Journal of Chemical Ecology, Vol. 30, No. 5, May 2004 (© 2004)

THE EFFECT OF EXOGENOUS JASMONIC ACID ON INDUCED RESISTANCE AND PRODUCTIVITY IN AMARANTH (*Amaranthus hypochondriacus*) IS INFLUENCED BY ENVIRONMENTAL CONDITIONS

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(Received May 13, 2003; accepted January 9, 2004)

Abstract—*Amaranthus hypochondriacus* is a C_4 pseudocereal crop capable of producing reasonable grain yields in adverse environmental conditions that limit cereal performance. It accumulates trypsin inhibitors and α-amylase inhibitors in seeds and leaves that are considered to act as insect feeding deterrents. Foliar trypsin and α-amylase inhibitors also accumulate by treatment with exogenous jasmonic acid (JA) in controlled laboratory conditions. Three field experiments were performed in successive years to test if two nonphytotoxic dosages of JA were capable of inducing inhibitor activity in *A. hypochondriacus* in agronomical settings, and if this induced response reduced insect herbivory and insect abundance in foliage and seed heads. The performance of JA-treated plants was compared to insecticide-treated plants and untreated controls. The effect of exogenous JA on the foliar levels of six additional putatively defence proteins was also evaluated. Possible adverse effects of JA induction on productivity were evaluated by measuring grain yield, seed protein content, and germination efficiency. The results present a complex pattern and were not consistent from year to year. To some extent, the yearly variability observed could have been consequence of growth under drought versus nondrought conditions. In a drought year, JA-treated plants had lower levels of insect herbivory-derived damage in apical leaves and panicle than control plants, whereas in nondrought years, there was an inconsistent effect on aphids, with no effect on lepidopteran larvae. JA

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0098-0331/04/0500-1001/0

treatments reduced the size of the insect community in seed heads. The effect varied with year. Exogenous JA did not adversely affect productivity, and in the absence of drought stress, the higher dosage enhanced grain yield. Induction of defensive proteins by JA, although sporadic, was more effective in nondrought conditions. The patterns of foliar protein accumulation observed suggest that they may be part of a constitutive, rather than inducible, chemical defense mechanism that is developmentally regulated and critically dependent on the environment. The results emphasize the difficulties that are often encountered when evaluating the performance of chemical elicitors of induced resistance in field settings.

Key Words—*Amaranthus hypochondriacus*, defense proteins, drought stress, field experiments, induced resistance, insect community, jasmonic acid, productivity.

INTRODUCTION

Amaranthus species are C_4 dicotyledonous pseudocereals. They are cultivated as leaf vegetables or as grain-producing crops (Teutonico and Knorr, 1985; Kauffman and Weber, 1990). Amaranth leaves are a good source of vitamins and minerals, whereas seeds have relatively high protein contents of superior quality due to their elevated percentage of lysine and sulphur amino acids (Downton, 1973; Segura-Nieto et al., 1992). The high nutritional quality of its seed proteins, which can supplement cereal and legume proteins, and the high rates of growth allowing up to six generations per year, make amaranth an attractive alternative crop for commercial application (Rawate, 1983). In addition, many amaranth species, including *Amaranthus hypochondriacus* L., can produce reasonable yields in poor soils and/or semiarid conditions characterized by low water availability, high light intensity and temperature (Dean, 1986; A. Borodanenko, personal communication). The cultivation of *A. hypochondriacus* in México is widespread, although most of the production concentrates in unirrigated land in the states of Puebla, Tlaxcala, Morelos, and the Federal District (Espitia-Rangel, 1990). Several insect pests affect this crop in the above regions and can significantly reduce yields if not controlled. Control is usually achieved by chemical insecticides (Aragón-García and López-Olguín, 2001). Little is known about defense mechanisms, whether constitutive or inducible, that *A. hypochondriacus* might employ to reduce insect-derived damage. Previous studies reported the isolation and characterization of protease and α-amylase inhibitors in seeds that were proposed to have a defensive role against grain-infesting insects (Valdés-Rodríguez et al., 1993; Chagolla-López et al., 1994). Subsequent studies revealed that protease and α-amylase inhibitors are also present in the leaves of *A. hypochondriacus*. Moreover, these inhibitors further accumulate by foliar application of nontoxic dosages of exogenous jasmonic acid (JA), under controlled conditions of temperature and light (Nagamatsu-López, 2004; Sánchez-Hernández, 2001). This suggests that JA could be used as a chemical elicitor of induced resistance in this crop.

JA and its volatile methyl ester are found in many species, and have been identified as endogenous regulators of wound-induced chemistry in plants and as signal molecules in the responses of plants to herbivory (Reinbothe et al., 1994; Baldwin, 1999; Karban, 1999; Staswick and Lehman, 1999; Thaler, 1999a). The ability of JA to stimulate the expression of proteins putatively involved in plant defense (e.g., polyphenoloxidases, peroxidases, and protease inhibitors), has been associated with an increase of natural plant resistance in field settings. Previous results with tomato, grape vines, lettuce, and carrots indicate that exogenous JA has the potential to be a useful tool for the control of herbivores in the field (Karban, 1999; Thaler, 1999a,b,c; Omer et al., 2000; Thaler et al., 2001). As aptly indicated by Thaler (1999b), the agricultural use of chemical elicitors that have the capacity to reduce insect herbivory, such as JA, should be subjected to a careful cost/benefit analysis, weighing any advantages brought about by induced resistance against possible penalties on productivity, mostly caused by the allocation of plant resources to the synthesis of defense chemicals.

In this investigation, the use of exogenous JA to induce resistance against insect damage in field cultivated *A. hypochondriacus* was evaluated. Three consecutive experiments were performed, and the effects of JA on induced resistance were measured by monitoring changes in the insect community in foliage and seed heads. The effect of JA on the levels of several defense-related proteins in leaves and seed of *A. hypochondricus*, as well as on productivity-related variables, such as yield, seed protein content, and percent germination, was also determined.

