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The Predatory Mite *Phytoseiulus persimilis* Does Not Perceive Odor Mixtures As Strictly Elemental Objects

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Abstract *Phytoseiulus persimilis* is a predatory mite that in absence of vision relies on the detection of herbivore-induced plant odors to locate its prey, the two-spotted spider-mite *Tetranychus urticae*. This herbivorous prey is feeding on leaves of a wide variety of plant species in different families. The predatory mites respond to numerous structurally different compounds. However, typical spider-mite induced plant compounds do not attract more predatory mites than plant compounds not associated with prey. Because the mites are sensitive to many compounds, components of odor mixtures may affect each other's perception. Although the response to pure compounds has been well documented, little is known how interactions among compounds affect the response to odor mixtures. We assessed the relation between the mites' responses elicited by simple mixtures of two compounds and by the single components of these mixtures. The preference for the mixture was compared to predictions under three conceptual models, each based on one of the following assumptions: (1) the responses elicited by each of the individual components can be added to each other; (2) they can be averaged; or (3) one response overshadows the other. The observed response differed significantly from the response predicted under the additive response, average response, and overshadowing response model in 52, 36, and 32% of the experimental tests, respectively. Moreover, the behav-

ioral responses elicited by individual compounds and their binary mixtures were determined as a function of the odor concentration. The relative contribution of each component to the behavioral response elicited by the mixture varied with the odor concentration, even though the ratio of both compounds in the mixture was kept constant. Our experiments revealed that compounds that elicited no response had an effect on the response elicited by binary mixtures that they were part of. The results are not consistent with the hypothesis that *P. persimilis* perceives odor mixtures as a collection of strictly elemental objects. They suggest that odor mixtures rather are perceived as one synthetic whole.

Key Words Olfaction · Synthetic odor perception · Odor mixtures · Binary mixture · Mite · Learning · Herbivore-induced plant volatiles

Introduction

Upon attack by herbivorous mites, plants attract predatory mites by changing the composition of the odor they emit (Dicke et al., 1998; Sabelis et al., 1999). These odors consist of a complex mixture of herbivore-induced and non-induced plant compounds. Predators use this complex chemical signal to locate herbivore-infested plants in an equally complex odor background produced by other (possibly herbivore-infested) plants (Schröder and Hilker, 2008). It is not known to what extent predatory mites perceive odor mixtures by their individual components (here referred to also as molecular elements or elemental objects) or as a synthetic whole. Consequently, it is not clear if individual herbivore-induced compounds are the key mediators of predatory mite attraction, or if the mites

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are instead attracted to the mixture as a whole. Here, we seek to disentangle these alternatives.

In the present study, we investigated the behavioral response of the predatory mite *Phytoseiulus persimilis* to individual volatile compounds and to binary mixtures thereof. This predatory mite feeds on the highly polyphagous two-spotted spider-mite *Tetranychus urticae* (Bolland et al., 1998). The predatory mite relies entirely on olfactory cues to locate distant spider-mite-infested plants (Sabelis and Van der Baan, 1983; Sabelis et al., 1984; Dicke et al., 1990; Dicke and Dijkman, 1992). Different plants produce qualitatively and quantitatively different odor mixtures in response to spider-mite feeding (van den Boom et al., 2004). Consistent with the chemical diversity of the information associated with its prey this predatory mite responds to a wide variety of spider-mite induced plant odor mixtures (van den Boom et al., 2002) and a wide range of structurally different volatile compounds (Dicke et al., 1990; de Boer and Dicke, 2004a, b; Kappers et al., 2005; van Wijk et al., 2008). Discriminating the odor of a spider-mite-infested plant from an uninfested conspecific is a challenging task because many components of both odor mixtures will be present in both. Spider-mite-infested tomato, for example, emits substantial amounts of 19 compounds, 5 of which change significantly in concentration after spider-mite infestation.

Discrimination of the target odor from similar odor sources essentially can be achieved in two ways. Odor perception could be elemental, i.e., the mites perceive and respond to individual components of the mixture. As the components in a mixture are recognized, a component that is an attractant when presented individually is also in a mixture recognized as an attractant. Hence, if odor perception is elemental, the additive effects of all individually recognized attractants and repellents in the mixture will form a likely estimate of the attraction to the mixture. However, in most olfactory systems, the different chemical components of odor mixtures are not detected as independent elements because different odorants of the same mixture may compete for the same receptor site (Oka et al., 2004; Rospars et al., 2008), or because they may affect each other's representation through cross-glomerular circuit interactions in the olfactory system (Kay and Stopfer, 2006; Riffell et al., 2009). Consequently, odor perception becomes synthetic, the individual components of a blend may no longer be recognizable in the mixture, and the mixture is perceived as a distinct odor different from its individual components. This may facilitate the ability to discriminate between mixtures that share many components such as the odor of herbivore-infested plants and uninfested conspecifics. This is because synthetic perception releases the brain from the constraint of representing an odor in the same way, whether it is part of a mixture or not. Hence,

correlated olfactory input can be decorrelated in such a way, that the ensemble of neurons encoding one odor, shares little or no overlap with the ensemble of neurons encoding the other. Consequently, when olfaction is strictly synthetic, the response elicited by a specific volatile compound may be entirely different from its contribution to the response elicited by a mixture that it is part of.

The central nervous system of *Phytoseiulus persimilis* consists of just 10,000 cells (van Wijk et al., 2006a). Its glomerular olfactory system is similar to the system found in insects and vertebrates (van Wijk et al., 2006b). The relative simplicity of this mite's olfactory system combined with its reliance on olfaction to locate distant prey makes it an ideal model organism to study how predators utilize the complex and variable source of olfactory information that herbivore-infested plants provide.

The olfactory response elicited by the individual components of an odor mixture serve as the input for three conceptual models that are used to predict the response elicited by the mixture. These model predictions were compared to the observed response elicited by the binary mixture in the predatory mite *P. persimilis*.

