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PLANT SECONDARY COMPOUNDS–A BASIS FOR NEW AVIAN REPELLENTS

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ABSTRACT: Bird repellents that are effective have tended to be toxic, while those that are relatively nontoxic have tended to be ineffective. There is a need for repellents that work well and safely. Interest has focused on the natural chemical defenses used by plants to defend themselves from herbivores. Preferences of bullfinches in orchards for different pear cultivars were correlated with biochemical differences between cultivars. A class of plant secondary compounds has been isolated and shown to be physiologically active against bullfinch and pigeon gut enzymes, and also to deter feeding in the laboratory. The physiological and biochemical mechanisms responsible for their repellency are under investigation.

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INTRODUCTION

Where animals in the field concentrate their feeding on a particular food type, then a plausible explanation is that it is more nutritious than available alternatives. It may also be less poisonous. Animals not only seek foods that benefit them, but also attempt to avoid foods that harm them. For example, bullfinches (Pyrrhula pyrrhula), a pest of fruit orchards in the UK, clearly choose Conference in preference to Cornice pear buds. Differences in major nutrients between cultivars do not appear to be responsible (Greig-Smith and Wilson 1983). Possibly the physical characteristics of the buds make Cornice less digestible or less profitable (Summers and Huson 1984), or it may be due to some underlying biochemical difference.

To investigate this possibility, Greig-Smith and Wilson (unpubl.) gave bullfinches seeds coated with a series of 26 pear-bud extracts and examined their behaviour while eating them. They found that measures of distaste (such as bill-wiping, head-shaking) were correlated with biochemical differences between varieties. Among other things, bullfinches tended to favour varieties that were low in chlorogenic acid. This compound belongs to the cinnamic acids, which in turn belong to the group of plant secondary compounds known as the phenolics. The tannins also belong to this group and have long been implicated in plant defense (see Rosenthal and Janzen 1979). Many animals avoid plants high in tannins and do not thrive when fed on high tannin diets (Rogler et al. 1985). It is known that tannins bind to proteins, causing them to precipitate out of solution, and causing an astringent sensation in the mouth. It has been suggested that they actively participate in plant defense by interfering with herbivores' digestion of proteins. The following experiments were designed to investigate the potential of cinnamic acids as bird repellents and to understand their biochemical effects.

EXPERIMENT 1

Previous work at the ADAS Central Science Laboratory (Crocker and Perry 1990; Wilson and Hennessy, unpubl.) has shown that the avian alkaline serine proteases trypsin and chymotrypsin-enzymes responsible for protein digestion in the intestine-may be inhibited by plant secondary compounds. The present experiment examined the effect of cinnamic acid derivatives on trypsin-like enzymes extracted from feral pigeon gut, and on the birds' feeding behaviour.

Methods

Nine easily procurable cinnamic acid derivatives were chosen for investigation. For comparison, a commercial trypsin inhibitor (purified from turkey egg white), sucrose octaacetate (which tastes bitter to humans), dimethyl anthranilate [believed to act as a nasal irritant in birds (Mason et al. 1989)] and a control were also included as treatments. Each treatment was tested for its inhibitory effect in the test tube on trypsin purified from feral pigeon gut extract. A detailed description of biochemical methods will be published elsewhere (Crocker, Perry and Wilson, in prep.).

The effects of cinnamic acid derivatives on feeding behaviour were tested on 35 feral pigeons. One group of 13 birds received all 13 treatments in a Latin square design. Chlorogenic acid and 3,5 dimethoxycinnamic acid were available in limited amounts, so the remaining subjects were divided into two Latin squares of 11 birds each and given 11 treatments. Preference was assessed by presenting each bird with a no-choice test (a single bowl of treated food) lasting 3 days, followed by a two-choice test (treated and untreated food simultaneously available) lasting 4 days. The treated food was a standard laboratory diet of turkey starter crumbs sprayed with the chemical compound dissolved in an acetone/water mixture, to achieve a concentration of 0.5% w/w. The control treatment was sprayed only with the acetone/water solvent. Food consumption was monitored daily and birds were weighed at the beginning and end of the no-choice test.

