Mechanisms of Feeding Deterrence by Ziziphins

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Feeding Deterrence, taste modifiers, video analysis, Lepidoptera, noctuidae, larvae, leaf extracts

The mechanism of feeding deterrence by ziziphins for southern armyworms (Spodoptera eridania; Lepidoptera: Noctuidae) was investigated using both long-term, two-choice and no-choice measures, and short term (20 min) high magnification video analysis. Ziziphus jujuba leaves and leaf extracts are non-preferred in two-choice tests, and only eaten sparsely and sporadically, with long initial delays, in no-choice tests. In short-term tests, treatment of mouth parts with ziziphins significantly reduced total time feeding and feeding bout duration, compared to classical deterrent and carrier treatments. Time feeding decreased after ziziphins treatment while time crawling, time searching, and quiescent increased. A taste modifying action of ziziphins may account for this.
A. STATEMENT OF THE PROBLEM

The research used larvae of southern armyworms, *Spodoptera eridania*, to investigate the mechanisms of feeding deterrence by ziziphins extracted from the leaves of *Ziziphus jujuba*. Evaluation of the possibility of taste modification as a deterrence mechanism was the primary objective. The selectivity, temporal properties, and categories of effective stimuli were studied.

The time course of deterrence was examined in behavioral investigations. To study immediate effects, a brief application of either ziziphins or control liquids directly to the mouthparts of *S. eridania* was followed by short duration measures of feeding behavior. This was done with high resolution video taping of contact with, and biting of, leaves or diet disks.

Feeding experiments distinguished between indirect (taste modification) and direct (stimulatory) effects. These experiments were two-choice or no-choice measurements of ingestion of artificial diet disks, to which phagostimulants, or ziziphins, were added.

B. SUMMARY OF MOST IMPORTANT RESULTS

1. Long Term Measures

Nine to 24 hour diet consumption experiments showed that *Z. jujuba* leaves are nonpreferred by *S. eridania* larvae relative to host plant (*Phaseolus vulgaris*) leaves in two-choice tests. In no-choice situations, *Z. jujuba* leaves are sparsely and sporadically eaten, with most intake starting 3 hours or more after the tests begin. Likewise, host-plant based test diets treated with *Z. jujuba* leaf powders or aqueous-alcohol or chloroform-ethanol extracts of *Z. jujuba* leaves are non-preferred in two-choice tests, and show suppressed feeding in no-choice tests. These observations confirm the deterrent action of *Z. jujuba* leaves and extracts, and suggest that a "true deterrence" is produced.
2. Short Term Measures

High magnification (56 diameters), 20 minute video tape records of individual, six hour starved, S. eridania larvae feeding on host plant (P. vulgaris) leaves showed that Z. jujuba extracts produced feeding behavior which differed from that seen after control treatments. In general, after Z. jujuba extract treatment, feeding stopped after a few bites, while feeding continued after treatment with water or with berberine, a "classical" deterrent.

More specifically, a 3 minute treatment with a 10 microliter volume was applied to the mouth parts. This was followed by a 10 second rinse with 100 microliters flowing distilled water, then a 10 second blot, and a 10 second rest. Treatments were either distilled water, 0.9% w/v aqueous-alcohol extract (in distilled water), 0.048% w/v berberine (in distilled water), or no treatment.

There were no significant differences between treatment groups for number of changes in behavior, or number of feeding bouts (p>0.1, Kruskal-Wallis one-way non-parametric analysis of variance). However, significant effects were found for total feeding duration (p<0.005), and for feeding bout duration (p=0.054). Median total duration of feeding decreased by 39% after ziziphins treatment, compared to the other treatments. Median feeding bout duration decreased by 46% after ziziphins treatment, compared to the other treatments. A detailed time budget analysis showed that highly significant effects (p<0.005) of extract treatment occurred in percent of time feeding, and percent of time quiescent. Significant effects also were found in percent of time crawling (p<0.025) and percent of time searching (p<0.05). After ziziphins treatment, time feeding decreased, while time crawling, searching, and quiescent increased.

For the ziziphins treated larvae, median time spent feeding is more variable that for the other groups during the first 3 minutes after treatment, is below the other treatment groups for much of the first 3 minutes, is reduced by 67% compared to the other groups by 225 seconds after treatment, and reaches zero at 450 msec after treatment. All other treatment groups are actively feeding at this time, and continue for several hundred seconds more.
3. Conclusions

The data strongly suggest that ziziphins change feeding behavior of southern armyworms in a manner different from a classical feeding deterrent. Taste modification may be the basis of this difference, and may account for the feeding deterrence of ziziphins for *S. eridania*.

C. LIST OF ALL PUBLICATIONS

I. Published


II. In Preparation


D. LIST OF ALL PARTICIPATING SCIENTIFIC PERSONNEL

1. Bruce P. Halpern, Professor of Psychology and Neurobiology and Behavior