SYNERGISTIC EFFECTS OF THREE *Piper* AMIDES ON GENERALIST AND SPECIALIST HERBIVORES

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Abstract—The tropical rainforest shrub *Piper cenocladum*, which is normally defended against herbivores by a mutualistic ant, contains three amides that have various defensive functions. While the ants are effective primarily against specialist herbivores, we hypothesized that these secondary compounds would be effective against a wider range of insects, thus providing a broad array of defenses against herbivores. We also tested whether a mixture of amides would be more effective against herbivores than individual amides. Diets spiked with amides were offered to five herbivores: a naïve generalist caterpillar (*Spodoptera frugiperda*), two caterpillar species that are monophagous on *P. cenocladum* (*Eois* spp.), leaf-cutting ants (*Atta cephalotes*), and an omnivorous ant (*Paraponera clavata*). Amides had negative effects on all insects, whether they were naïve, experienced, generalized, or specialized feeders. For *Spodoptera*, amide mixtures caused decreased pupal weights and survivorship and increased development times. *Eois* pupal weights, larval mass gain, and development times were affected by additions of individual amides, but increased parasitism and lower survivorship were caused only by the amide mixture. Amide mixtures also deterred feeding by the two ant species, and crude plant extracts were strongly deterrent to *P. clavata*. The mixture of all three amides had the most dramatic deterrent and toxic effects across experiments, with the effects usually

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surpassing expected additive responses, indicating that these compounds can act synergistically against a wide array of herbivores.

INTRODUCTION

Manipulative experiments examining the effects of plant defenses on herbivores often examine individual defenses in isolation, despite evidence that many defenses are additive or synergistic (Hay et al., 1994; Nelson and Kursar, 1999). Synergistic plant defenses are broadly defined as effects of multiple compounds that are greater than expected based on projected additive values of each individual compound (Berenbaum et al., 1991; Jones, 1998; Nelson and Kursar, 1999). Some authors use the term "potentiation" to distinguish those synergistic effects where compounds are each separately toxic to the insect but the toxicity of the combination is greater than expected additive effects (Stewart, 1998). Synergisms between different chemical defenses have been documented where deterrence, toxicity, or other fitness effects of one compound are increased dramatically (i.e., significantly greater than additive effects) in the presence of other compounds (Kumar and Parmar, 1996; Jones, 1998; Scott et al., 2002). One documented mechanism of synergy is that inactive metabolites impede an herbivore's or pathogen's ability to metabolize or eliminate toxins (Berenbaum and Neal, 1985; Kubo and Muroi, 1993; Stermitz et al., 2000). Secondary metabolites are always present in plants as simple or complex mixtures, and there are many hypotheses for this so called phytochemical redundancy (Jones and Firn, 1991; Romeo et al., 1996). Since synergy may be a common phenomenon in plant defenses and one important reason for phytochemical redundancy, it warrants investigation in any system where plant–herbivore interactions are well studied. We chose to study synergy in *Piper cenocladum* C. DC. (Piperaceae), which is emerging as a model system for studies of plant defense and herbivory.

A wide array of secondary compounds has been isolated from *Piper* spp., including amides of a characteristic type referred to as "*Piper* amides" (reviewed by Parmar et al., 1997). These amides contain a phenyl moiety with a variable length carbon side chain (typically with at least one unsaturation) ending in a carbonyl carbon. The nitrogen containing portion of the amide is derived from piperidine, pyrrole, or an isobutyl group and may contain an unsaturation, an epoxide, or a carbonyl group. *Piper cenocladum* contains three amides at high concentrations (total amide content can be as high as 3.8% dry weight): piplartine, $4'$-desmethylpiplartine, and cenocladamide (Figure 1; Dodson et al., 2000). Many *Piper* amides that have been investigated for biological activity have been demonstrated to be insecticidal (Su and Horvat, 1981; Miyakado et al., 1989; Gbewonyo
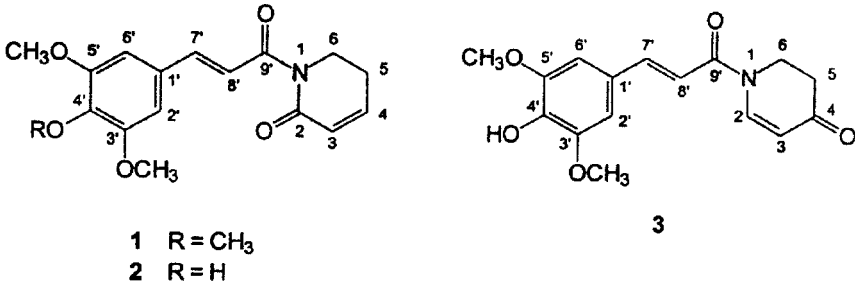
FIG. 1. *Piper cenocladum* amides: piplartine (1), 4′-desmethylpiplartine (2), and cenocladamide (3).