METHODS AND MATERIALS

Field Experiments. The study was performed in the experimental fields of the Institute of Agricultural Sciences (ICA) of the University of Guanajuato, México. The fields are situated in the locality of "El Copal," municipality of Irapuato, state of Guanajuato (24°44′44″ North latitude and 101°19′19″ West longitude) at 1745 m above sea-level. The climate is classified as semiwarm subhumid, with average annual temperatures of 17.4–18.8°C and rainfall around 700 mm. The soil is alkaline (pH 8.1) with the first 30 cm layer classified as loam–sand–clay, and the second 30 cm layer as clay–loam–sand. The organic matter content of the soil was 2.32% (first 30 cm layer) and 2.80% (30–60 cm layer). Nitrogen content was 0.056% in both layers, whereas potassium and phosphorus in the first 30 cm layer were 210 ppm and 2.73 meq/100 g, respectively. Amaranth plants (*A. hypochondriacus* var. San Antonio) were grown from seed during the summer and fall of the years 2000–2002. This improved variety was developed by A. Borodanenko (ICA) and is particularly well suited for growth in the semiarid conditions prevalent in this region. The fields were fertilized with 120–60–0 kg/ha (N–P–K) starter fertilizer. In 2001 and 2002, the soil was also mixed with organic compost (2 ton/ha).

Fields were irrigated, before sowing. No additional irrigation was applied, and the experimental plants were dependent on rainfall for subsequent water. Sowing and seedling thinning were performed manually. Plants were spaced at ≈0.2 m intervals and divided into experimental units consisting of 1.56× 5 m plots having three furrows separated by 0.76 m. The field was flanked by two additional rows of plants. Each furrow had an average of 40 plants, giving a density of ≈16 plants/m^2. Fields were weeded manually throughout the experiment. In 2000 and 2001, the experimental plots were organized into blocks in which 16 different treatments were randomly distributed. Each treatment was replicated four times: (1) untreated control; (2) low JA (0.5 mM JA; ≈0.545 μmoles JA per plant); (3) high JA (1.5 mM JA; ≈1.635 μmoles JA per plant); and (4) chemical insecticide (125 ml/100 l; Rogor™; Agricultura Nacional, México). Twelve treatments consisted of a single application of low JA, high JA, or insecticide at either one of four different phenological stages. Three treatments consisted of the repeated application of low JA, high JA, or insecticide in the four stages. The phenological stages selected were young plants (15 d after seedling emergence and thinning (det)), developing plants (35 det), adult flowering plants (60 det), and senescing plants in the process of grain filling (90 det). The JA dosage applied was based on previous reports (Thaler et al., 1996; Thaler, 1999a) and on preliminary laboratory results that showed induction of trypsin and α-amylase inhibitor activities in leaves of young *A. hypochondriacus* plants sprayed with similar nonphytotoxic concentrations of JA (Nagamatsu-López, 2001; Sánchez-Hernández, 2001). JA was produced from the alkaline hydrolysis of methyl jasmonate (Sigma Chemical Co., St. Louis, MO), as described by Farmer et al. (1992). JA was dissolved in 1 ml of acetone and subsequently dispersed in an appropriate volume of distilled water containing 0.1% (v/v) of a nonionic surfactant (INEX-A® ; Cosmocel S.A., México) and buffered to ≈pH 5.0 with a pH and water hardness regulator (Buffex® ; Cosmocel S.A., México). The final volume of the solutions was adjusted throughout the experiments to ensure that different-sized plants received the same JA dosage. The chemical insecticide was similarly dispersed in identical volumes of water plus additives. Plants were sprayed with a backpack sprayer. A separate sprayer was used for each treatment. Neighboring plants were shielded from the spray with a large piece of plastic supported by wood poles. Spraying was always performed in the morning, between 8 and 10 A.M., to minimize evaporation and dispersion by wind. Leaf samples from five plants per experimental plot were collected 48 hr after the first three applications (15, 35, and 60 det); plants treated 90 det were not sampled since they already showed signs of senescence. Sampling was performed by cutting four leaves per plant from the upper third section of the plant, at different distances from the apex. These younger leaves were less damaged than older leaves. While on the field, leaf samples were temporally stored in plastic ice boxes filled with solid CO_2. Once in the laboratory, they were stored at –80˚C, ground with liquid nitrogen, and lyophilized. Lyophilized samples were utilized for biochemical assays (see below).

Effect on Insect Herbivory and the Insect Community in Foliage and Seed Heads. In 2000, folivory by lepidopteran caterpillars, mostly by *Spodoptera* sp., was determined. This was performed by visually inspecting the damage produced to the apical end of the plants, including the youngest leaves and the nascent and developing panicule. Sampling was performed thrice, on the 4th and 27th day of August and on the 9th day of September. In the first sampling, 60 plants per treatment, from one replicate only, were examined, whereas in the latter two, 60 plants per treatment in all four replicates were examined, giving a total of 2160 observations. Damage was scored according to a scale involving four different levels: (I) low damage (0–10% of plant area damaged); (II) moderate damage (10–25% of plant area damaged), (III) extensive damage (25–50% of plant area damaged); and (IV) severe damage ($>$50% of plant area damaged).

To sample the insect community in seed heads, five seed heads per experimental plot were collected 2–3 wk after the last spaying (90 det), giving a total of 20 seed heads per treatment per year. The sampling procedure was performed by quickly covering each randomly chosen grain head with a large transparent plastic bag, which was subsequently severed from the rest of the plant by bending at its base. This was done in order to trap the insects present in the seed heads during the seed maturation stage. The bags were frozen at -20°C (year 2000) or stored in a well-ventilated room after their treatment with tablets of aluminium phosphide (Agro-Fum^{TM} 57; Centro Agroindustrial S.A., México; year 2001), prior to insect collection, counting, and storage (in 30% (v/v) aqueous ethanol solutions). Grain was harvested and cleaned with an air stream (mid November and early December). Yields of grain per hectare were extrapolated from yields produced by the experimental 7.8 m^2 plots. Samples of grain were stored at 4°C until required for biochemical and protein content analyses and germination assays (see below).

A third field experiment, following a similar design, was performed in 2002. In this experiment, only the high dosage of JA (1.5 mM) was repeatedly applied, at approximately the same four developmental stages, and was compared to insecticide-treated and control plants. No leaf or seed samples were taken for biochemical assays and productivity measurements, nor was yield determined. However, plants were sampled five times to determine the abundance of lepidopteran caterpillars and aphids infesting the foliage. Sampling was performed 1 wk after each of the first four sprayings. The final sampling was performed 2 wk after the last spraying. Because of the low level of lepidopteran caterpillar infestation in 2002, all experimental plants were examined on each sampling date, and an additional sixth sampling was performed close to the end of the growing season. All sampled caterpillars were taken alive to the laboratory where they were monitored for parasitism or allowed to develop into adults to facilitate identification (results not shown). Sampling for aphid abundance in foliage was performed by thoroughly searching the abaxial surface of all leaves of four plants per experimental plot. A total number of 16 plants per treatment per sampling date were examined. Aphid

specimens were also taken to the laboratory for identification. For insect counts in seeds heads, 12 seed heads per experimental plot were sampled, as above. A higher number of samples per plot was taken to compensate for the fact that only two replicates were sampled this year. A total of 24 seed heads per treatment were sampled. Insects were aspirated directly from the plastic bags used to trap them by means of portable insect Vacs (BioQuip®, Gardena, CA). They were subsequently frozen at -20°C and placed in 30% ethanol solutions prior to identification. Identification was done by M.D. Salas, at the Entomology Laboratory of the Institute for Agricultural Sciences (ICA) of the University of Guanajuato, México. For analysis, the insect community in seed heads was divided into phloem feeding (PF) insects, chewing (CH) insects, and predaceous and parasitoid (PP) insects.