- (1) One model is additive and therefore tests the assumption that the response elicited by each of the mixture's components contribute equally to the response elicited by the mixture.
- (2) In a second model, the response elicited by an odor mixture is equal to the response elicited by the mixture's component that elicits the strongest response. Hence, this model is consistent with the frequently observed phenomenon of overshadowing, i.e., an olfactory system that receives competitive inputs by the different chemical components in the odor mixture. The odor that elicits the strongest response inhibits the perception of or the response to the other components in the mixture.
- (3) The third model is intermediate between the other two models. Here, we assume that the response to a mixture is equal to the mean response elicited by its components. Hence, it is assumed that all of the mixture's components contribute equally to the response elicited by the mixture, while the perception of or the response to each component is reduced by the fraction of the total number of components in the mixture.

Because odors occur at different concentrations, we do not only investigate how the different components of odor mixtures affect the response that these mixtures elicit, but also, whether the same mechanism applies at different concentrations of the same odor mixture. This was examined in a separate series of experiments where we asked if a function that describes the response to a binary mixture as a function of the response to its components at

one concentration also describes the response to the same mixture at other concentrations.

Methods and Materials

Plants and Mites Lima bean plants (*Phaseolus lunatus*) were grown from seeds in a climate room (22°C, 60% RH, 16:8 LD). After 2 week, these plants were infested with two spotted spider-mites (*Tetranychus urticae* Koch) to establish a culture of this mite. Predatory mites (*Phytoseiulus persimilis* Athias-Henriot) were reared in a climate room (25°C, 60% RH, 16:8 hL:D) on detached, spider-mite-infested Lima bean leaves. The predatory mites received fresh, spider-mite-infested Lima bean leaves every day, and the culture was harvested every day except for the weekend. This frequent harvesting of adult female mites ensured that most mites used in the experiments were one to a few days old after their molt to the adult phase. Predatory mites were obtained originally in 2001 from various locations near the coast of Sicily, Italy. Before choice tests, female predatory mites were kept in Eppendorf tubes and were deprived of water and food for 16–22 hr.

Odor Odors were selected from a panel of pure volatile compounds used to assess the olfactory response of *P. persimilis* (van Wijk et al., 2008). The odors selected for this study ensure that all possible combinations of repellents, attractants, and odors that elicited no response could be made. Most odors were obtained from Fluka with the exception of 2,3-dimethyl pyrazine that was obtained from Aldrich. Octan-1-ol and butan-1-ol were obtained from Sigma, dodecyl acetate from Aldrich, methyl salicylate (MeSA) from Sigma-Aldrich, and β -ocimene (70% *E*- and 30% *Z*- isomers) from R. C. Treat & co. The terpenoids (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) were provided by Dr. W. Boland of the Max Planck Institute for Chemical Ecology, Jena, Germany.

Olfactory Response Tests Choice tests were conducted as described by van Wijk et al. (2008). In short, the response to the odors was assessed by using an experimental arena constructed from a Petri dish (diam 9 cm) positioned upside down. A radial airflow was established by the connection of a vacuum pump (flow 0.42 l/min) to an opening at the center of the bottom (lid) of the Petri dish. Prior to the experiment, groups of about 25 predatory mites were placed in cartridges that could be fitted between the vacuum pump and the experimental arena. For each replicate, the arena was provided with fresh odor sources and a new cartridge with a new group of predatory mites. Each odor was tested in a different set-up. To restrain predatory mites

after their choice on each side of the arena, an insect glue barrier divided the arena into two halves except for an opening of 3 cm at the center of the bottom of the arena. This opening allowed mites to move from the cartridge to the side of their choice, and it allowed mites to move from one side to the other. One side contained filter paper (diam. 1 cm) with 0.5 μ l of the odor (dissolved in hexane), and the other side contained a control filter paper with 0.5 μ l of the solvent (hexane). Odor sources were prepared in a fume hut, and the solvent was allowed to evaporate for 1 min before the odor source was placed in the set-up. Mites were released from the cartridge, and after 3 min the mites at both halves of the choice dish were counted.

Control Experiments To assess whether there is a difference between the measured olfactory preference of *P. persimilis* released in groups and *P. persimilis* released individually, a control experiment was conducted. In this experiment, the olfactory preferences of mites released individually (40 mites) and mites released in groups (six groups each with ca. 20 individuals) were compared. Four different combinations were tested: 1) no odor at both sides of the Petri-dish; 2) a Lima bean leaf discs (1,5 cm) vs. no odor at the control side; 3) a leaf disc at both sides; 4) a spider-mite infested leaf disc (web, eggs and mites removed) vs. an uninfested leaf disc.

Experimental Design and Statistics The preference of nine groups, each consisting of ca. 25 mites was tested for each of the two odors and their binary mixture. The experimental design followed a 3 \times 3 Latin square; the odors (A, B, and the mixture AB) formed one side of the square while the other consisted of the experimental sequence. Hence, each odor was perceived by three groups first, by three groups second, and so on, thereby ensuring that three clusters of three groups of ca. 25 mites received the three odors in three different sequences. All experiments in one Latin square were completed in ca. 75 min. The mites were held ca. 20 min. in Eppendorf tubes between subsequent choice tests.

Assuming individual mites make independent choices, the response to each of the three odors compared to the control was analyzed using the replicated *G*-test for goodness of fit (Sokal and Rohlf, 1995). Differences among choice experiments were determined by analysis of variance (*ANOVA*) of the arcsine square root transformed frequencies of the olfactory preference, followed by Bonferroni *post-hoc* testing. This not only allowed us to test if there was a difference between the responses to the odors, but also if there was an effect of the position in the sequence and whether there was a difference among the experimental sequences as a whole.

The experimental results were compared to three model predictions. These models predict the response that a binary mixture elicits as a function of the response to each of the mixture's components. The first model had the following additive structure:

$$E_{(AB)} = (0.5 + (O_{(A)} - 0.5) + (O_{(B)} - 0.5)) * 100\%$$

Here, $E_{(AB)}$ is the expected attraction to the mixture AB in%, and $O_{(A)}$ and $O_{(B)}$ are the observed fractions of mites attracted to odor A and B, respectively. If $E_{(AB)}$ became greater than 100%, it was assumed to be 100%. Under the second model, it was assumed that the response that a mixture elicits was equal to the response elicited by the mixture's component that elicited the strongest response. Hence, there is always a complete overshadowing. Under the third model, the expected response elicited by the binary mixture was predicted to be equal to the mean of the response elicited by its components [$E_{(AB)} = ((O_{(A)} + O_{(B)}) / 2 * 100\%)$]. The observed attraction to the binary mixture was compared to the expected attraction under each of the three models using a χ^2 for goodness of fit test.