et al., 1993; Scott et al., 2002) or deterrent to leaf-cutting ants (Capron and Wiemer, 1996; Dyer and Dodson, unpublished data). One of the amides in *P. cenocladum*, piplartine, is cytotoxic *in vitro* (Duh et al., 1990). Amides are found in higher concentrations when mutualistic ants are absent from *P. cenocladum* (Dodson et al., 2000; Dyer et al., 2001), and measures of herbivory (Dyer and Letourneau, 1999a,b) have suggested that amides in *P. cenocladum* are deterrent to arthropods.

As with most apparently redundant chemical defenses in plants, it is unknown whether amides are enemy-specific in their deterrence or toxicity (as in Lindroth and Hwang, 1996) or whether they act in a synergistic fashion, as has been indicated for furanocoumarin defenses in apiaceous plants by Berenbaum and colleagues (Berenbaum and Neal, 1985; Berenbaum et al., 1991; Berenbaum and Zangerl, 1993). To test the hypothesis that *Piper* amides act synergistically, we performed a series of experiments examining the preference and performance of generalist and specialist insects on amide-manipulated diets.

METHODS AND MATERIALS

Natural History. Piper cenocladum are understory shrubs common in lowland wet forests throughout Costa Rica (Burger, 1971). Opalescent food bodies, rich in lipids and proteins, are produced on the adaxial side of sheathing leaf bases (hollow petioles) when occupied by *Pheidole bicornis* Forel ants (the plants are not always occupied by these ants). There are dozens of herbivores associated with *P. cenocladum* (Dyer and Letourneau, 2003). The most common external folivores include specialist and generalist lepidopterans (Geometridae, Hesperiidae, Apatelodidae, and Limacodidae), specialist coleopterans (Chrysomelidae and Curculionidae), generalist orthopterans (Tettigoniidae), and leaf-cutting ants (*Atta cephalotes* L.). More extensive natural history of this system, relevant to this paper, is described in Dyer and Letourneau (1999b) and Letourneau (1998).

Experiments—Overview. Four primary experiments were performed to examine the synergistic effects of *P. cenocladum* amides on four different arthropod herbivores: *Spodoptera frugiperda* (Lepidoptera: Noctuidae), the leaf-cutting ant *Atta cephalotes* (Hymenoptera: Formicidae), two species in the genus *Eois* (Lepidoptera: Geometridae), and the omnivorous ant *Paraponera clavata* Fabricius (Hymenoptera: Formicidae). The experiments with lepidopteran herbivores tested the performance of generalists and specialists on diets containing amides from *P. cenocladum*, and the experiments with ants assessed whether these amides were deterrent to a generalist herbivore and an omnivore. To assess the potential role of synergistic interactions between amide compounds, the effects of single amides on herbivore choice or performance were compared to the effects of mixtures in each of these experiments. Controls for all experiments were administered along with experimental diets and the methods for control and experimental diet applications were identical. For experiments that required synthetic material, amides were synthesized at Mesa State College, Colorado (Richards et al., 2001).

Experiment 1—Naïve Generalist. *Spodoptera frugiperda* JE Smith is a naïve (to *P. cenocladum*) generalist herbivore native to tropical regions of the western hemisphere and is a major crop pest in several regions of North America. Eggs of *S. frugiperda* were acquired from Agripest, North Carolina. Upon hatching, 50 larvae were placed on one of eight artificial diet treatments. The control diet consisted of 35 g powdered Fall Armyworm Diet (Southland Products, Lake Village, AR) mixed with 201 ml distilled water and 1.5 ml linseed oil. For experimental diets, a single amide or a combination of amides was added to the powdered control diet. Amide quantities added were 0.203 g of commercially available isolated piplartine (Sigma-Aldrich, St. Louis, MO), 0.1575 g of synthetically produced 4′-desmethylpiplartine, and 0.1155 g of synthetically produced cenocladamide. The three-way and two-way amide combinations contained additive amounts of the amides (e.g., the three-way combination contained the addition of 0.476 g of dry amide material). The quantity of amides added represents the upper concentration limits found in non-ant containing plants of *P. cenocladum* (piplartine: 0.58% leaf dry mass, 4′-desmethylpiplartine: 0.45% leaf dry mass, cenocladamide: 0.33% leaf dry mass; Dodson et al., 2000). First instars were placed in 1.25 oz opaque plastic cups and reared in a growth chamber (28°C, 14 hr L). Larval mass was measured every fourth day, and larvae were given fresh food every second day. At time of pupation, a final mass was obtained and gender determined. Date of adulthood was recorded upon eclosion.

