These results are similar to those obtained by Carpita *et al.* (*6*). When cells were exposed to a mixture of 0.2*M* sucrose plus 5.0 percent (weight to volume) ovalbumin, plasmolysis was observed. In this case, most of the osmotic potential required for plasmolysis was produced by a small solute (sucrose) which equilibrates rapidly through the cell wall and does not cause wall shrinkage. The presence of a small amount of the large solute (ovalbumin) should cause only minimal osmotic shrinkage. Under these conditions it appears that the ovalbumin was able to penetrate the cell wall and hence cause plasmolysis. We suggest that cell wall shrinkage in the presence of high concentrations of large solutes may have caused Carpita *et al.* (*6*) to underestimate significantly the size of molecule capable of penetrating the cell wall.

When Carpita *et al.* (*6*) exposed plant cells to extremely high concentrations of macromolecules, they found that only molecules whose diameter was less than 17 Å penetrated the cell wall in detectable amounts. They suggested that, over a longer period of time, somewhat larger molecules might have penetrated the cell wall. In our cell wall column experiments we have exposed cell walls to much lower concentrations of macromolecules; this should more closely reflect the conditions to which plant cells are exposed in nature (*7*). Under these conditions the cell wall of bean hypocotyl tissue is permeable to proteins of molecular weight up to 60,000. It is reasonable to expect that enzymes of moderate size could permeate enough of the cell wall matrix in vivo to play a significant role in cell wall biochemistry. Since we have shown that cell wall shrinkage can reduce the permeability of the wall matrix, it is possible that our results also underestimate the permeability of the wall in vivo, since the wall is considerably stretched by the turgor of the cells that it encloses.

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Antibiotic-Induced Inhibition of Pheromone Synthesis in a Bark Beetle

Abstract. *Ingestion of diet containing streptomycin inhibited the conversion of myrcene, a host plant terpene, to the male-specific pheromones ipsenol and ipsdienol in* Ips paraconfusus. *Synthesis of* cis*-verbenol, which is not a sex-specific pheromone, from the host plant terpene (−)-α-pinene and other metabolites from these two terpenes was not inhibited by the antibiotic.*

The bacterium *Bacillus cereus* isolated from hindguts of male and female *Ips paraconfusus* (Coleoptera: Scolytidae) produced the pheromone *cis*-verbenol when exposed in culture to vapors of α-pinene, a terpene found in the host, ponderosa pine (*1*). Exposure of male and female beetles to (−)-α-pinene vapor resulted in the production of *cis*-verbenol and myrtenol in the hindgut, indicating a relation between precursor and product (*2*). Another host plant chemical, myrcene, was shown to be converted to the pheromones ipsenol and ipsdienol, but only in male *I. paraconfusus* (*3–5*). Therefore, we added antibiotics to the diets of male *I. paraconfusus* and then exposed the insects to vapors of (−)-α-pinene and myrcene to determine if pheromone synthesis could be inhibited, thereby implicating symbiotic microorganisms.

Male *I. paraconfusus* were reared from naturally infested logging debris (Sierra and Eldorado National Forests, California) shortly before their use. The diets consisted of 62 ml of water, 34.5 g of powdered cellulose (Alpha-Cel, ICN Pharmaceuticals), 8 g of sucrose, and 22 g of freshly ground phloem (< 0.5 mm in diameter) from recently felled ponderosa pines. Streptomycin and penicillin G (1667 U/mg; both Sigma) were added to the diets at 10 mg each per milliliter of water in experiment 1 (17 November 1976) and each separately at 10 mg/ml in experiment 2 (16 November 1977). The diet ingredients were mixed and divided among three 10-cm petri dishes, and the mixture was tamped in each dish to 50 ml (air pockets < 1 mm in diameter) with a final concentration of each antibiotic in the diet estimated to be 4.1 μg/μl. Holes (3 mm in diameter by 8 mm deep) were made in the tamped diet mixes to promote rapid boring and feeding. In both experiments, 21 males were added to each dish of diet.