Binary Mixtures with the Same Component Ratio at Different Concentrations Dose response curves (pure odor, 10, 100, and 1000 times diluted odor in hexane) were generated in a way similar to the experiments described by van Wijk et al. (2008). The response to each concentration of each odor was tested in 6 replicate experiments, each based on ca. 20 predatory mites. Dose response curves for the binary mixtures described here were measured simultaneously with the dose response curves for their components published earlier (Van Wijk et al., 2008).

Results

Control Experiments To examine if *P. persimilis* mites released in groups show the same preference for odors in the experimental set-up as *P. persimilis* released individually, 4 control experiments were conducted. The olfactory responses by *P. persimilis* assayed in groups did not differ from those tested individually (Table 1).

To examine whether the repetitive testing affected the responsiveness of the mites, a meta-analysis was performed on all experiments listed in Table 2. We regressed the responsiveness defined as absolute fraction of mites attracted to each odor minus 0.5, on the position in the experimental sequence. We found that the responsiveness did not vary with the position in the experimental sequence (equation of the regression line: $y = -0.0017x + 0.1244$,

$r^2 = 0.0002$ with y being the responsiveness and x being the position in the experimental sequence). Additionally, we checked for each experiment whether there were differences among the different experimental sequences and among the positions of odors in the experimental sequence using ANOVA on the arcsine square root transformed frequencies of the olfactory preference followed by Bonferroni post-hoc analysis (Supplemental data Table 1). We did not detect any effect of the sequential testing on the olfactory preference except for experiment 1 (α -humulene and linalool). In this experiment, a significant effect of the position in the experimental sequence was found. This was due to the significant heterogeneity in the response to α -humulene (Table 2). In 9 choice tests (three times as the first, the second, and the third odor in the sequence), significant repellence was measured only once, when the odor was presented as the first odor in the sequence. Significant attraction was measured twice, when the odor was presented as the third odor in the sequence. The other six replicates revealed neither attraction nor repellence.

The Contribution of Each Component to the Mixture's Attractiveness We investigated the response of *P. persimilis* to 25 binary mixtures and to their individual components. Table 2 reports the fraction of mites (9 replicate experiments of ca. 25 mites per mixture) that were attracted to each binary mixture and its components. Table 2 also reports the predicted response under each of the proposed models, i.e., the additive response, the averaged response, and the overshadowing response model.

Based on the response to the mixtures' components, the experimental results can be divided into three groups: (1) experiments in which both components did not elicit a significant choice (G_p^{ns}) (experiment 1 to 7, Table 2); (2) experiments in which one of the components elicited a response (G_p^*) whereas the other did not (experiment 8 to 17, Table 2); and (3) experiments in which both components elicited a response (G_p^*) (experiment 18 to 25, Table 2). Figure 1 depicts the difference between the observed attraction to each binary mixture and the predicted attraction under each of the models.

In experiment 1 to 7 both components of the mixture elicited no response. Hence, in these experiments, the averaged response model and the overshadowing response model predict no significant attraction to or repellence of the mixture. Likewise, the additive response model predicts no response except when the response that each odor elicits borders significance and has the same direction. This latter case explains the significant difference between the observed absence of a response (G_p^{ns}) for the mixture and the expected response under the additive response model in experiments 2 (octan-1-ol and TMTT) and 5 (farnesol and

Table 1 Olfactory preference measured with mites released in groups and mites released individually

Group/ individual	No odor (%)	No Odor (%)	Replicated <i>G</i> -test for goodness of fit							<i>G</i> -test		Difference between mites released in groups and mites released individually (χ^2 test) <i>P</i> (χ^2) <i>d.f.</i> =1
			<i>N</i>	<i>G_p</i>	<i>P</i> (<i>G_p</i>) <i>d.f.</i> =1	<i>G_h</i>	<i>P</i> (<i>G_h</i>) <i>d.f.</i> =5	<i>G_t</i>	<i>P</i> (<i>G_t</i>) <i>d.f.</i> =6	<i>G</i>	<i>P</i> (<i>G</i>) <i>d.f.</i> =1	
6 groups	47.64	52.45	118	0.08	0.78	2.81	0.73	2.89	0.82			
Individuals	42.50	57.50	40							0.90	0.34	0.28
	Leaf disc (%)	Leaf disc (%)										
6 groups	48.74	51.26	119	0.08	0.78	2.81	0.73	2.89	0.82			
Individuals	52.50	47.50	40							0.10	0.75	0.41
	Leaf disc (%)	No odor (%)										
6 groups	52.54	47.46	118	0.31	0.58	5.21	0.39	5.52	0.48			
Individuals	45.00	55.00	40							0.40	0.53	0.10
	Infested leaf disc (%)	Leaf disc (%)										
6 groups	79.00	21.00	119	42.62	0.00	6.82	0.23	49.44	0.00			
Individuals	80.00	20.00	40							15.42	0.00	0.78

dodecyl acetate) (Fig. 1). The significant response to the mixtures (G_p^*) in experiment 3 (dodecyl acetate, and 2,3-dimethyl pyrazine) and 6 (hexyl acetate and *cis*-3-hexen-1-ol) was significantly different from the predicted response under all three models. Given the variances among replicates, this does not necessarily imply that the difference among the components and the mixture differ significantly. As the experimental design followed a 3×3 Latin square, differences among odors were determined by analysis of variance (*ANOVA*). In this *ANOVA*, the arcsine square root transformed frequencies of the 27 individual choice experiments were analyzed for significant differences among the response to odors, position in the experimental sequence, and the three groups of different experimental sequences in the experiment. This analysis revealed that there were significant differences between the odors in experiment 6 ($F_2=4.33$, $P<0.05$) and in experiment 3 ($F_2=18.34$, $P<0.05$). *Post hoc* analysis revealed that the response to the mixture was significantly different from the response to its individual components. Hence, we conclude that in these experiments a mixture of two compounds that individually elicited no response, elicited a response when blended together in a binary mixture.