Pupal mass and development times of *S. frugiperda* were analyzed with ANOVAs, using amide treatment as the independent variable. We were unable to include caterpillars fed the mixture of all three amides and the piplartine-4′-desmethylpiplartine combination in these analyses because of the almost complete mortality within these treatments. Treatment responses were compared with *post hoc* pairwise comparisons of each treatment employing modified Bonferroni

correction (McDonald et al., 2002). Survival data were analyzed with a series of log-rank tests, also employing a modified Bonferroni α to correct for multiple comparisons.

Experiment 2—Leaf-Cutting Ants. To assess deterrent effects of *P. ceno-cladum* amides on a generalist herbivore that commonly encounters this plant, we performed a series of field choice experiments with the leaf-cutting ant, *Atta cephalotes* at the La Selva Biological Station, Heredia Province, Costa Rica, (10°25'N 84°05'W). In these experiments (modeled after Folgarait et al., 1996), we presented foraging *A. cephalotes* colonies with a selection of leaf disks coated with amides in 2 mg/ml methanol solutions. The treatments included piplartine, 4'-desmethylpiplartine, cenocladamide, a mix of all three compounds, and a control containing only methanol. *Hyeronima alchorneoides* Allemao (Euphorbiaceae), a species shown to be palatable to *A. cephalotes* (Folgarait et al., 1996), was used for all leaf disks. Young leaves were cut into 1×0.5 cm disks and coated with 5 μl of one of the treatment solutions, creating a disk with total amide content of about 1% dry weight. The disks were allowed to air-dry and were presented to the ants soon after preparation to ensure freshness.

Twenty-five distinct (spatially separated by at least 200 m) *A. cephalotes* colonies were presented with five disks of each of the five treatments (for a total of 25 leaf disks per trial). Disks were offered simultaneously on a piece of paper (to control for the background on which the disks were offered) to foraging ants in a cafeteria-style display. Trials continued for 15 min or until all five disks of one of the treatments had been removed by the ants. We performed each trial at least 30 m from the entrance to the nest to ensure that any single ant did not participate in the choice test multiple times. Thus, each leaf disk or each leaf-cutting ant could be considered a replicate.

Because of the difficulties in analyzing results from multiple choice preference tests (Lockwood, 1998 and references therein), we analyzed the feeding preference of leaf-cutting ants (*A. cephalotes*) using the method advocated by Folgarait et al. (1996) that takes into account lack of independence in leaves simultaneously offered to ants. Each of the five treatments was included as a dichotomous variable (disk taken or not taken) in a loglinear model, along with appropriate interactions (i.e., all interactions with the control and all interactions that were necessary for the model to fit the data). We utilized nonhierarchical models for testing specific hypotheses, a maximum likelihood method to estimate parameters, and chi-square statistics for hypothesis testing (Dyer, 1995).

Experiment 3—Specialist Herbivores. To test the effects of elevated *P. cenocladum* amide concentrations on a specialized herbivore we conducted a performance experiment with two undescribed species of geometrid larvae in the genus *Eois* that appear to be oligophagous (Dyer, personal observations) at La Selva Biological Station in Costa Rica. In pilot feeding studies, the two species did not exhibit significant differences in development times, pupal weights, parasitism

