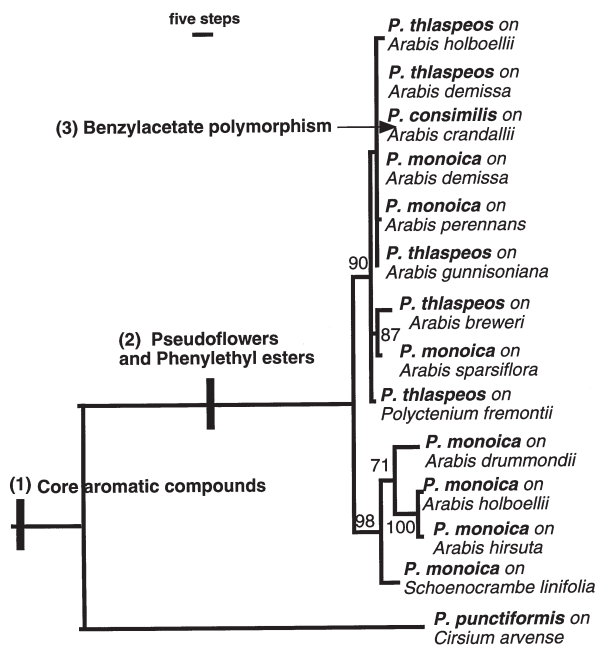


Fig. 2 Phylogram based on rDNA sequences from the ITS I, 5.8S and ITS II regions of the rust fungi studied for fragrance chemistry. The tree shown is the strict consensus of three trees found in a heuristic search with midpoint rooting. All characters were evenly weighted because the ti:tv ratio was essentially 1:1. Length, 234 steps; CI, 0.98; HI, 0.02; RI, 0.92. Bootstrap values for the branches are based on 1000 replicates. The hypothesized evolution of fragrance chemistry has been attached to the phylogram in the appropriate places (bold numbers in parentheses). For the crucifer rusts (all but *Puccinia punctiformis*) the molecular methods and phylogenetic analyses can be found in Roy *et al.* (1998). The sequence for *P. punctiformis* was obtained in the same manner as the crucifer rust sequences and is deposited in GenBank under Accession no. AF043476. *P. punctiformis* remains basal to the crucifer rusts when additional sequences from GenBank are included in the analysis (see Kropp *et al.* 1997).

Pseudoflower scent does not represent a simple amplification of host floral or vegetative emissions, nor does it appear to mimic the floral scent of four coflowering plants. The benzenoid compounds that characterize pseudoflower fragrance are emitted by numerous true flowers with diverse morphologies, pollinator spectra and phylogenetic affinities, including orchids pollinated by euglossine bees (Hills *et al.* 1972; Whitten & Williams 1992), tubular, night-blooming flowers pollinated by hawkmoths (Knudsen & Tollsten 1993), and especially small, nectar-rich flowers visited by noctuid moths (Cantelo & Jacobson 1979; Haynes *et al.* 1991; Landolt *et al.* 1991; Heath *et al.* 1992a). Phenylacetaldehyde and 2-phenyl ethanol are attractive to many species of noctuid moths and appear to be mating pheromone constituents of male hair pencil glands for at least some of these species (Creighton *et al.* 1973; Jacobson *et al.* 1976; Birch

et al. 1989; but see Landolt & Heath 1990; Heath *et al.* 1992b). Noctuid moths did not visit *Puccinia* pseudoflowers at our study sites in the Rocky Mountains, presumably because cool evening temperatures above 2700 m limit the moths' foraging activities after dark (Cruden *et al.* 1976), but they may be important visitors to pseudoflowers in warmer settings where *Puccinia* pseudoflowers are found, such as Moab, Utah, USA and Tucson, Arizona, USA. Perhaps fungal pseudoflowers are functionally most similar to the relatively unspecialized flowers of Edelweiss (*Leontopodium alpinum*; Asteraceae), which attract alpine flies, bees and other insects as pollinators and have hexose-dominated nectar, accessible, actinomorphic flowers and a fragrance that contains 2-phenylethanol, benzaldehyde and benzyl benzoate as well as terpenoids, GLVs and other 'goaty'-smelling aliphatic acids (Erhardt 1993).

The interactions between *Puccinia* fungal pseudoflowers and coblooming plants are complex, depending on relative population densities, floral species and class of insect visitor (Roy 1994a, 1996). Both pseudoflowers and true flowers benefit from each other's presence in attracting greater numbers of insects, but mixed visitation bouts by insects may have a negative impact via the improper placement of spermatia and pollen (Roy 1994a, 1996; see Inouye *et al.* 1994). While nectar rewards and visual cues such as colour, size, shape and habit suggest that fungal pseudoflowers are generalized mimics of true flowers (Roy 1993a, 1994a), we have demonstrated that their fragrances are chemically distinct from at least four species of coblooming flowers and from the flowers of their *Arabis* hosts. Even if subsequent analyses prove that low levels of 2-phenylethanol or similar aromatic compounds are present in *Ranunculus inamoenus*, its probable combination with terpenoid compounds (see Bergström *et al.* 1995) render it olfactorily distinct from the pseudoflower fragrance blends. Clearly, the emission of benzaldehyde, 2-phenylethanol and phenylacetaldehyde by spermatia is an ancestral condition among insect-fertilized *Puccinia* rust fungi, and is independent of the floral community that we describe from the Rocky Mountains of Colorado. However, it is possible that these chemically distinct fragrances may be important in the mimicry system if insects initially choose to forage in a patch due to density-dependent visual cues, but then home in on specific taxa based on their distinct fragrances. Distinctive fragrances may thus reduce gamete loss via olfactory reinforcement of visitor constancy among foraging flower visitors.