Results

Figure 1 shows that cinnamic acid was a powerful trypsin inhibitor. In vitro, it reduced trypsin activity by 93%, exceeding that of the commercial turkey egg white inhibitor (64%). The remaining compounds had little or no effect.

Figure 2 shows food consumption during the two-choice tests and no-choice tests and weight loss during no-choice tests. By all measures there were significant ($p<.001$) differences between treatments with birds avoiding cinnamamide most strongly, followed by 3,5 dimethoxycinnamic acid and DMA.

Cinnamic acid was a potent inhibitor of trypsin activity in the test tube, while cinnamamide significantly reduced food consumption by birds in the laboratory. However, there was no correlation between inhibition of trypsin and feeding deterrence in pigeons (Fig. 3).

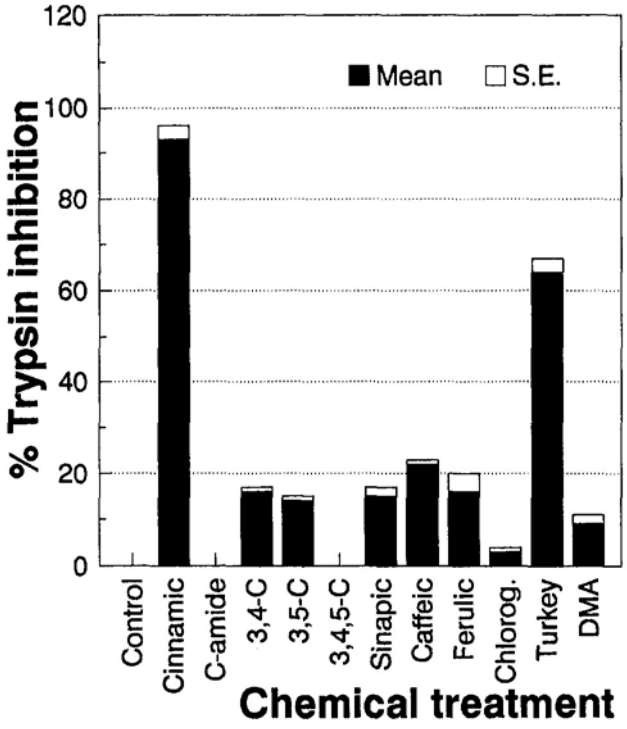

Fig. 1. Inhibition of feral pigeon trypsin activity by the cinnamic acid derivatives-cinnamic acid, cinnamamide, 3,4 dimethoxycinnamic acid, 3,5 dimethoxycinnamic acid, sinapic acid, caffeic acid, ferulic acid chlorogenic acid-and by turkey egg white trypsin inhibitor and dimethyl anthranilate.

Discussion

The cinnamic acids are relatively low molecular weight phenolics and would not be expected to behave as the heavier tannins do, causing general protein precipitation. Nevertheless, some of them are powerful inhibitors of specific proteolytic enzymes. Cinnamic acid was a powerful inhibitor of trypsin and might be expected to be aversive, but it was not. In feeding experiments, pigeons were indifferent to cinnamic acid but strongly avoided cinnamamide, which was not a good enzyme inhibitor.

Several explanations of this result are worth considering. It is possible that in vitro conditions in the test tube do not match the in vivo environment in the gut (Mole and Waterman 1987a,b): a compound's ability to inhibit enzymes in the test tube may not be maintained in the animal. Perhaps the cinnamic acids inhibit other enzyme systems which do correlate with behavioural preferences. Perhaps the cinnamamide and 3,5 dimethoxycinnamic acid depend for their repellency on entirely different mechanisms, acting on sites other than the gut, as an unpleasant taste in the mouth for example, or causing sensations of nausea, or as a toxin in the liver or central nervous system. We have evidence that these compounds are small enough to pass into the bloodstream.