rates, or mortality (Dyer and Gentry, unpublished data), so they were lumped together for this experiment. We collected 508 early instars (primarily second) from the field, measured their length and mass, and randomly assigned them to petri dishes with 9-cm disks of fresh leaf material harvested from ant-containing *P. cenocladum* plants growing in the field. Before adding the caterpillars, we made a series of 100 ml methanol–amide solutions and pipetted 1 ml onto the underside of each leaf disk, covering the entire surface, and let the methanol evaporate. Treatments consisted of 0.17 mg (per leaf) piplartine ($N = 109$), 0.14 mg 4′-desmethylpiplartine ($N = 70$), 0.1 mg cenocladamide ($N = 79$), a mixture of the three amides (with each amide added at the same mass as for individual treatments; $N = 82$), and a control with only methanol ($N = 168$). Shrubs with ants have a mean concentration of 0.41% dry weight total amides in the leaves, thus the concentrations that we used supplemented existing amide concentrations so that they were within the range of concentrations found in leaves of plants in the field without ants (the mean value for total amides in fragments without ants is 1.74% dry weight; Dyer et al., 2001) and were based on mean dry weights of leaf disks this size (30.4 mg, $N = 20$). Caterpillars were reared at ambient temperature and light. All larvae were reared until they eclosed as adults. The status of individuals was checked each day, and mortality, the time until pupation, and pupal mass were recorded.

Chi-square tests were used to determine whether mortality and parasitism differed among each of the amide treatments and controls, and ANCOVAs were used to assess treatment effects on total weight gain, development time, and pupal mass with initial length (mm) as a covariate. Tukey's HSD studentized range tests were used to conduct *post hoc* comparisons of means for each treatment group incorporating corrections for the elevated probability of Type I error.

Experiment 4—Bioassay with Omnivorous Ant. From January 2000 to July 2001 in rainy season months (June–August, October–December), we conducted a bioassay in which we offered amides in artificial nectaries (sugar water in a 2.5 ml microcentrifuge tube) along with control nectaries to *P. clavata* colonies at La Selva. Detailed methods for this bioassay are in Dyer et al. (2003). We assessed responses of ants to piplartine (0.44 and 1.06% dry weight of the nectary), 4′-desmethylpiplartine (0.36 and 1.06%), cenocladamide (0.26 and 1.06%), and a mixture of all three amides at the lower concentration (1.06% total amide concentration). The lower amide concentrations used in these nectaries were adjusted to mimic those that are normal for *P. cenocladum* shrubs with ants at La Selva (Dodson et al., 2000; Dyer et al., 2001). The higher concentrations (i.e., 1.06%) were used in assays to assess whether the amides act additively, where 1.06% concentrations of the mixture should exhibit a similar response to 1.06% of any amide alone, or synergistically, in which case the mixture may be far more deterrent than the high concentration of any single compound. In addition to the tests

of synthetic amides, methanol extracts of *P. cenocladum* plants without ants were also tested. The response variable in this bioassay is the adjusted consumption difference (ACD), which ranges from -1 to 1, with values from 0 to 1 indicating an unpalatable extract (i.e., ants consume more control than experimental nectar) and values from 0 to -1 indicating a palatable extract (Dyer et al., 2003). Two tailed t-tests were used to determine if ACD values were significantly different from zero. The sample size for these tests was always 15 (based on the number of ant colonies, not on the number of extracts made) for each extract type.

RESULTS

Experiment 1. Spodoptera frugiperda exhibited a variety of responses to *P. cenocladum* amides, but it was clear that amides caused dramatic increase in caterpillar development time ($F_{5,171} = 96.9$, $P < 0.001$), reduction in adult mass ($F_{5,171} = 38.0$, $P < 0.001$), and decreased survival of *S. frugiperda*. In general, diets containing piplartine by itself or in any combination with other amides were the most detrimental in terms of survival, pupal mass, and development times (Figure 2). However, 4′-desmethylpiplartine also reduced pupal mass, especially in mixtures (Figure 2), and appeared to increase development time (though this was not significant at a modified Bonferroni corrected α of 0.0035). Analysis of survival was complicated by the fact that early instar mortality was high on the control diet because of drowning of early instars in condensate. However, log-rank comparisons of mortality curves beginning at day 10 showed that caterpillars reared on the mixture of all three amides, piplartine alone, and the piplartine—-4′-desmethylpiplartine mixture all experienced significantly greater mortality than those on control food (Figure 3). In contrast, those feeding on diets containing only cenocladamide experienced significantly lower probabilities of mortality (Figure 3). Regressions of days to development, pupal mass, and relative growth rates against total amide concentration were all highly significant ($F_{1,180} = 39.5$, $F_{1,157} = 63.9$, $F_{1,221} = 109.2$; all $P < 0.001$) but the variance explained was relatively low ($R^2 = 0.180, 0.289, 0.335$, respectively).