In experiment 8 to 16, one component of the binary mixture elicited no response whereas the other did. The overshadowing response model predicts that the response to the mixture is equal to the response elicited by the mixture's component that elicited a response. The additive response model predicts a response similar to that. Only the response to the mixture in experiment 8 (α -pinene and *cis*-3-hexen-1-ol) was significantly different from that predicted by the overshadowing response model. Experiments

8, 10 (dodecyl acetate and octan-1-ol) and 16 (linalool and MeSA) revealed a significant difference between the observed and the predicted response under the additive response model. In experiment 16, this appears to result from the fact that the repellence by the mixture was greater than the additive repellence by its components. *ANOVA* and the subsequent *post hoc* analysis revealed that this difference tended to be significant at $P=0.07$, $d.f.=6$. The reverse is seen in experiments 8 and 10. Here, the mixture elicited no response (G_p^{ns}) whereas one of its components did (G_p^*). The *ANOVA* revealed that in both experiments the response to the mixture was not significantly different from the response to its components. Hence, although the responses to the mixture and its components were not significantly different, the replicated *G*-test indicates that the mites made no choice when provided with the mixture, whereas they did when offered one of its components. Moreover, the observed response in experiment 8 was significantly different from both the overshadowed response and the additive response model. In conclusion, in experiments in which only one of the mixtures' components elicited a response, the two models that predict overshadowing for the response to the mixture are most consistent with the observed response. There are, however, several cases where the component that elicited no response contributed to the response elicited by the binary mixture (experiment 8, 10, and 16).

In the last group of experiments, components in each mixture elicited a response. As in the previous groups of experiments, none of the models accurately predicts the response to all binary mixtures in this group. On one side of the spectrum, there are cases of overshadowing by the

Table 2 Observed and expected response to binary mixtures and their components

Experiment	Odor	Dilution factor	odor	Fraction mites attracted	Replicated <i>G</i> -test for goodness of fit				Difference between observed response and model prediction <i>P</i> (χ^2), <i>d.f.</i> =1	
					<i>Gp</i> <i>d.f.</i> =1	<i>Gh</i>	<i>P</i> (<i>Gh</i>) <i>d.f.</i> =8	<i>Gt</i>		<i>P</i> (<i>Gt</i>) <i>d.f.</i> =9
Where both components elicit no response										
1	α -Humulene	100	0.489	0.160	0.690	23.555	0.003	23.715	0.005	
	Linalool	100	0.541	1.731	0.188	9.508	0.301	11.240	0.260	
	α -Humulene+Linalool	100	0.502	0.004	0.951	10.118	0.257	10.121	0.341	0.697
	Averaged response model		0.51							0.237
	Overshadowing response model		0.54							0.402
	Additive response model		0.53							
	Octan-1-ol	100	0.455	2.425	0.119	12.770	0.120	15.196	0.086	
	TMTT	100	0.465	1.629	0.202	16.098	0.041	17.727	0.038	
	Octan-1-ol + TMTT	100	0.520	0.474	0.491	4.579	0.801	5.053	0.830	0.072
	Averaged response model		0.46							0.052
2	Overshadowing response model		0.46							0.002
	Additive response model		0.42							
	Dodecyl acetate	10	0.518	0.387	0.534	10.578	0.227	10.965	0.278	
	2,3-Dimethyl pyrazine	10	0.492	0.081	0.776	5.852	0.664	5.933	0.747	
	Dodecyl acetate + 2,3-Dimethyl pyrazine	10	0.295	50.806	0.000	15.442	0.051	66.248	0.000	0.000
	Averaged response model		0.50							0.000
	Overshadowing response model		0.52							0.000
	Additive response model		0.51							0.000
	DMNT	100	0.537	1.626	0.202	14.432	0.071	16.058	0.066	
	TMTT	100	0.540	2.025	0.155	8.494	0.387	10.519	0.310	
3	DMNT + TMTT	100	0.548	2.961	0.085	8.707	0.368	11.668	0.233	0.786
	Averaged response model		0.54							0.828
	Overshadowing response model		0.54							0.368
	Additive response model		0.58							
	Dodecyl acetate	100	0.549	3.166	0.075	33.353	0.000	36.519	0.000	
	Farnesol	100	0.526	0.826	0.363	12.615	0.126	13.441	0.144	
	Farnesol+Dodecyl acetate	100	0.495	0.029	0.864	8.831	0.357	8.860	0.450	0.201
	Averaged response model		0.54							0.102
	Overshadowing response model		0.54							0.015
	Additive response model		0.58							
4	Cis-3-hexen-1-ol	100	0.487	0.226	0.635	8.331	0.402	8.556	0.479	
	Hexyl acetate	100	0.520	0.610	0.435	10.105	0.258	10.715	0.296	

7	Cis-3-hexen-1-ol+Hexyl acetate	100	0.395	17.079	0.000	11.244	0.188	28.323	0.001	0.001
	Averaged response model		0.50							0.000
	Overshadowing response model		0.52							0.001
	Additive response model		0.51							
	Trans-2-hexen-1-ol	100	0.502	0.003	0.956	20.905	0.007	20.908	0.013	0.094
	α -Terpinene	100	0.494	0.044	0.833	3.097	0.928	3.141	0.958	0.117
	Trans-2-hexen-1-ol+ α -Terpinene	100	0.442	4.746	0.029	33.538	0.000	38.284	0.000	0.107
	Averaged response model		0.50							
	Overshadowing response model		0.49							
	Additive response model		0.50							
Where one of the components elicits a response										
8	α -Pinene	10	0.458	1.864	0.172	4.942	0.764	6.805	0.657	0.150
	Cis-3-hexen-1-ol	100	0.404	9.676	0.002	2.835	0.944	12.511	0.186	0.023
	Cis-3-hexen-1-ol+ α -Pinene	100+10	0.478	0.478	0.489	9.940	0.269	10.419	0.318	0.000
	Averaged response model		0.43							
	Overshadowing response model		0.40							
	Additive response model		0.36							
	TMTT	10	0.468	1.091	0.296	7.262	0.509	8.353	0.499	0.099
	Linalool	10	0.589	8.047	0.005	7.049	0.531	15.096	0.088	0.864
	TMTT+Linalool	10	0.583	7.368	0.007	4.542	0.805	11.909	0.218	0.424
	Averaged response model		0.53							
10	Overshadowing response model		0.59							
	Additive response model		0.56							
	Dodecyl acetate	10	0.541	1.871	0.171	10.398	0.238	12.269	0.199	0.570
	Octan-1-ol	10	0.579	7.662	0.006	22.416	0.004	30.078	0.000	0.246
	Octan-1-ol + Dodecyl acetate	10	0.541	2.065	0.151	9.150	0.330	11.215	0.261	0.015
	Averaged response model		0.56							
	Overshadowing response model		0.58							
	Additive response model		0.62							
	Octan-1-ol	10	0.569	5.640	0.018	8.234	0.411	13.874	0.127	0.383
	α -Pinene	10	0.519	0.468	0.494	17.314	0.027	17.782	0.038	0.893
11	Octan-1-ol+ α -Pinene	10	0.573	5.860	0.015	17.094	0.029	22.954	0.006	0.646
	Averaged response model		0.54							
	Overshadowing response model		0.57							
	Additive response model		0.59							
	Cis-3-hexen-1-ol	100	0.521	0.514	0.473	9.319	0.316	9.833	0.364	0.000
	MeSA	100	0.369	20.475	0.000	11.625	0.169	32.100	0.000	0.000
	Cis-3-hexen-ol+MeSA	100	0.365	20.563	0.000	10.156	0.254	30.719	0.000	0.015
	Averaged response model		0.45							