Meteorological Conditions. Experiments were performed from late spring to late autumn of the years 2000, 2001, and 2002. Seeds were sown in the 2nd week of June (year 2000) and 4th week of June (years 2001 and 2002). Total monthly rainfall, average insolation and evaporation per month, and maximum, mean, and minimum monthly values for relative humidity and ambient temperature were recorded by a weather station manned by the ICA of the University of Guanajuato (Appendix). The station is situated less than 1 km from the experimental fields.

Enzyme and Inhibitor Assays. Crude extracts were prepared by mixing lyophilized leaf samples or ground seed flour samples in appropriate buffers (see below). Leaf extracts were employed to measure the levels of activity of the following putative defense-related proteins: trypsin inhibitors, chymotrypsin inhibitors, α-amylase inhibitors, polyphenol oxidases, peroxidases, leucine aminopepetidases, β-1,3-glucanases, chitinases, and polygalacturonases. Seed extracts were used to measure trypsin, chymotrypsin, and α-amylase inhibitor activity. Trypsin and chymotrypsin inhibitor levels of activity (TIA and CTIA) were determined according to Erlanger et al. (1961) and Gervaix et al. (1991), using $N\alpha$-benzoyl-L-arginine-*p*-nitroanilide hypochloride (BApNA) and *N*-benzoyl-L-tyrosine-*p*-nitroanilide (BTpNA) as substrates, respectively. Alpha amylase inhibitor activity (AAIA) was measured according to Bird and Hopkins (1954), using starch as substrate. TIA, CTIA, and AAIA were determined as inhibitor units per milligram of dry weight. Leucine aminopeptidase activity (LAPA) was determined according to Appel (1974), using leucine-*p*-nitroanilide as substrate. Polyphenol oxidase (PPO) and peroxidase (PRX) activities were measured according to Thaler et al. (1996), using caffeic acid and guaiacol as substrates, respectively. Polygalacturonase activity (PGA) was assayed according to Gross (1982), based on the measurement of reducing sugars released from the hydrolysis of polygalacturonic acid. Chitinase activity (CHIA) was determined according to Villagómez-Castro et al. (1992), using 4-methylumbelliferyl-β-D-$N,N^{\mathrm{I}},N^{\mathrm{II}}$-triacetylchitotrioside hydrate as substrate. β-1,3-Glucanase activity (BGA) was assayed according to Zheng and Wozniak (1997) using laminarin as substrate. All activity assays, except the chitinase assay, were modified to fit a microplate format. The activity of foliar proteins

(except enzyme inhibitors) was calculated per milligram total protein. Protein content was measured according to the Bradford method (Bradford, 1976), employing a commercial kit (Bio-Rad Laboratories, USA). The enzyme controls employed were trypsin and chymotrypsin from bovine pancreas, laminarinase from *Trichoderma* sp., peroxidase from horseradish, tyrosinase from mushroom, polygalacturonase from *Aspergillus japonicus*, and chitinase from *Streptomyces griseus* (all from Sigma-Aldrich Chemical Co., USA). Alpha-amylases and leucine aminopeptidases were extracted from larvae of the red flour beetle (*Tribolium castaneum* Herbst) and the large grain borer (*Prostephanus truncatus* Horn), respectively (Sandoval-Cardoso, 1991; Chagolla-López et al., 1994). All enzyme substrates employed were also from Sigma-Aldrich.

Protein and Germination Assays. Seed protein content was determined by multiplying total seed nitrogen content by a 5.85 conversion factor (Becker et al., 1981). Seed nitrogen content was determined using the Kjeldahl method (AOAC, 2000). To evaluate percent germination, 100 seeds per treatment per replicate were surface sterilized by washing in a 1% solution of sodium hypochlorite for 10 min. The seeds were subsequently rinsed with running water to eliminate excess hypochlorite and placed on humidified filter paper contained in sterile petri dishes. Seeds were incubated in darkness at 28°C for 2 d. Germination was scored by counting the number of germinated seeds, and they were classified into five groups based on seedling length (0–1, 1–2, 2–3, 3–4, and 4–5 cm, respectively) in order to determine seed vigor. All assays were repeated at least thrice.

Statistical Analysis. Herbivory by noctuid larvae in 2000 was analyzed to determine if the degree of damage was independent of the treatment applied (null hyphothesis). To test this, data were cross-tabulated, and the chi-square (χ^2) test was applied to the resulting table (Sokal and Rohlf, 1969). To understand further the relation between level of damage and treatment, the cross-tabulated data were analyzed taking into account all possible pairs of treatments, resulting in each case in a 2×4 contingency table, to which the χ^2 test was applied. To test for differences in aphid and lepidopteran larvae counts in foliage in 2002, a general lineal model, assuming a Poisson distribution for the number of insects, was fitted using treatment (for aphids and larvae) and sampling date (for aphids) as explanatory variables. Data were analyzed to test for independence of treatment and sampling date on the insect counts obtained. For aphid counts in each sampling date, all pairs of treatments were contrasted and subjected to analyses of deviance. For insect counts in seed heads, an analysis of deviance was performed independently for each insect community. Treatment, Year, and Treatment $\times$ Year interaction were used as explanatory variables. In each Year and for each insect community, all pairs of treatments were contrasted and subjected to analyses of deviance. In each case, χ^2 tests were applied to test for independence between insect counts and treatment.

Chemical and plant productivity variables were examined by analyses of variance (ANOVA). For seed chemistry and plant productivity variables, the overall

effect of treatment, year, and its interaction were analyzed. For foliar chemistry, the overall effect of treatment, year, and development stage, as well as their interactions were analyzed. A separate ANOVA was performed per year to test the effect of treatment on seed chemistry and productivity variables, and an additional ANOVA was performed per year to test the effect of treatment on foliar chemistry at each development stage.