Experiment 2. Analysis of the *A. cephalotes* choice tests revealed that control and piplartine disks were the most attractive to the ants, and the piplartine by control interaction was the strongest of all effects that included the control (Figure 4, Table 1), reflecting the fact that if high numbers of control disks were taken, then high numbers of piplartine disks were also taken. The 4′-desmethylpiplartine, cenocladamide, and mixture disks were taken less frequently than controls, which was reflected in the significant interaction terms (i.e., there were higher than expected cell frequencies for control disks taken and no amide disks taken). Leaves coated with the mixture of all three *P. cenocladum* amides were removed least often by
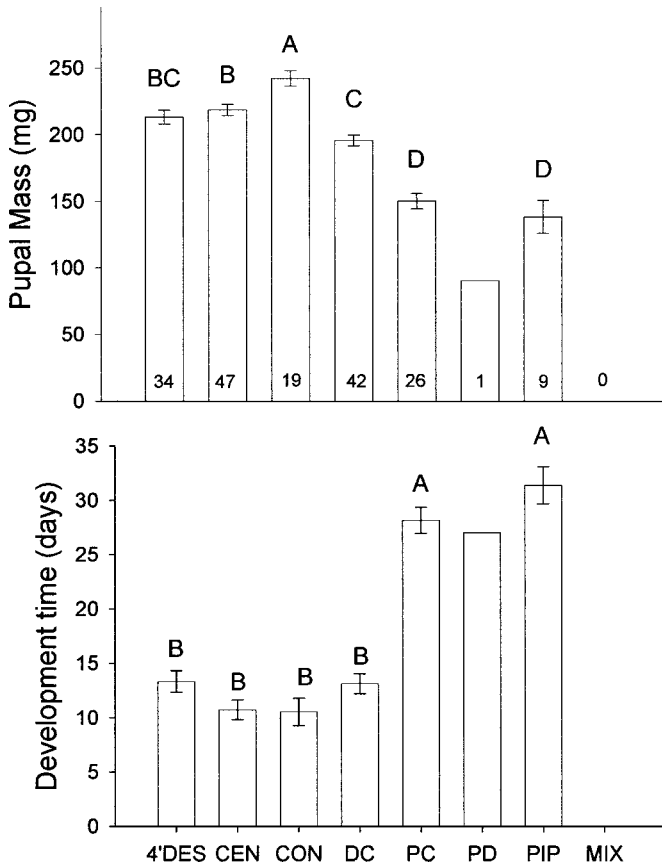
FIG. 2. *Spodoptera frugiperda* pupal mass and development times when reared on artificial diet spiked with a control solvent, a single amide compound, or combinations of the compounds. MIX = mixture; CEN = cenocladamide; CON = control; PIP = piplartine; 4′DES = 4′-desmethylpiplartine; DC = 4′DES + CEN; PC = PIP + CEN; PD = PIP + 4′DES. Numbers on the bars indicate sample sizes (not all larvae survived to pupation). Different letters indicate significant differences.

foraging ants (Figure 4), which was evident from the fact that the three mixture interaction terms contributed the most to the fit of the loglinear model (Table 1).

Experiment 3. All measures of fitness for the specialist geometrids, *Eois* spp., were significantly affected by leaf diets spiked with amides, but the mixture of amides was the only leaf diet associated with increased parasitism (16% vs. 7% or lower for controls and individual amides; $\chi^2 = 5.5$, DF = 1, $P = 0.02$), and increased mortality (65% vs. 37% or lower for controls and individual amides; $\chi^2 = 17.1$, DF = 1, $P < 0.001$). Mortality and parasitism were not different for
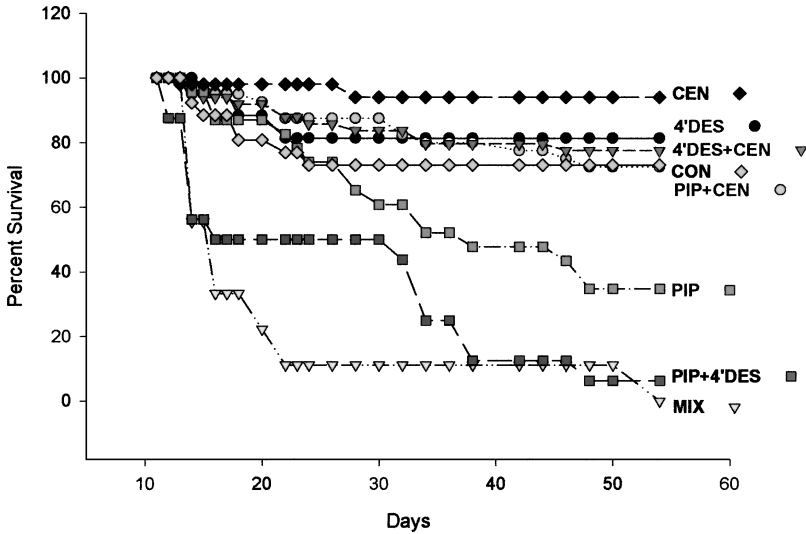
FIG. 3. Percent survival of *Spodoptera frugiperda* larvae feeding on artificial diet spiked with either a control solvent (CON), a single amide compound (piplartine (PIP), 4′-desmethylpiplartine (4′DES), cenocladamide (CEN)), or combinations of the compounds (MIX).