Table 2 (continued)

Experiment	Odor	Dilution factor	Dilution odor	Fraction mites attracted	Replicated <i>G</i> -test for goodness of fit				Difference between observed response and model prediction		
					<i>Gp</i>	<i>P</i> (<i>Gp</i>) <i>d.f.</i> =1	<i>Gh</i>	<i>P</i> (<i>Gh</i>) <i>d.f.</i> =8	<i>Gt</i>	<i>P</i> (<i>Gt</i>) <i>d.f.</i> =9	<i>P</i> (χ^2), <i>d.f.</i> =1
13	Overshadowing response model			0.37							0.902
	Additive response model			0.39							0.435
	s-Limonene	100		0.483	0.379	0.538	23.261	0.003	23.640	0.005	
	MeSA	100		0.405	11.261	0.001	22.664	0.004	33.925	0.000	
	s-Limonene+MeSA	100		0.404	12.013	0.001	18.355	0.019	30.368	0.000	
	Averaged response model			0.44							0.225
	Overshadowing response model			0.41							0.965
	Additive response model			0.39							0.626
	Linalool	10		0.496	0.025	0.876	13.094	0.109	13.119	0.157	
	MeSA	10		0.251	91.282	0.000	6.587	0.582	97.869	0.000	
	Linalool+MeSA	10		0.216	129.532	0.000	21.959	0.005	151.491	0.000	
	Averaged response model			0.37							0.000
	14	Overshadowing response model			0.25						
Additive response model				0.25							0.292
DMNT		100		0.506	0.033	0.855	18.408	0.018	18.441	0.030	
MeSA		100		0.406	9.985	0.002	21.611	0.006	31.596	0.000	
DMNT+MeSA		100		0.395	11.587	0.001	5.894	0.659	17.482	0.042	
Averaged response model				0.46							0.068
Overshadowing response model				0.41							0.739
15	Additive response model			0.41							0.616
	Linalool	100		0.493	2.350	0.125	4.985	0.759	7.335	0.602	
	MeSA	100		0.297	48.432	0.000	8.749	0.364	57.181	0.000	
	Linalool + MeSA	100		0.171	72.499	0.000	18.999	0.015	91.498	0.000	
	Averaged response model			0.42							0.000
	Overshadowing response model			0.30							0.000
	Additive response model			0.34							0.002
	Where both components elicit a response										
	Octan-1-ol	10		0.580	7.572	0.006	18.565	0.017	26.137	0.002	
	3-Octanone	10		0.402	11.439	0.001	8.871	0.353	20.310	0.016	
16	Octan-1-ol+3-Octanone	10		0.404	10.204	0.001	15.082	0.058	25.286	0.003	
	Averaged response model			0.49							0.009
	Overshadowing response model			0.40							0.944
	Additive response model			0.48							0.019

18	MeSA	10	0.317	36.344	0.000	17.687	0.024	54.032	0.000	0.004
	3-octanone	10	0.307	42.757	0.000	6.463	0.595	49.220	0.000	0.006
	MeSA + 3-Octanone	10	0.222	85.316	0.000	12.805	0.119	98.121	0.000	0.000
	Averaged response model		0.31							
19	Overshadowing response model		0.31							
	Additive response model		0.12							
	Dodecyl acetate	10	0.603	12.819	0.000	11.106	0.196	23.925	0.004	
	MeSA	10	0.268	62.738	0.000	16.640	0.034	79.378	0.000	
	Dodecyl acetate+MeSA	10	0.388	15.538	0.000	6.687	0.571	22.225	0.008	
	Averaged response model		0.44							0.156
	Overshadowing response model		0.27							0.000
20	Additive response model		0.37							0.579
	MeSA	10	0.264	67.894	0.000	14.436	0.071	82.330	0.000	
	β -Ocimene	10	0.385	15.369	0.000	8.478	0.388	23.847	0.005	
	MeSA + β -Ocimene	10	0.288	53.349	0.000	17.414	0.026	70.764	0.000	
	Averaged response model		0.32							0.249
	Overshadowing response model		0.26							0.404
	Additive response model		0.15							0.000
21	β -Ocimene	10	0.567	5.884	0.015	6.869	0.551	12.753	0.174	
	Octan-1-ol	10	0.574	7.380	0.007	11.133	0.194	18.512	0.030	
	β -Ocimene +Octan-1-ol	10	0.605	15.874	0.000	10.027	0.263	25.901	0.002	
	Averaged response model		0.57							0.290
	Overshadowing response model		0.57							0.339
	Additive response model		0.64							0.272
	Benzyl benzoate	100	0.560	5.495	0.019	8.369	0.398	13.864	0.127	
22	Farnesol	100	0.395	17.370	0.000	18.078	0.021	35.448	0.000	
	Benzyl benoate+Farnesol	100	0.471	1.320	0.251	12.704	0.122	14.023	0.121	
	Averaged response model		0.48							0.859
	Overshadowing response model		0.39							0.019
	Additive response model		0.45							0.611
	Farnesol	10	0.447	4.286	0.038	14.368	0.073	18.655	0.028	
	2,3-Dimethyl pyrazine	10	0.424	8.412	0.004	21.891	0.005	30.303	0.000	
23	Farnesol+2,3-Dimethyl pyrazine	10	0.179	168.757	0.000	11.333	0.184	180.090	0.000	
	Averaged response model		0.44							0.000
	Overshadowing response model		0.42							0.000
	Additive response model		0.37							0.000
	Farnesol	100	0.442	4.800	0.028	18.151	0.020	22.951	0.006	
	3-Octanone	100	0.447	4.152	0.042	10.424	0.237	14.576	0.103	
	Farnesol + 3-Octanone	100	0.454	3.163	0.075	13.067	0.110	16.230	0.062	
24										