The values of the F statistic, as well as the probability of that value under the null hypothesis of equality of treatments, are reported in the results. For ANOVA tables where the F test was significant at 0.05 or lower, the Tukey method was used to obtain 95% simultaneous confidence intervals for the differences among treatment means. All data were analyzed by using the S-plus statistical package (S-PLUS Professional Edition, Version 6.0.2 Release 1 for Microsoft Windows).

RESULTS

In 2000 and 2001, the complete field experiments consisted of 16 different treatments; in 12 of these JA, low (0.5 mM) or high (1.5 mM), or insecticide were applied only once in each of four phenological stages. The effects observed did not differ much from those obtained from plants in which sprayings were applied repeatedly in all four stages. Therefore, for clarity, only those treatments in which JA or insecticide were applied continuously are included here. A simplified experiment was performed in 2002; only the high JA dosage was used, which was applied four times throughout the growing season, and the effects were likewise compared to those obtained in insecticide-treated and untreated controls.

Effect of Exogenous JA on Herbivory by Noctuid Caterpillars. Plants were sampled three times through August and September 2000 to test for caterpillar damage, in the apical section of the plant, which included the youngest leaves and the emerging and developing panicles. The sampling period coincided with the onset and development of flowering and with a high population density of noctuid larvae. A χ^2 test applied to the cross-tabulated data derived from the 2160 observations (Table 1) was highly significant ($\chi^2 = 25.42$, $P = 0.002$), indicating that the levels of damage were dependent on treatment. A χ^2 test, taking into account all possible pairs of treatments (Table 2), indicated that the dependence observed between damage level and treatment could be attributed to the heterogeneity of effect between untreated controls (more damaged) and the insecticide and JA treatments (less damaged). No significant difference in herbivory was found between insecticide- and JA-treated plants, nor between the two dosages of JA employed.

Effect on Abundance of Insect Herbivores in Foliage. The number of lepidopteran larvae and aphids infesting the foliage of *A. hypochondriacus* plants was monitored in 2002. In contrast to the two previous years, the level of infestation by

TABLE 1. NUMBER OF *A. hypochondriacus* PLANTS CROSS-CLASSIFIED BY LEVEL OF DAMAGE BY LEPIDOPTERAN LARVAE[a] AND TREATMENT[b] (YEAR 2000)

	Level of damage				
Treatment	Low (0–10%)[c]	Moderate (10–25%)[c]	Extensive (25–50%)[c]	Severe (>50%)[c]	Total
Control	453	36	38	13	540
JAL	479	35	15	11	540
JAH	487	28	20	5	540
INS	489	25	14	12	540
Total	1908	124	87	41	2160

[a] Damage produced by lepidopteran larvae was evaluated in apical leaves and panicles.
[b] Cultivated plants were untreated (control) or treated with insecticide (INS) or with 0.5 mM (JAL) or 1.5 mM jasmonic acid (JAH).
[c] Percent area damaged.

lepidopteran larvae was low. Consequently, six independent samplings of all experimental plants yielded a total of 18, 14, and 10 specimens in untreated controls, JA-treated, and insecticide-treated plants, respectively. Considering the scarcity of lepidopteran insects, all larval counts were combined and analyzed as one sample. The difference in larval abundance between treatments was not statistically different ($\chi^2 = 0.00$, $P = 0.313$). Conversely, aphid numbers in foliage were significantly affected by treatment (T) ($\chi^2 = 345.5$, $P < 0.001$), sampling date (S) ($\chi^2 = 313.82$, $P < 0.001$), and T $\times$ S interaction ($\chi^2 = 243.53$, $P < 0.001$). Highly significant differences were detected when aphid counts were analyzed per sampling date (Table 3, Figure 1). JA treatment significantly reduced aphid numbers only on the first sampling date (08/13/2002), whereas aphid counts were higher than controls on all other sampling dates, except the last one (10/8/2002). Curiously, insecticide treatment did not reduce aphid numbers below levels found

TABLE 2. CHI-SQUARE TEST FOR INDEPENDENCE OF ALL TREATMENT PAIRS ON DEGREE OF DAMAGE PRODUCED BY LEPIDOPTERAN LARVAE IN *A. hypochondriacus* PLANTS (YEAR 2000)[a]

Treatment contrast	χ^2	P
Control vs. INS*	14.47	0.002
Control vs. JAL*	10.88	0.012
Control vs. JAH*	11.37	0.001
INS vs. JAL	1.85	0.605
INS vs. JAH	4.12	0.249
JAL vs. JAH	3.00	0.283

[a] Data from Table 1 were used for the analysis.
*Significant differences at $P < 0.05$

TABLE 3. ANALYSIS OF DEVIANCE OF CONTRASTS AMONG TREATMENT PAIRS ON APHID ABUNDANCE PER SAMPLING DATE IN FOLIAGE OF UNTREATED *A. hypochondriacus* PLANTS (C) OR PLANTS TREATED WITH INSECTICIDE (INS) OR A HIGH (1.5 MM; JAH) DOSAGE OF JASMONIC ACID

Sampling date	Treatment contrast	df	Deviance residual	df	Residual deviance	*P*
08/13/2002	Null			7	51.62	0.002
	C vs. JAH	1	9.33	6	42.29	
	Null			7	39.26	0.536
	C vs. INS	1	0.38	6	38.87	
	Null			7	19.20	0.015
	INS vs. JAH	1	5.97	6	13.22	
08/27/2002	Null			7	47.91	<0.001
	C vs. JAH	1	18.29	6	29.62	
	Null			7	52.34	0.021
	C vs. INS	1	5.31	6	47.03	
	Null			7	57.61	0.042
	INS vs. JAH	1	4.14	6	54.46	
09/10/2002	Null			7	55.60	<0.001
	C vs. JAH	1	13.41	6	42.20	
	Null			7	58.87	0.746
	C vs. INS	1	0.11	6	58.76	
	Null			7	50.49	<0.001
	INS vs. JAH	1	11.2	6	39.29	
09/24/2002	Null			7	23.60	0.050
	C vs. JAH	1	3.86	6	19.74	
	Null			7	54.76	<0.001
	C vs. INS	1	30.61	6	24.15	
	Null			7	28.28	<0.001
	INS vs. JAH	1	13.15	6	15.13	
10/08/2002	Null			7	20.49	0.893
	C vs. JAH	1	0.018	6	20.47	
	Null			7	36.89	0.021
	C vs. INS	1	5.33	6	31.56	
	Null			7	25.19	0.015
	INS vs. JAH	1	5.96	6	19.23	

in controls and clearly promoted aphid abundance towards the end of the sampling period (Table 3, Figure 1).