individual amides vs. the controls ($\chi^2 < 1$, DF $= 1$, $P > 0.05$ for all comparisons). Total weight gain, development time (days to pupation), and final pupal mass all differed among treatments (Table 2). Caterpillars feeding on leaves to which
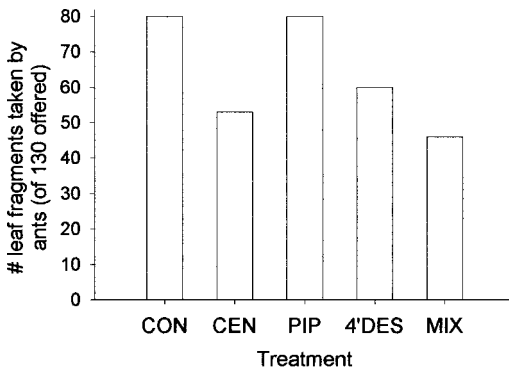

FIG. 4. Frequency of individual leaf disks taken by *Atta cephalotes* foraging ants. Disks were spiked with a control solvent, a single amide compound (piplartine, 4′-desmethylpiplartine, cenocladamide), or a mixture of the compounds.

TABLE 1. LEAF-CUTTING ANT (*Atta cephalotes*)
RESPONSES TO COMBINATIONS OF LEAF DISKS SPIKED
WITH DIFFERENT AMIDES

Source[a,b]	Parameter estimate[c]
Mixture × 4′-desmethylpiplartine	5.7064**
Cenocladamide × Mixture	5.0720**
Control × Piplartine	4.2127**
Control × 4′-desmethylpiplartine	3.8747**
Control × Cenocladamide	3.4456**
Control × Mixture	2.4048*

[a] Results are from a parsimonious log-linear model, which was a significant fit to the data ($\chi^2 = 7.5$, DF = 7, $P = 0.38$).
[b] The mixture included all three amides, piplartine, cenocladamide, and 4′-desmethylpiplartine.
[c] Parameter estimates are standardized, and P values are **$P < 0.001$, *$P < 0.02$, based on chi-square statistic and 1 degree of freedom for each.

cenocladamide was added gained half the weight of those on leaves with no amide addition. Development time exhibited a different pattern, with caterpillars feeding on piplartine laced leaves developing more slowly than those on 4′-desmethylpiplartine laced leaves (Figure 5). Finally, pupal mass was lowered on leaf diets with all amide combinations except piplartine alone (Figure 5).

Experiment 4. None of the single amide sugar solutions were deterrent to *P. clavata* ants, regardless of their concentration. In fact, concentrated solutions of cenocladamide (1.06%), appeared to be more attractive to ants than the straight sugar solution controls (Table 3). However, the sugar solution containing all three amides was avoided relative to controls, and *P. clavata* strongly avoided sugar solutions containing whole plant extracts.