Table 2 (continued)

Experiment	Odor	Dilution factor odor	Fraction mites attracted	Replicated G-test for goodness of fit				Difference between observed response and model prediction	
				Gp	P (Gp) d.f.=1	Gh	P (Gh) d.f.=8	Gt	P (Gt) d.f.=9
	Averaged response model		0.44						0.778
	Overshadowing response model		0.44						0.718
	Additive response model		0.39						0.045
25	Dodecyl acetate	100	0.568	6.386	0.012	18.570	0.017	24.956	0.003
	Linalool	100	0.573	7.437	0.006	16.838	0.032	24.275	0.004
	Dodecyl acetate+Linalool	100	0.547	3.234	0.072	19.978	0.010	23.212	0.006
	Averaged response model		0.57						0.492
	Overshadowing response model		0.57						0.445
	Additive response model		0.64						0.004

component that elicited the strongest response, which was most obvious in experiment 17 (octan-1-ol and 3-octanone) and 20 (MeSA and β -ocimene) but this also applies to experiment 21 (β -ocimene and octan-1-ol), 24 (farnesol + 3-octanone), and 25 (dodecyl acetate and linalool). The additive response model accurately predicts the results obtained in experiment 19 (dodecyl acetate and MeSA), 20, 21, 24, and 25 (dodecyl acetate and linalool). Experiment 18 (MeSA + 3-octanone) revealed a weak additivity, which was significantly different from all three proposed models. There also are cases that are consistent with the additive response model experiment 19, 21, and 22 (benzyl benzoate and farnesol). On the other side of the spectrum, there was clear synergism in experiment 23 (farnesol and 2,3-dimethyl pyrazine), which was significantly different from the predictions under all three proposed models.

Binary Mixtures with the Same Component Ratio at Different Concentrations Here, we examined the hypothesis that a function that describes the attraction to an odor mixture as a function of the attraction to its components at one concentration also applies to other concentrations of the mixture. For example, if a mixture of two compounds is as attractive as the sum of the attractiveness of its components, we asked if this relation holds over a range of concentrations where the two components occur in the same ratio.

Dose response curves for the response to binary mixtures and the response to their components were constructed (Fig. 2). Apart from propionic acid, none of the odors elicited a significant response at the lowest concentration (1000 \times diluted). Since the individual components hardly elicited a response by the *P. persimilis* at this low concentration (van Wijk et al., 2008), and since the binary mixtures also hardly elicited a response (except for octan-1-ol + decan-1-ol), we only considered the three higher concentrations to test the null hypothesis. The null hypothesis was rejected in five (Fig. 2e to i) out of nine examined dose response curves of binary mixtures and their components.

In two experiments in which the null hypothesis was not rejected, the response elicited by one component overshadowed the response elicited by the other at all concentrations (Fig. 2a and b). Hence, the possibility that α -pinene was not perceived cannot be excluded (Fig. 2b). A mixture of two equally attractive components was not more attractive than either of its components (at all concentrations tested). Figure 2d shows a mixture of two compounds that were neither attractive nor repellent at any concentration. At the two highest concentrations, i.e. pure and 10 \times dilution, the response to the mixture was not significantly different from the response to its components. At the 100 \times

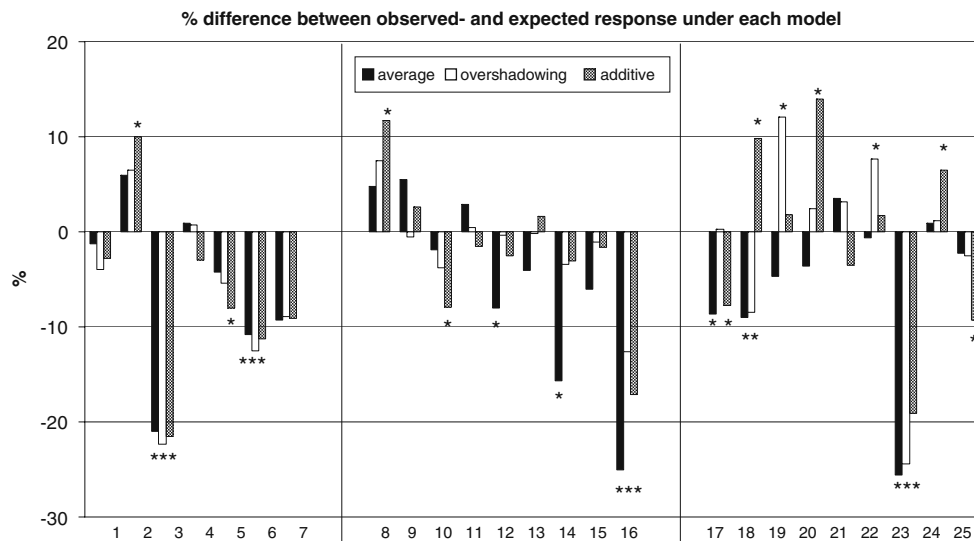


Fig. 1 Behavioral responses by *Phytoseiulus persimilis* to various odors. Below each group of three bars is the reference number of the experiment (also present in Table 2). Depicted is, for each experiment, the difference between the observed attraction ($N=225$ mites divided over 9 replicate choice tests) and predicted attraction under the averaging, the overshadowing and the additive response model

respectively. A * indicates a significant difference ($\chi^2_{df=1} P < 0.05$) between the observed response and the response under the model. The results are divided in three blocks representing experiments with both components of the mixture not eliciting a response, one component eliciting a response and both components eliciting a response, respectively

dilution, this difference was significant ($F_2=4.72$, $P=0.026$), however, the mixture was significantly attractive (G_p^*) only at this dilution level while it tended to become significant at the $10\times$ dilution ($P(G_p)=0.055$). Hence, this is an example where a mixture of two components that elicited no response when presented individually, triggered significant attraction when offered in combination. Although statistical significance for the difference between the mixture and its components was reached only at the $100\times$ dilution, this result does not violate the null hypothesis tested here, as the qualitative contribution of each of the mixture's components to the response of the mites was the same at all concentrations.