Effect on the Insect Community in Seed Heads. A total of 38 different insect species were identified in seed heads of *A. hypochondriacus* sampled in three consecutive growing seasons. They were identified at least to family, and to species when possible. For the analysis of the community in seed heads, all insects were further divided into three groups: phloem feeding (PF), chewing (CH), and predaceous and parasitoid (PP) insects (Table 4). An analysis of deviance indicated that the abundance of the three communities in seed heads was dependent on

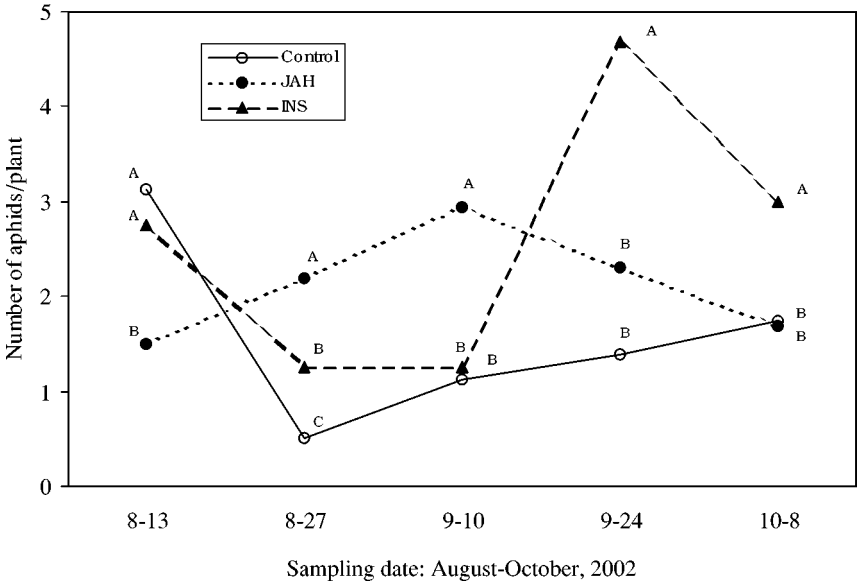


FIG. 1. Aphid abundance in foliage of untreated, field grown *A. hypochondriacus* plants (control) and plants treated with 1.5 mM jasmonic acid (JAH) or insecticide (INS). Different letters above the symbols in each sampling date represent statistically different aphid counts (at $P < 0.05$), obtained after applying a χ^2 test to all contrasted pairs of treatments (see Table 3).

the effect of time (T), year (Y), and its interaction (Table 5). PF and PP insects were most abundant in 2000, whereas CH insects were in 2001. An analysis of deviance of contrasts among all treatment pairs (Table 6) revealed that in 2000, JA-treated plants had the lowest counts of PF insects, which were significantly different from those found in untreated controls and insecticide-treated plants. In 2000, the effect of JA dosage was also significant, with low JA-treated plants having the smallest PF insect counts. This behavior was partially repeated in 2002, year in which high JA-treated plants had lower PF insect counts than controls (but higher than insecticide-treated plants). In contrast, no significant differences in PF insect counts between control plants and JA-treated plants (high and low) were detected in 2001 (Table 6, Figure 2a). CH insect counts were lower than controls in JA-treated plants only in 2000; in 2001 and 2002, no significant differences between controls and JA-treated plants were detected (Table 6, Figure 2b). With respect to PP insects, low JA-treated plants had lower counts than controls and high JA-treated plants in 2000. Similar to PF and CH insects, PP insect counts in seed heads of JA-treated plants were not different from untreated controls in 2001, whereas in 2002, high JA-treated plants had lower PP counts than controls (Table 6,

TABLE 4. INSECT SPECIES COLLECTED IN SEED HEADS OF *Amaranthus hypochondriacus* DURING THREE CONSECUTIVE FIELD EXPERIMENTS (2000 TO 2002) IN IRAPUATO, GTO., MEXICO

	Year		
	2000	2001	2002
Phloem feeding insects			
Hemiptera			
Miridae			
Lygus lineolaris (Palisot de Beauvois)	237	240	95
Litomiris debilis (Uhler)			21
Pentatomidae			
Euschistus sp.	37	61	
Holcosthethus sp.	54		
Brochymena arborea (say)	23		
Undetermined		32	
Coreidae			
Ceraleptus sp.	133	108	33
Catorhintha sp.		94	
Thyreocoridae			
Galgupha sp.	63		
Corimelaena sp.	20	21	
Lygaeidae			
Oedancala sp.	1372	98	
Tingidae			
Physatocheila sp.	34		83
Undetermined			16
Largidae			
Largus succinctus (L.)		4	
Homoptera			
Membracidae			
Micrutalis sp. + *M. malleifera* (Fowler)	181	140	45
Cicadellidae			
Undetermined	13		
Chewing Insects			
Coleoptera			
Cleridae			
Undetermined	20		
Chrysomelidae			
Diabrotica balteata (Le Conte)		24	19
D. undecinpunctata (Mannerheim)		9	
Epitrix cucumeris (Harris)			8
Disonycha sp.	21	10	
Gastrophysa sp.			32
Bruchidae			
Acanthoscelides obtectus (Say)	19	3	
Phalacridae			
Undetermined		5	

TABLE 4. CONTINUED

	Year		
	2000	2001	2002
Tenebrionidae			
Undetermined		68	
Dermoptera			
Forficulidae			
Doru taenatium (Dorhn)	18	125	35
Lepidoptera			
Pyralidae			
Undetermined	20		
Hymenoptera			
Eurytomidae			
Harmolita tritici (Fitch)			13
Predaceous and parasitoid insects			
Hemiptera			
Anthocoridae			
Orius insidiosus (say)	12		49
Pentatomidae			
Podisus maculiventris (say)	19		
Saldidae			
Undetermined	77		
Coleoptera			
Coccinelidae			
Hippodamia convergens (Guerin)	13		19
Epilachna tredecimnotata (Latreille)		2	
Scymnus sp.	197		
Carabidae			
Lebia viridis (Say)	34	15	
Lampiridae			
Undetermined		3	
Hymenoptera			
Eulophidae			
Undetermined			4
Formicidae			
Formica sp.			13

Figure 2c). The results also indicate that the PP component of the seed head insect community in *A. hypochondriacus* was particularly sensitive to insecticide treatment (Figure 2c).