TABLE 2. EFFECTS OF HEIGHTENED *P. cenocladum* AMIDE
CONCENTRATIONS ON GROWTH AND DEVELOPMENT OF SPECIALIST
Eois spp. CATERPILLARS[a]

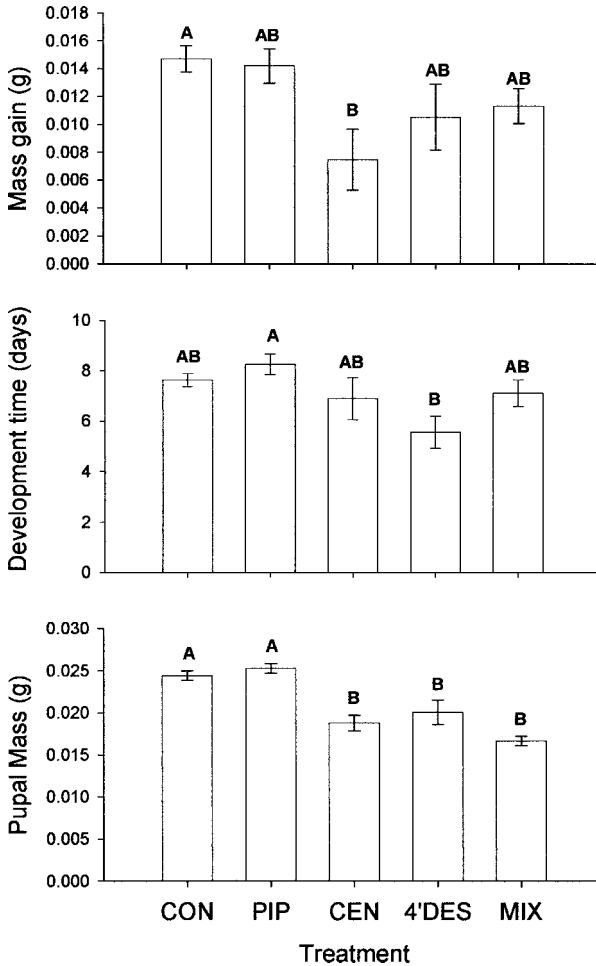| Response variable	Source	F	DF	P
Weight gain	Amide treatment	3.36	4, 260	0.0106
	Initial length	19.97	1, 260	<0.001
Development time	Amide treatment	4.03	4, 260	0.004
	Initial length	27.80	1, 260	<0.001
Pupal mass	Amide treatment	17.45	4, 313	<0.001
	Initial length	1.20	1, 313	0.274

[a] Based on survivors of 508 individual rearings.

FIG. 5. Mass gain, pupal mass, and development times of *Eois* spp. larvae feeding on leaf diet spiked with a control solvent, a single amide compound (piplartine, 4′-desmethylpiplartine, cenocladamide), or a mixture of the compounds. Different letters indicate significant differences.

DISCUSSION

Amides in *P. cenocladum* are deterrent and toxic to both specialist and generalist herbivores. We detected several responses to amides including: (1) toxicity, (2) repellency, (3) attractance, (4) developmental effects, and (5) fitness effects. The first two were synergistic, while the latter were either independent (attractance)

TABLE 3. RESULTS OF BIOASSAY USING *Paraponera clavata*
TO ASSESS THE RELATIVE PALATABILITY OF THREE SYNTHETIC
AMIDES AND THE PLANT FROM WHICH THEY ARE DERIVED

Compound (% dry mass) or plant	ACD[a]	SE[b]
Cenocladamide (1.06)	*−0.10*	0.002
Piplartine (0.44)	*−0.049*	0.004
Cenocladamide (0.26)	*−0.047*	0.01
4′-Desmethylpiplartine (1.06)	*−0.04*	0.002
Piplartine (1.06)	*−0.03*	0.002
4′-Desmethylpiplartine (0.36)	*−0.019*	0.008
Mixture of the three amides (1.06)	**0.065**	0.01
Piper cenocladum without ants	**0.3**	0.01

[a] ACD = adjusted consumption difference (described in the text). Italic
ACD values are attractant to ants while those in bold are deterrent.
[b] SE = 1 standard error of the mean.

or additive (developmental and fitness effects). The most compelling support for the synergy hypothesis came from the *P. clavata* bioassay, in which the mixture was deterrent, while individual amides at both the natural concentrations and at the increased mixture concentration were neutral or attractive to the ants. The deterrence value for the mixture was greater than three times the values (i.e., expected additive effects) for individual amides (Table 3). The strong preference of *P. clavata* for controls relative to whole plant extracts of *P. cenocladum* suggests that synergistic interactions may also occur among the three amides studied and other unidentified compounds in *P. cenocladum*.