EXPERIMENT 2

The cinnamic acid derivatives in Experiment 1 were chosen more or less arbitrarily. They have very similar molecular structures, but it is clear that small differences between them cause large differences in their repellency. For example, 3,5 dimethoxycinnamic acid (3,5-C) differed from 3,4 dimethoxycinnamic acid (3,4-C) only in a shift of a methoxy group from the fourth to the fifth carbon atom of the benzene ring (Fig. 4). Yet birds avoided 3,5-C and were

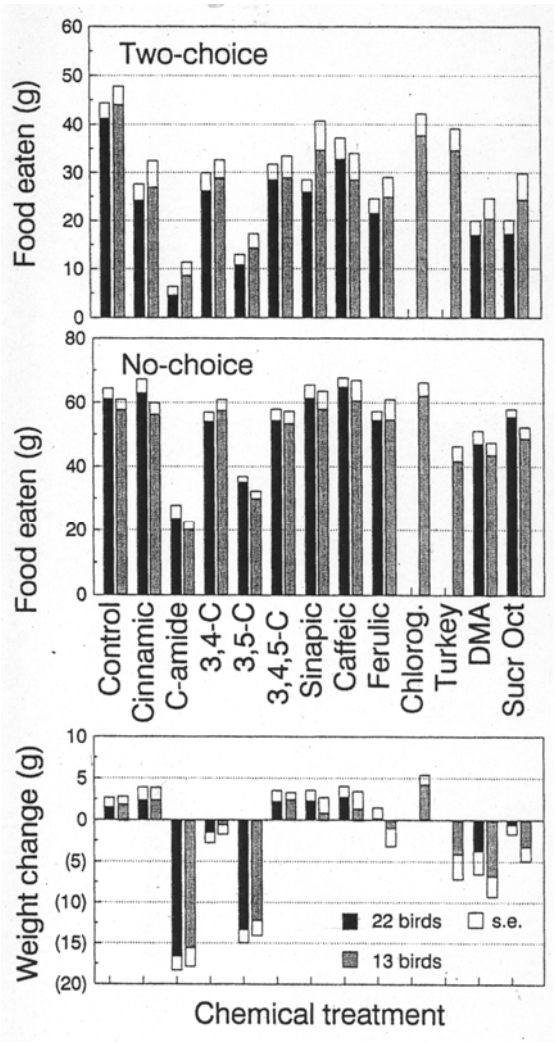

Fig. 2. Food consumption and weight loss by 35 feral pigeons during two-choice and no-choice tests of cinnamic acid derivatives and other repellents (Experiment 1).

indifferent to 3,4-C and 3,4,5-C. To further examine the importance of the precise positioning of methoxy groups, it was decided to test 3- and 4-methoxycinnamic acid. Similarly, cinnamamide (a good repellent) differs from cinnamic acid (a poor repellent) only by the substitution of an amide group for a hydroxy group. The present experiment therefore investigated the effects of two more substituents, alcohol and aldehyde, at this position. Cinnamic acid differs from benzoic acid by having an extra unsaturated carbon atom on the side chain. It was decided to include two benzoic acid derivatives, 3,4 and 3,5 benzoic acid. Finally, two nonphenolic compounds were included for comparison: thiram has a long history as a seed dressing with bird-repellent properties, while safrole was identified by Schafer and Jacobson (1983) as a naturally occurring chemical with repellent properties.

Methods

The repellency of 10 treatments (including cinnamamide and a control) was tested on two groups of 10 feral pigeons in a Latin square design. Each bird received each treatment in a 3-day no-choice test.