Effect on Seed and Leaf Chemistry and Plant Productivity. Seed TIA and AAIA levels and yield were affected by the overall effect of T, Y, and its interaction. CTIA was affected by T and Y, whereas the effect of Y was significant for seed protein content and germination efficiency (Table 7). The effect of T was subsequently analyzed separately each year. The results are summarized in

TABLE 5. ANALYSIS OF DEVIANCE OF THE EFFECT OF TREATMENT, YEAR, AND ITS INTERACTION ON THE INSECT COMMUNITY IN SEED HEADS OF UNTREATED *A. hypochondriacus* PLANTS[a]

Variable	Factor	df	Deviance residual	df	Residual deviance	*P*
Phloem feeders	Null			43	4755.21	
	Treatment (T)	3	1102.51	40	3652.69	<0.001
	Year (Y)	2	1483.06	38	2169.63	<0.001
	T×Y	5	404.50	33	1765.14	<0.001
Chewing insects	Null			43	280.56	
	Treatment (T)	3	32.04	40	248.51	<0.001
	Year (Y)	2	67.87	38	180.64	<0.001
	T×Y	5	15.28	33	165.35	0.009
Predators	Null			43	529.69	
and parasitoids	Treatment (T)	3	69.10	40	460.58	<0.001
	Year (Y)	2	788.21	38	172.27	<0.001
	T×Y	5	10.62	33	161.55	0.059

[a] The analysis includes data from three consecutive field experiments (2000 to 2002).

Table 7 and Figure 3. In 2000, JA-treated plants had higher levels of TIA (high JA, Figure 3b) and AAIA (low JA, Figure 3d) than untreated control plants. On the other hand, JA-treated plants (low JA) produced lower yields than insecticide-treated plants (Figure 3a). In 2001, differences were obtained only in plants treated with high JA: these produced higher grain yields than insecticide-treated plants (Figure 3a) and higher levels of AAIA than low JA-treated and control plants (Figure 3d). The highly significant effect that Y had on seed chemistry and plant productivity variables was evident in the differences detected between both years. Thus, in 2001, overall seed protein content was reduced 20%, seed yield was doubled, seed germination efficiency increased from 64.4 to 83.7%, TIA and AAIA levels were reduced 3- and 1.4-fold, respectively, and CTIA levels increased 5.3-fold.

The ANOVA shown in Table 8 indicates that most foliar protein activity levels were affected by Y, development stage (DS), and their interaction. The exceptions were LAPA, which was only affected by Y and Y × DS interaction, and PRX and PPO activities, which were not detected at all (results not shown). The overall effect of T was significant for CTIA, BGA, and CHIA, whereas the levels of TIA, BGA, and CHIA were affected by the T × DS interaction. Only BGA and AAIA levels were affected by the T × Y × DS interaction.

The effects of treatment on foliar chemistry were also analyzed separately per year (Table 9 and Figure 4). They indicate that in 2000, exogenous JA treatments produced few significant differences in foliar protein activity levels, which were never detected in young plants. Thus, the only significant changes detected in JA-treated plants, with respect to untreated controls and/or insecticide-treated plants,

TABLE 6. ANALYSIS OF DEVIANCE OF CONTRASTS AMONG TREATMENT PAIRS ON THE INSECT COMMUNITY IN SEED HEADS OF UNTREATED *A. hypochondriacus* PLANTS (C) OR PLANTS TREATED WITH INSECTICIDE (INS) OR WITH LOW (0.5 mM; JAL) OR HIGH (1.5 mM; JAH) DOSAGES OF JASMONIC ACID

		Year 2000				Year 2001				Year 2002			
Variable	Treatment contrast	df	Deviance residual	df	Residual deviance	*P*	df	Deviance residual	df	Residual deviance	*P*	df	Deviance residual	df	Residual deviance	*P*
Phloem feeders	Null			7	2357.44	<0.001			7	71.56	<0.001			7	90.24	<0.001
	C vs. INS	1	758.13	6	1599.31		1	28.14	6	43.42		1	64.99	6	25.25	
	Null			7	2229.79	<0.001			7	53.17	0.226			7	41.00	<0.001
	C vs. JAH	1	865.89	6	1363.9		1	1.46	6	51.70		1	11.45	6	29.55	
	Null			7	2405.61	<0.001			7	27.16	0.547					
	C vs. JAL	1	1009.00	6	1396.51		1	0.36	6	26.79				n.d.		
	Null			7	255.47	0.038			7	74.52	<0.001			7	34.64	<0.001
	INS vs. JAH	1	4.32	6	251.15		1	16.84	6	57.78		1	22.75	6	11.88	
	Null			7	305.93	<0.001			7	67.70	<0.001					
	INS vs. JAL	1	22.17	6	283.76		1	34.83	6	32.87				n.d.		
	Null			7	55.3	0.008			7	44.44	0.070					
	JAH vs. JAL	1	6.96	6	48.55		1	3.29	6	41.16				n.d.		
Chewing insects	Null			7	20.44	0.162			7	88.22				7	28.52	<0.001
	C vs. INS	1	1.95	6	18.49		1	18.11	6	70.11	<0.001	1	15.93	6	12.58	
	Null			7	28.23	0.009			7	86.83				7	26.73	0.753
	C vs. JAH	1	6.91	6	21.37		1	0.75	6	86.08	0.386	1	0.10	6	26.63	
	Null			7	13.91	0.009			7	76.06						
	C vs. JAL	1	6.91	6	7.01		1	2.37	6	73.70	0.124			n.d.		
	Null			7	33.02	0.215			7	36.41				7	31.01	<0.001
	INS vs. JAH	1	1.54	6	31.48		1	11.57	6	24.85	<0.001	1	13.59	6	17.42	
	Null			7	18.65	0.215			7	19.95						
	INS vs. JAL	1	1.55	6	17.11		1	7.48	6	12.47	0.006			n.d.		

TABLE 6. CONTINUED

Variable	Treatment contrast	Year 2000					Year 2001					Year 2002				
		df	Deviance residual	df	Residual deviance	*P*	df	Deviance residual	df	Residual deviance	*P*	df	Deviance residual	df	Residual deviance	*P*
	Null			7	20	1.000			7	28.89						
	JAH vs. JAL	1	0.00	6	20		1	0.05	6	28.44	0.502			n.d.		
Predators	Null			7	99.08	<0.001			7	8.88	0.160			7	45.14	<0.001
and parasitoids	C vs. INS	1	29.06	6	70.02		1	1.97	6	6.90		1	35.66	6	9.47	
	Null			7	80.4	0.593			7	2.88	0.295			7	17.31	0.017
	C vs. JAH	1	0.29	6	80.12		1	1.10	6	1.78		1	5.69	6	11.62	
	Null			7	48.31	<0.001			7	3.68	0.819					
	C vs. JAL	1	17.38	6	30.93		1	0.05	6	3.63				n.d.		
	Null			7	129.06	<0.001			7	13.95	0.015			7	19.91	<0.001
	INS vs. JAH	1	23.68	6	105.57		1	5.88	6	8.06		1	13.68	6	6.23	
	Null			7	57.75	0.213			7	12.56	0.103					
	INS vs. JAL	1	1.55	6	56.19		1	2.33	6	9.91				n.d.		
	Null			7	79.54	<0.001			7	5.46	0.413					
	JAH vs. JAL	1	13.25	6	66.29		1	0.67	6	4.79				n.d.		