Fungal odours and their biochemical origins

The volatiles emitted by *Puccinia* pseudoflowers are intriguing in that all are benzyl alcohols, aldehydes or esters, structurally related metabolites of a single biosynthetic pathway that is abundantly expressed in other

basidiomycetes, including *Polyporus tuberaster* (Kawabe and Morita 1994) and *P. hispidus* (French *et al.* 1976), and the genera *Agaricus*, *Armillaria*, *Hygrophoris* and *Lepiota* (Arora 1986). Although pseudoflower fragrance is chemically distinct from vegetative and floral volatiles produced by host *Arabis* plants (Fig. 1), it remains possible that fungal spermatial tissue produces fragrance by modifying host-plant metabolites, rather than synthesizing their own fragrance compounds *de novo*. This hypothesis follows from Connick & French's (1991) study, which demonstrated that the three benzenoid scent compounds emitted by *P. punctiformis* also were present in the insect-pollinated flowers of the host thistle plant (*Cirsium arvense*). A fourth compound, the nitrogen-bearing aromatic indole, was the major volatile detected in *P. punctiformis* (Connick and French 1991), and is a ubiquitous metabolite of tryptophan and auxin in all plants (reviewed by Romero *et al.* 1995). Indole was either not detected or present at low, inconsistent levels in our survey of the *P. monoica* complex (Tables 2 and 3). Rather than an evolutionary loss, this pattern may reflect a methodological artefact, in that Connick & French (1991) used thermal desorption, a more sensitive method, to elute trapped scent compounds from *P. punctiformis*, while we used hexane solvent desorption, and indole is poorly soluble in hexane (Raguso & Pellmyr 1998).

In contrast to the *Cirsium*-*P. punctiformis* relationship, most of the *Arabis* hosts of the *P. monoica* rust complex produce small, nearly scentless flowers that are either apomictic (*A. holboellii*, *A. gunnisoniana* and *A. lignifera*) or predominantly self-pollinating (*A. drummondii* and *A. crandallii*) (Roy 1995). From a synomonal standpoint, floral scent would be superfluous in these flowers. However, benzaldehyde, phenylacetaldehyde and 2-phenylethanol have been detected as floral fragrance constituents in larger, insect-pollinated cruciferous flowers (Tollsten & Bergström 1988; Evans & Allen-Williams 1992; Jakobsen *et al.* 1994; Nielsen *et al.* 1995), and they are common products of the ubiquitous shikimate biosynthetic pathway, leading from phenylalanine to benzoic acid and phenylpropanoid products (reviewed by Dixon & Paiva 1995; Schmid & Amrhein 1995). The presence of benzaldehyde and benzyl alcohol in bolting *Arabis* foliage (Table 1) suggests that their precursors, and those of the phenolic acetates and esters, are doubtless available within the tissues of *Arabis* host plants. On the other hand, fungi are noteworthy for their biochemical versatility, producing a wide assortment of volatile organic compounds, including phenolics (Arora 1986; Kawabe & Morita 1994), derived mono- and sesquiterpenoids (Hanssen 1985; Bock *et al.* 1986), fatty acid derivatives (Tressl *et al.* 1982; Grosh & Wurzenberger 1984; Armstrong 1986; Lin & Phelan 1992; Noël-Suberville *et al.* 1996) nitrogen- (indolic) and sulphur-bearing organic compounds (Bau 1981; Chen

et al. 1986; Talou *et al.* 1989; Pelusio *et al.* 1995). Thus, *Puccinia* fungal pseudoflowers are, in theory, capable of synthesizing their own fragrance compounds. The qualitative and quantitative differences in scent emissions between 'conspecific' fungal taxa on different *Arabis* hosts (*P. thlaspeos* on *A. demissa* vs. *A. gunnisoniana* [Table 2], *P. consimilis* on *A. crandallii* vs. *A. lignifera* [Table 3]) suggest that differences in host physiology may translate into differences in host metabolite pools available for scent production. Alternatively, the presence of cryptic host race 'species' within the *P. monoica* complex (Roy *et al.* 1998), combined with the potential for sampling error in our surveys, undermines the strength of such comparisons. Radiolabel tracer experiments or tissue-specific enzymatic assays are recommended for future efforts to distinguish between these conflicting hypotheses.

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Robert Raguso and Bitty Roy are interested in the roles of olfactory and visual signals in insect–plant and insect–fungal interactions, and the mechanisms by which such interactions evolve. A major goal of our research is to track the sequence of evolutionary modifications in a floral-mimicry system, to understand how pathogenic fungi have ‘reinvented’ the rewards and advertisements used by flowering plants to attract pollinators and effect outcrossing. This paper describes chemical analyses of fungal ‘floral scent’, which were used previously to guide behavioural bioassays and field experiments, within a phylogenetic framework provided by the analysis of independent molecular markers.