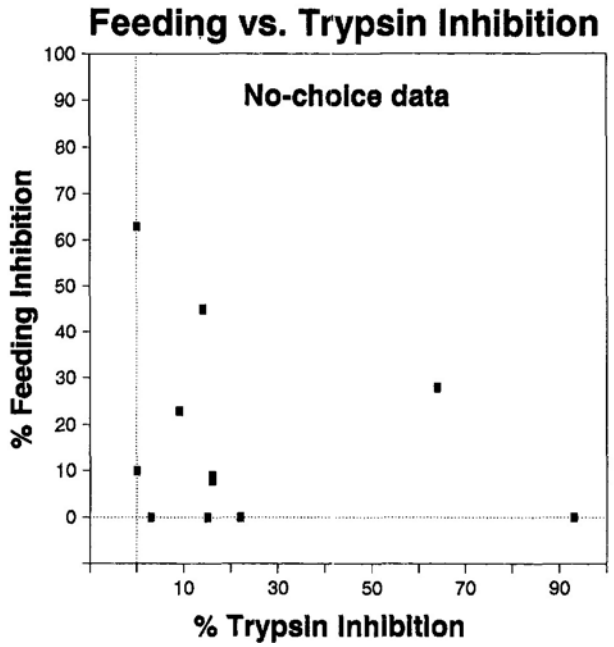

Fig. 3. Correlation between inhibition of trypsin activity in vitro, and feeding inhibition of caged pigeons.

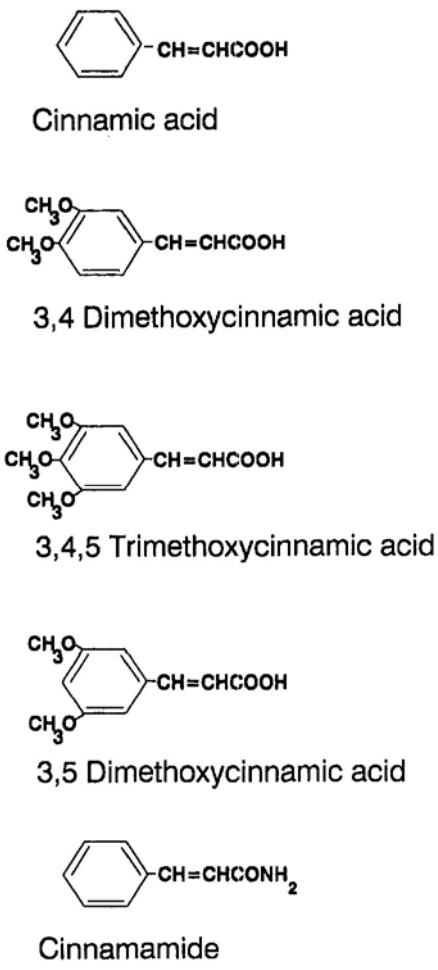

Fig. 4. Molecular structures of some closely related cinnamic acid derivatives.

Results and Discussion

Analysis of variance showed a significant treatment effect ($p<.001$), but none of the new cinnamic or benzoic acid derivatives came close to cinnamamide as a potential feeding deterrent (Fig. 5). Safrole, however, significantly reduced food consumption, as did thiram.

Safrole is a suspected carcinogen and can probably be ignored as a prospective bird repellent. Thiram, however, is well established in this role. Cinnamamide did not perform as well as thiram in these trials but the difference in food consumption was not significant (post hoc Scheffe test, $p >.05$), and initial studies suggest that it may have other advantages such as reduced toxicity.

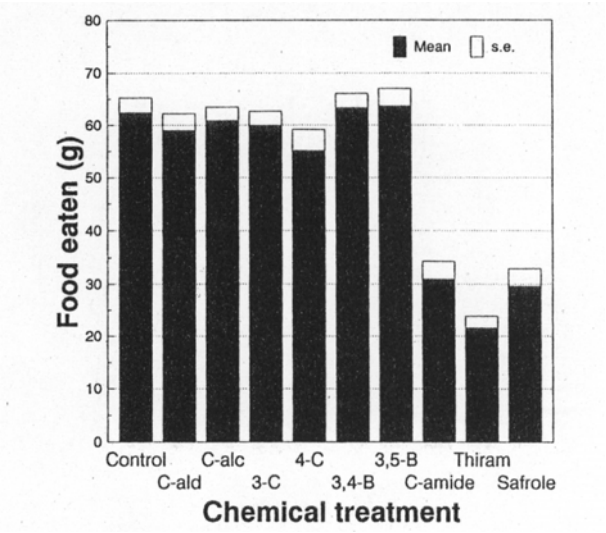

Fig. 5. Consumption, during 3-day no-choice tests by 20 feral pigeons, of food treated with cinnamic acid derivatives, thiram or safrole (Experiment 2).