The graphs depicted in Fig. 2e to i, all contain at least two points in the dose–response curve that require a different function to describe the attraction to the mixture as a function of the attraction to its components at different odor concentrations. Hence, we conclude that in five out of nine examined dose–response curves of binary mixtures of a constant component ratio, the relative contribution to the behavior elicited by each of the mixture's components varies with the odor concentration. Consistent with the results reported in Table 2 both components of the mixtures used in the dose–response experiments (Fig. 2) also do not appear to contribute equally to the response elicited by the mixtures. Sometimes the response to the mixture is very different from the response to both components. Figure 2e ($100\times$ dilution, 2,3-dimethyl pyrazine [$(G_p^{ns}$, $G_h^{ns})$, α -terpinene (G_p^* , G_h^*)] shows an example of a mixture

consisting of a neutral and an attractive compound that together are repellent (G_p^*). Figure 2f ($10\times$ dilution: propionic acid (G_p^*) and octan-1-ol, (G_p^*)) shows an example of two attractive compounds that together are repellent (G^*).

Discussion

Models based on neurophysiological principles have provided interesting insights in olfaction (Cleland and Linster 2005) and ideally are used to predict the response to odor mixtures. However, the neurophysiology of olfaction in organisms like *P. persimilis* is too poorly understood to construct models of olfactory information processing that incorporate morphological traits and physiological processes. This is why we used phenomenological models to test underlying general principles of information processing as they become apparent from behavioral responses.

The additive response model was based on the assumption that odors presented as individual compounds or as part of mixtures always elicit the same response. This requires that these odors are perceived strictly as elemental objects, and that each recognized object always triggers the same response. The responses to each compound are assumed to sum up to a response towards a mixture of these compounds. In the majority of mixtures studied (13 out of 25), the model prediction was significantly different from the observed results. This is consistent with results of

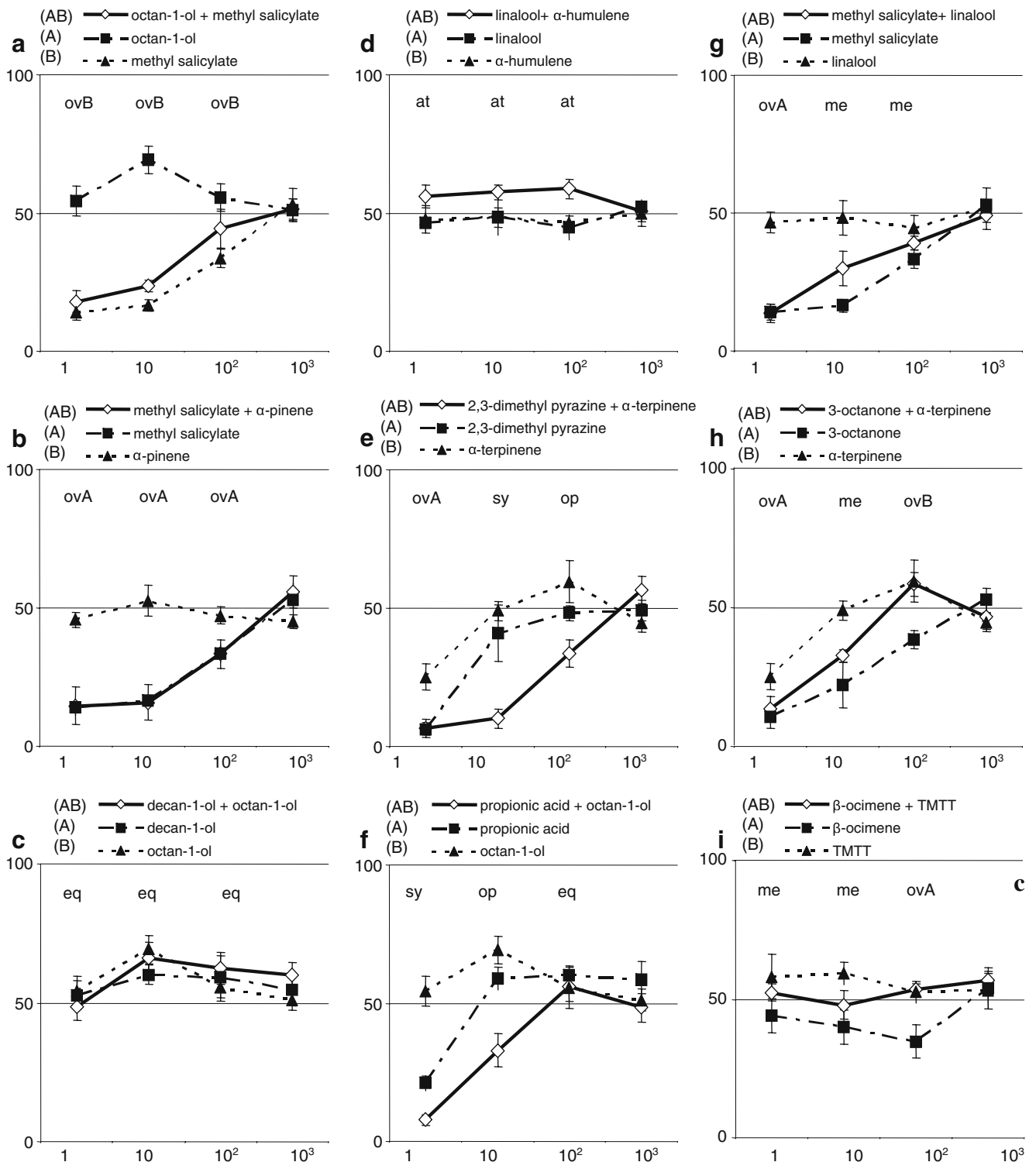


Fig. 2 The dose response relation of binary mixtures and their components. The experiments were conducted to assess if a function which describes the response of *Phytoseiulus persimilis* to a binary odor mixture as a function of the response to its components at one concentration also describes the response to the mixture at a different concentration. The y-axis represents the attraction of *P. persimilis* to the odor ($N=120$ mites divided over 6 replicates = 100%), the x-axis represents the four odor concentrations in decreasing sequence (pure, $10\times$, $100\times$ and $1000\times$ –diluted in hexane). The lowest concentration