In the first experiment, 126 males were fed the diet that did not contain antibiotic, and 63 males were fed the antibiotic diet for 96 hours at 21°C under natural light. In the second experiment, 84 males were fed the nonantibiotic diet, 84 males were fed the penicillin diet, and 84 males were fed the streptomycin diet for 96 hours under similar conditions. At the end of the feeding period, groups of 20 males (experiment 1) or 10 males (experiment 2) were each placed in an aluminum screen cage inside a 70-ml glass jar containing 3 μl of (−)-α-pinene ($[\alpha]_D^{20}$ = −47.5°; Aldrich) purified by gas-liquid chromatography (GLC) and 3 μl of GLC-purified myrcene (Chem Samples) (each > 99.8 percent) for 18 hours at 21°C under natural light. Two groups of ten males in each experiment that had been fed the respective diets were not exposed to host terpene vapors to determine amounts of pheromone that were obtained from the ground phloem. In the second experiment, six groups of ten unfed males were exposed to host terpene vapors and compared to fed insects (*6*) (Table 1). The concentration of (−)-α-pinene and myrcene vapors in the exposure chamber were determined by withdrawing samples with a gastight syringe for GLC analysis [glass column, 1.8 m by 2 mm (inside diameter); 3 percent Apiezon L on 100/120 Gas-Chrom Q at 100°C; N_2 flow, 12 ml/min] (*4*) (Table 1).

After the exposure period, the mid- and hindguts were dissected out and extracted with diethyl ether (0.15 ml per ten males). Authentic samples of ipsenol, ipsdienol (each > 97 percent) and *cis*-verbenol (> 95 percent) (both Chem Samples), and myrtenol (> 99 percent; Aldrich) were compared to the gut extracts by GLC on the Apiezon L column. These compounds can be resolved by this GLC column. In previous studies unfed males were exposed to these terpenes by aeration. Metabolic products were identified in extracts of the hind gut by gas chromatography–mass spectrometry (*2, 4, 5*), nuclear magnetic resonance (*2, 5*), and by on-column hydrogenation (*4*). The percent mortality after feeding and after exposure to (−)-α-pi-

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Table 1. Pheromones and metabolites produced in male *I. paraconfusus* exposed to myrcene and (−)-α-pinene vapors after being fed diets with and without streptomycin sulfate or penicillin G (or a combination thereof) at 4.1 mg per milliliter of diet. In experiment 1, the beetles were exposed to myrcene at 21.1 ± 1.2 × 10^{-7}g/ml and to (−)-α-pinene at 47.1 ± 2.8 × 10^{-7}g/ml. In experiment 2, beetles were exposed to myrcene at 17.6 ± 0.6 × 10^{-7}g/ml and to (−)-α-pinene at 51.0 ± 3.0 × 10^{-7}g/ml.

Treatment	Mean amount of pheromone per male (10^{-8} g ± S.E.M.)				
	Ipsdienol	Ipsenol	*cis*-Verbenol*†	Myrtenol†	Compound B†‡
			Experiment 1		
No antibiotic	3.9 ± 0.6	9.5 ± 1.8	294.2 ± 40.0	96.0 ± 12.5	609.8 ± 42.7
Streptomycin and penicillin G	< 0.1§	< 0.1§	345.8 ± 51.7	123.5 ± 15.0	467.4 ± 44.2
			Experiment 2		
No antibiotic	6.2 ± 1.8†	45.1 ± 8.8†	1179.4 ± 228.7	379.3 ± 66.5	1123.0 ± 106.0
Streptomycin	< 0.5§	< 0.5§	1098.6 ± 195.6	351.5 ± 72.4	996.6 ± 55.7
Penicillin G	3.6 ± 0.7†	26.3 ± 5.2†	1145.5 ± 182.9	380.1 ± 27.6	991.4 ± 76.9
Unfed	3.4 ± 0.8†	24.4 ± 5.0†	998.3 ± 142.4	335.6 ± 38.7	1208.1 ± 180.6

*Amounts expected to include 5 percent *trans*-verbenol from 5 percent of the (+) enantiomer impurity in the (−)-α-pinene (*2*). †All means in same column of each experiment or as indicated were not significantly different at $\alpha > .05$ (*t*-test). ‡Compound B was quantified by comparison with an authentic sample of ipsenol. §Significantly different from animals on other diets and the unfed animals within each experiment at $\alpha < .001$ (*t*-test).

nene and myrcene vapors (Yate's chi-square test) and the number of fecal pellets defecated during the exposure period (*t*-test) were recorded to assess possible differences between the diets.