Note. n.d. = not determined.

were in AAIA (Figure 4c), and in PGA (Figure 4e) in developing plants (35 det), and in CHIA (Figure 4g) in mature plants (60 det). All other differences detected involved control and insecticide-treated plants (Figure 4a, b, and g).

In 2001, the number of differences detected in JA-treated plants increased slightly. In contrast to the previous year, most differences were observed in young and developing plants. Accordingly, significant changes in JA-treated plants, with respect to controls and/or insecticide-treated plants, were detected in CHIA in young plants (Figure 4f), AAIA and BGA in young and mature plants (Figure 4c and g), and TIA and CTIA in developing plants (Figure 4a and b).

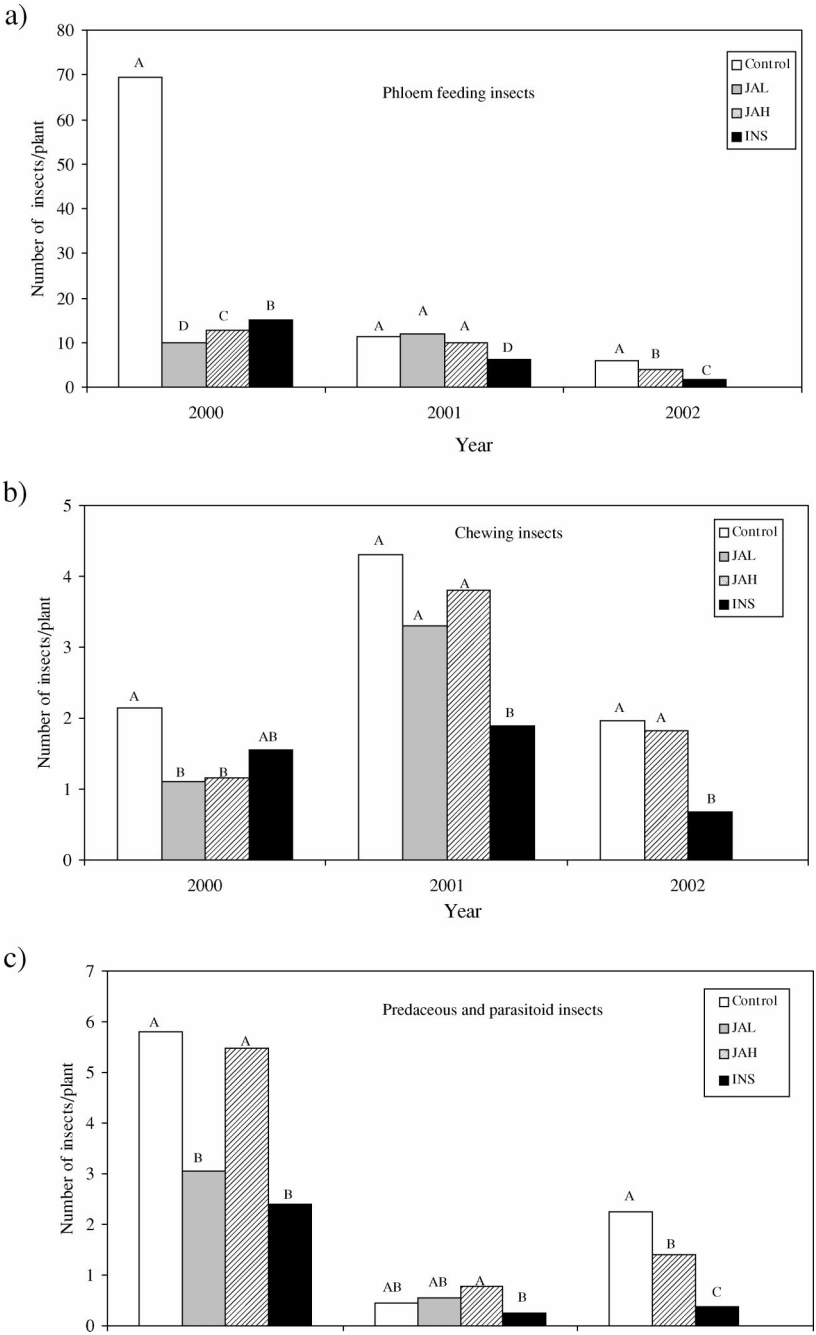
The effects of Y, DS, and Y $\times$ DS interaction on foliar protein activity are seen in Figure 4. Except for PGA levels (Figure 4e) that increased concomitantly with development to reach a maximum point in mature plants in both years, all other foliar protein activities showed variations between year and development stage, having in some cases (e.g., LAPA and CTIA) completely opposite patterns of accumulation during development.

The most pronounced changes in overall foliar protein activity levels detected between years involved a 4.8-fold reduction in TIA levels in mature plants in 2001, and 11.7- and 4.1-fold increases in CHIA levels in leaves of young and mature plants, respectively.

DISCUSSION

The results present a complex pattern and were not consistent from year to year. Nevertheless, they indicate that exogenous JA (1) significantly affected the insect community in *A. hypochondriacus*, (2) had no negative effect on productivity, and (3) had only a sporadic effect (both enhancing and inhibitory) on foliar and seed protein activities. Furthermore, environmental conditions and ontogeny were significant factors affecting most of the chemical variables analyzed. Thus, it is likely that multiple biotic and abiotic stimuli produced responses on *A. hypochondriacus* plants that were superimposed with those induced by exogenous JA.