For the caterpillar and *Atta* experiments, mixtures of amides offered to herbivores were triple the concentration of single amide treatments, suggesting that the observed effects were additive rather than synergistic. This could be true for some important response variables such as caterpillar pupal weights and development times; it is clear that individual amides do affect these attributes. However, there are several strong results that justify rejecting this alternative hypothesis for the most important variable measured—caterpillar mortality. For *Eois*, only the mixture affected parasitism and mortality, with χ^2 values being more than triple the values for individual amides. For the *Spodoptera* experiments, linear regressions of performance measures against total amide concentrations leave the majority of variance unexplained, which suggests that effects were more than additive. Furthermore, the mixture caused 100% mortality, which was more than triple the mortality of all treatments except piplartine alone and the piplartine—4′-desmethyl piplartine mixture; all the double amide treatments with cenocladamide had no effects. These results suggest that the synergy involved in *Spodoptera* mortality requires the presence of piplartine. These potent effects of amide mixtures are comparable to effects of commercial pesticides as well as other natural products. For example,

other studies using *S. frugiperda* report the same (100%) or lower (0–85%) mortality for diets spiked with compounds present at concentrations varying from 0.0025–1% dry weight (Belofsky et al., 1995; Cespedes et al., 2001; Wheeler et al., 2001).

One important example of synergy that requires comment is the work of Scott et al. (2002). They documented synergistic insecticidal effects of *Piper tuberculatum* amides against the mosquito *Aedes atropalpus* L. Our work corroborates theirs but differs in two important ways. We tested the amides of *P. cenocladum* against four different ecologically relevant herbivores instead of a human parasite. These synergistic amide mixtures probably evolved to defend *Piper* spp. from herbivores, not mosquitoes; thus, our system is relevant to the chemical ecology of *Piper* spp. Second, Scott et al. (2002) hypothesize that synergy in *P. tuberculatum* amides may be due to the presence of methylene dioxyphenyl groups (MDP). Inactive molecules containing MDP groups are commonly added to pesticides to synergistically enhance their lethality. The MDP group is known to inhibit P450 mixed function oxidases and allow the insecticide to avoid metabolic inactivation. The amides used in this study do not contain this moiety (Figure 1), thus, the synergy observed is not due to the effect of MDP groups on mixed function oxidases.

Plant chemical defense theory has generated many hypotheses on differences in responses of specialist versus generalist herbivores to specific defenses. It is interesting to consider the same hypotheses for synergy. Did synergistic compounds evolve in response to specialist herbivores that have circumvented the toxicity of individual defenses? Do the mechanisms of synergy usually involve general physiological reactions that are the same for all herbivorous and omnivorous insects? In our experiments, specialists were better equipped to circumvent the synergistic toxicity and developmental effects of the amides than generalists, nonetheless, amides functioned as a synergistic defense against specialists. While mixed diets caused the greatest mortality for both specialists and generalists, the mechanism of mortality was different; for specialists, the amides enhanced parasitism, while for the generalists, there was a more direct toxicity.

Individual, additive, and synergistic effects are possible for all categories of animals tested in this study: generalists, specialists, herbivores, and omnivores, but the mechanisms involved are likely to be different. Differences in the effects of individual *P. cenocladum* amides on the different organisms were interesting. For *S. frugiperda*, any mixture containing piplartine was detrimental, while for *A. cephalotes* and *Eois* spp., piplartine was ineffective. Cenocladamide was effective on its own against *Eois* spp. and *A. cephalotes*, but it had no effect on *S. frugiperda*. No individual compounds were effective against *P. clavata*. These differences support the idea that there is no "magic bullet" of defensive chemistry. Broad synergistic defense in combination with toxicity of individual compounds to targeted species may be the best adaptation to surviving attack by multiple species of herbivores and pathogens (but see Jones and Firn 1991 for an alternative hypothesis).

The importance of synergistic interactions among plant secondary compounds is only beginning to be explored (Berenbaum and Neal, 1985; Berenbaum et al., 1991; Kubo and Muroi 1993; Stermitz et al., 2000; Calcagno et al., 2002; Scott et al., 2002). The scarcity of examples of synergistically acting secondary compounds is likely due to the lack of research on this topic or weak statistical methods (Nelson and Kursar, 1999), and we suggest synergistic effects may be the rule rather than the exception. This may explain the apparent lack of defensive properties that have been indicated for a variety of plant secondary compounds, which have no known function (Harborne, 1988; Ayres et al., 1997) and the explanation for the redundancy observed in chemical defenses. Tests of antiherbivore (or other) activity of specific plant secondary metabolites must be supplemented with appropriate tests (e.g., Jones 1998; Nelson and Kursar 1999) of pertinent mixtures and whole plant extracts.

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