Conversion in vivo of myrcene to ipsdienol was inhibited by streptomycin in the diet (Table 1). The effect of streptomycin on the subsequent conversion of ipsdienol to ipsenol that occurs only in males (*3, 7*) could not be determined since ipsdienol synthesis was inhibited. Streptomycin did not appear to affect synthesis of *cis*-verbenol, myrtenol, or compound B (unidentified metabolite from myrcene; the retention time was 3.66 times that of ipsenol on Apiezon L), an indication of the highly specific effects of the antibiotic on the ipsdienol and ipsenol biosynthetic system. There were no significant differences in mortality between any of the diet-fed beetles ($P > .1$), and excretion of fecal pellets during the exposure to precursor vapors (from 7.4 to 8.5 pellets per male; unfed beetles did not defecate) was also not significantly different ($P > .1$). Males that had been fed on diets with and without antibiotics but were not subsequently exposed to host terpene vapors did not contain detectable pheromones (< 0.5 × 10^{-8} g per male) 18 hours after they were taken off the diets. This result indicates that the pheromones in the males were synthesized from the terpene vapors and not from precursors in the ground phloem diet.

Our study indicates that symbiotic bacteria sensitive to streptomycin (*8, 9*) are involved in pheromone synthesis in *I. paraconfusus*. Streptomycin causes malfunctioning of enzyme synthesis in 70*S* bacterial ribosomes (*8*). Insect cells are not affected by streptomycin in this way since they do not have this type of ribosome except in their mitochondria. However, streptomycin, a trisaccharide, probably does not interact with mitochondria, whose inner membrane is not permeable to disaccharides (*10*). Even if the insect mitochondria somehow were damaged by streptomycin, one would not have expected inhibition of only the ipsenol and ipsdienol enzyme system. The inhibition of pheromone synthesis could still be due to effects of streptomycin on other than bacterial systems (*11*). We have demonstrated a relation between the amount of precursor vapors and pheromones so that subsequent comparisons between bacterial populations and pheromone biosynthesis can be made in vivo and in vitro. In order to demonstrate a male-specific role for microsymbionts in pheromone biosynthesis, it is still necessary to isolate the organism and explain the mechanism of sex specificity.

Our study does not support earlier in vitro work suggesting that *B. cereus* synthesizes *cis*-verbenol from α-pinene in the hindgut of this beetle (*1*). Penicillin G is an effective antibiotic against many Gram-positive bacteria (*8*) such as *B. cereus*. Furthermore, this species is reported to be sensitive to streptomycin (*9*). Our results suggest that *cis*-verbenol is formed by the insect cells, or that the concentration of antibiotic was not high enough to affect the pheromone-producing microorganisms.

Earlier evidence indicated that production of ipsenol, ipsdienol (*6, 12*), and *cis*-verbenol (*6*) in *I. paraconfusus* may be under hormonal control. Gut stretching due to feeding stimulates release of brain hormone, which causes juvenile hormone release and subsequent stimulation of pheromone synthesis (*6*). However, in our study the amounts of pheromones produced in unfed males exposed to host terpenes were not significantly different from that produced in similarly treated males fed the diet without antibiotics (Table 1, experiment 2). Thus gut stretching was not a prerequisite for pheromone synthesis.

One other report suggests that symbionts are involved in pheromone synthesis. A bacterial culture obtained from collaterial glands of the female grass grub beetle, *Costelytra zealandica*, when grown on nutrient medium synthesized the pheromone phenol (*13*). However, the relation between the bacterial population and pheromone production in vivo remains to be determined in order to establish that these microorganisms are the primary source of the pheromone (*1, 13*). Several other species of *Ips* utilize ipsenol, ipsdienol, or *cis*-verbenol (or a combination of both) as components of their aggregation pheromone (*14*). Further investigation is needed to determine if biosynthetic systems similar to that in *I. paraconfusus* occur in the genus *Ips* which has a circumpolar distribution in the Northern Hemisphere.

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