EXPERIMENT 3

The two most promising candidates, cinnamamide and 3,5 dimethoxycinnamic acid, appear to have their key substituents at different ends of the basic cinnamic acid molecule. This invites the possibility of combining the two to form a single molecule with two key substituents-3,5 dimethoxy-cinnamamide. If the compounds each have different mechanisms of action, competing for different active sites in the body, then synthesising them into a single compound may enhance its repellency. Similarly, a physical mixture of the two compounds should have additive (or synergistic) effects if the compounds are not competing for the same active site. The following experiment compared the effects of cinnamamide and 3,5 dimethoxycinnamic acid alone, physically combined (two concentrations), and chemically combined as 3,5 dimethoxycinnamamide.

Methods

Thirty feral pigeons were divided into five Latin squares, each bird receiving each of six treatments in a series of 3-day no-choice tests.

Results and Discussion

The hybrid compound 3,5 dimethoxycinnamamide was the least effective of the treatments, differing significantly from the control treatment only on day 1 ($p <.05$) (Fig. 6). That

it performed worse than either cinnamamide or 3,5 dimethoxycinnamic acid alone suggests that important features of the two molecules are lost upon their chemical combination.

The result of physically mixing the two compounds suggests that their effects are not additive. A factorial analysis of variance gave a significant interaction effect ($p<.001$) indicating that the compounds were masking each other. The repellency of the mixture did not exceed that of its components. Nevertheless, the results suggest that there may be benefits to be gained by mixing the compounds. Figure 6 shows that whereas cinnamamide was effective on the first day, its repellency attenuated with time. The opposite appears to be true of 3,5 dimethoxycinnamic acid. Pigeons appeared to increasingly dislike it as the trial progressed. Thus a mixture of the two compounds may allow cinnamamide to produce a strong initial aversion while 3,5 dimethoxycinnamic acid maintains it in the longer term.

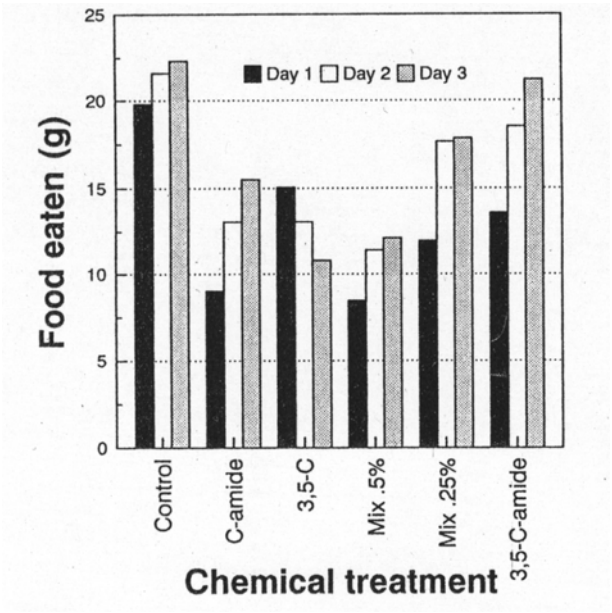

Fig. 6. Consumption, during 3-day no-choice tests by 30 feral pigeons, of food treated with cinnamamide, 3,5 dimethoxycinnamic acid, physical mixtures of the two, and their chemical combination–3,5 dimethoxycinnamamide (Experiment 3).

CONCLUSION

Several compounds related to naturally occurring plant secondary compounds have been identified as promising bird repellents. Similar compounds have been shown to inhibit the action of avian protein-digesting enzymes in vitro. Unfortunately, the ability of a compound to deter birds from feeding in the laboratory appears to be unrelated to its ability to inhibit enzymes in the test tube. Small manipulations of the molecular substituents have large effects on their repellency. A simple explanation of these differences has not presented itself. A more comprehensive study of structure-activity relationships is under way using computer modelling techniques. It is hoped to identify the key chemical, physical, and topological features of the repellent compounds and to design these features into new, more potent repellents.

ACKNOWLEDGMENTS

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