was omitted from the analysis as the mites hardly responded to the mixture. Above each odor concentration is an abbreviation which qualitatively describes the response to the odor mixture as a function of the response to its components. Abbreviations: ovA/ovB: overshadowing by compound A or B, eq: equal to the mixture's components, me: mean, sy: synergism, op: opposite of its components, at: a mixture of neutral compounds which is attractive, TMTT: (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene

studies in spiny lobsters (Lynn et al., 1994; Derby et al., 1996), beetles (Thiery and Visser, 1986), and humans (Schiet and Cain, 1990; Laing et al., 1994) where the response to odor mixtures also was found to be different from the additive response to their components. We conclude that in the majority of the assessed mixtures, *P. persimilis* does not respond to components in odor mixtures as if each of these components is experienced in isolation.

The overshadowing response model is based on the assumption that a component that elicits the strongest response suppresses the detection of or the response to all other components in the odor mixture. This model was best applicable to the specific situation where only one of the mixture's components elicited a response. If both components elicited a response, it fared much more poorly, however. We conclude that the response to an odor mixture usually does not result from absolute overshadowing by the mixture's component that elicits the strongest response.

In its assumption that all components equally contribute to the response elicited by the mixture, the averaged response model retains a key feature of the elemental perception of odors. On the other hand, the contribution of each of the mixture's components to the elicited response is reduced by a factor equal to the number of other components in the mixture. This latter assumption states clearly that each component of an odor mixture affects the perception of or the response to all other components that are part of the mixture. The predicted response under the overshadowing and the averaged response model were significantly different from the observed response in respectively 8 and 9 out of 25 mixtures examined. We conclude that these two models, which assume that components of odor mixtures affect each other's perception, explain the observed response better than the additive model, which assumes an elemental perception of odor mixtures.

Our results suggest that there is no simple rule that will predict accurately the response of *P. persimilis* to binary mixtures as a function of the response to their components. For the simple binary mixtures tested here, the model that predicts the responses to mixtures best still fails to explain 32% of the observed responses. Although the three selected models together span a large part of the possible response spectrum we found that the observed response was significantly different from all three models in 20% of the mixtures assayed.

If we consider the results of both the experiments that tested model predictions and the dose response experiments, we find almost all possible interactions between the components in odor mixtures. There are examples of overshadowing, averaging, synergies, and even a case of attractive components that became repellent when offered in a mixture. There were components of mixtures that in isolation elicited no response, whereas they clearly affected

the response elicited by mixtures they were part of. Furthermore, mixtures of the same two components at a constant concentration ratio gave—in the majority of the dose response curves assessed—rise to different interactions at different concentrations (Fig. 2). We conclude that the effect that different components in mixtures exert on the mite's response varies both with component identity and odor concentration. Consequently, knowledge about the repellence or the attractiveness of a specific odor has a limited predictive or explanatory value if this information is used in the context of the response that odor mixtures elicit in *P. persimilis*.

The response to some mixtures was so different from the response elicited by their components that they were probably perceived as something quite different from their components. Some species appear to perceive binary odor mixtures as elemental objects whereas, depending on the combination of odors in the mixture, binary mixtures appear to possess more synthetic properties in other species. Humans, for example, perceive the components of binary mixtures, while they fail to do so in mixtures with a complexity of three to four components (Laing and Francis, 1989; Livermore and Laing, 1996; Marshall et al., 2006). Binary mixtures appear to possess a varying degree of synthetic properties in other species such as rabbits (Coureaud et al., 2009), rats (Staubli et al., 1987; Linster and Smith, 1999; Wiltrout et al., 2003), and slugs (Hopfield and Gelperin, 1989; Sekiguchi et al., 1999).

Phytoseiulus persimilis is well known to acquire a preference for the odor of spider-mite-infested plants over the odor of uninfested plants. However, more remarkably, this predatory mite also learns to prefer odor from uninfested plants if this is associated with food (Drukker et al., 2000; van Wijk et al., 2008). If, in contrast to the results reported here, olfaction were strictly elemental, the mites could in theory associate each of the detectable components in an odor mixture with the presence of their prey. *Phytoseiulus persimilis* has been reported to acquire a preference for MeSA after it has been given the opportunity to associate MeSA containing plant odors with prey (de Boer and Dicke, 2004a), while other components of food-associated odors do not appear to be associated with prey (van Wijk et al., 2008). Parasitoids and bees also behaviorally generalize their conditioned response from a mixture to a small number of its components, while they do not generalize this response to other detectable components in the mixture (Meiners et al., 2003; Reinhard et al., 2010). Other arthropods may not generalize their response to any of a mixture's components. The black bean aphid is repelled by nine host compounds, while a mixture of these repellents is an attractant (Webster et al., 2010). Similarly, the moth *Manduca sexta* responds only to a full floral odor blend and not to its components (Riffell et al., 2009).

There is evidence that representations of odors become more distinct from their components with increasing mixture complexity and with the degree of similarity of the mixture's components. Measurements of the odor induced activity in second order neurons in the olfactory pathway of zebrafish have revealed that binary mixtures of pure odorants are often dominated by the responses induced by one of the components, while complex mixtures of food extracts that have many components in common are more distinct from their components (Tabor et al., 2004). Rats perceive mixtures of similar compounds as dissimilar from their components, while mixtures of dissimilar compounds are perceived as similar to their components (Wiltrout et al., 2003). In our experiments using simple binary mixtures, the mites responded to some mixtures as if they were odors distinct from their components. With increasing mixture complexity, there are more ways for components of odor mixtures to affect each others perception, thereby facilitating the mixtures distinctness from its components. This may well explain why the predatory mites do not generalize their response from the highly attractive odor of a spider-mite infested plant to most spider-mite induced components (van Wijk et al., 2008).

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