In 2000, JA-treated plants had lower levels of damage by noctuid larvae that feed on the young leaves and panicle of the apical portion of *A. hypochondriacus* plants (Tables 1 and 2). JA treatment also reduced the number of PF insects in seed heads in 2 years of the 3-year study, particularly in 2000. CH insects were also negatively affected by JA treatments in 2000 (Table 6, Figure 2a and b). However, the observed negative effect on insects considered to be important pests of *A. hypochondriacus* in the Irapuato area and other amaranth producing regions of México (see Table 4; Salas-Araiza, 1999; Aragón-García and López-Olguín, 2001) was not translated into larger grain yields in 2000 (Figure 3a). Moreover, high JA-treated plants produced higher yields than controls and insecticide-treated plants in 2001, a year in which no effects on PF and CH insects in seed heads were


a)
Phloem feeding insects
Number of insects/plant
Year
2000
2001
2002
Control
JAL
JAH
INS
b)
Chewing insects
Number of insects/plant
Year
c)
Predaceous and parasitoid insects
Number of insects/plant

detected (Table 6, Figure 2a and b). The lack of any benefits, in terms of yield, in *A. hypochondriacus* plants showing an induced resistance response mediated by exogenous JA, suggests that this species is tolerant to insect herbivory and, therefore, that productivity may not be seriously affected by insect-derived damage. In tomato plants, the lack of a positive effect on yield in JA-treated plants showing increased levels of defense-related proteins and lower levels of herbivory and herbivore numbers was also observed. In that case, however, this effect was attributed to nondamaging levels of herbivores during experimentation rather than to an inherent tolerance to herbivory in tomato (Thaler, 1999a,b; Thaler et al., 2001).

The above suggests that JA was able to induce chemical changes in *A. hypochondriacus* that made plants less attractive to lepidopteran larvae and PF and CH insect pests. However, the lack of a consistent induction of any of the defense-related proteins analyzed, especially in 2000, when the negative effect was more pronounced, strongly suggests that other unidentified JA-induced chemicals could have been responsible for the effect observed. Thus, *A. hypochondriacus* might rely, similar to barley and sorghum, on chemical compounds such as alkaloids and phenolics for defense (Corcuera, 1993), which might have accumulated, as has been observed in several other plant species, in response to exogenous JA (Blechert et al., 1995; Memelink et al., 2001). This is in contrast to other plants, such as tomato, in which a causal link between JA-induced accumulation of proteins and a reduction in herbivore performance has been firmly established (Orozco-Cardenas et al., 1993; Felton et al., 1994; Stout et al., 1994; Thaler et al., 1996; Cipollini and Redman, 1999; Thaler 1999a,b).

On the other hand, the results obtained from exogenous JA may be related to the finding that insecticide treatment either reduced, or had a tendency to show the lowest levels in most of the foliar proteins tested. This was probably an indication that insect herbivory was an important inducing factor and that JA treatment was incapable of further increasing levels above those induced by insect damage. Another possible scenario is that the foliar proteins assayed in amaranth were insensitive to exogenous JA due to their constitutive and developmentally regulated pattern of accumulation discussed below.

←

FIG. 2. The abundance of (a) phloem feeding insects, (b) chewing insects, and (c) predaceous and parasitoid insects in seed heads of untreated, field grown *A. hypochondriacus* plants (control) and plants treated with insecticide (INS) or with low (0.5 mM, JAL) or high (1.5 mM, JAH) dosages of jasmonic acid (JA). The results obtained from seed heads sampled in three consecutive field experiments are shown (years 2000–2002). Different letters above the bars represent significantly different counts (at $P < 0.05$), obtained after applying a χ^2 test to all contrasted pairs of treatments (see Table 6).

TABLE 7. ANOVA OF THE EFFECT OF TREATMENT, YEAR, AND ITS INTERACTION ON CHEMICAL AND PRODUCTIVITY RELATED VARIABLES IN SEED OF *A. hypochondriacus* PLANTS SUBJECTED TO FOUR DIFFERENT TREATMENTS (0.5 mM JA, 1.5 mM JA, INSECTICIDE, AND UNTREATED), DURING TWO CONSECUTIVE FIELD EXPERIMENTS (2000 AND 2001)

	TIA			CTIA			AAIA			Yield			Protein content (%)			Germination (%)		
Factor	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Treatment (T)	3	3.19	0.044	3	3.34	0.037	3	5.39	0.006	3	4.16	0.017	3	n.s.		3	n.s.	
Year (Y)	1	191.13	<0.001	1	1910.90	<0.001	1	51.11	<0.001	1	75.63	<0.001	1	215.77	<0.001	1	47.10	<0.001
T×Y	3	6.89	0.002	3	n.s.		3	6.54	0.002	3	6.82	0.002	3	n.s.		3	n.s.	
Residuals	22			23			24			24			24			23		

Note. TIA = trypsin inhibitor activity; CTIA = chymotrypsin inhibitor activity; AAIA = α-amylase inhibitor activity; n.s. = not significant at $P < 0.05$.

TABLE 8. ANOVA OF THE EFFECT OF TREATMENT, YEAR, DEVELOPMENT STAGE AND THEIR INTERACTIONS ON FOLIAR CHEMISTRY IN *A. hypochondriacus* PLANTS SUBJECTED TO FOUR DIFFERENT TREATMENTS (0.5 MM JA, 1.5 MM JA INSECTICIDE, AND UNTREATED), DURING TWO CONSECUTIVE FIELD EXPERIMENTS (2000 AND 2001)

	TIA			CTIA			AAIA			LAPA			PGA			BGA			CHIA		
Factor	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Treatment (T)	3	n.s.		3	4.40	0.007	3	n.s.		3	n.s.		3	n.s.		3	4.68	0.005	3	3.81	0.014
Year (Y)	1	137.15	<0.001	1	199.07	<0.001	1	76.67	<0.001	1	n.s.		1	40.24	<0.001	1	10.45	0.002	1	153.40	<0.001
Development stage (DS)	2	167.31	<0.001	2	61.82	<0.001	2	104.09	<0.001	2	8.92	<0.001	2	224.70	<0.001	2	125.30	<0.001	2	194.10	<0.001
T × Y	3	8.21	<0.001	3	n.s.		3	n.s.		3	n.s.		3	n.s.		3	13.58	<0.001	3	9.22	<0.001
T × DS	6	3.04	0.014	6	n.s.		6	n.s.		6	n.s.		6	n.s.		6	12.84	<0.001	6	4.09	0.002
Y × DS	2	299.72	<0.001	2	63.87	<0.001	2	55.10	<0.001	2	94.08	<0.001	2	13.29	<0.001	2	82.14	<0.001	2	117.16	<0.001
T × Y × DS	6	2.18	0.054	6	n.s.		6	n.s.		6	n.s.		6	n.s.		6	4.17	0.001	6	n.s.	
Residuals	70			64			72			71			55			63			63		

Note. TIA = trypsin inhibitor activity; CTIA = chymotrypsin inhibitor activity; AAIA = α-amylase inhibitor activity; LAPA = leucine aminopeptidase activity; PGA = polygalacturonase activity; BGA = β-1,3-glucanase activity; CHIA = chitinase activity; n.s. = not significant at $p<0.05$.

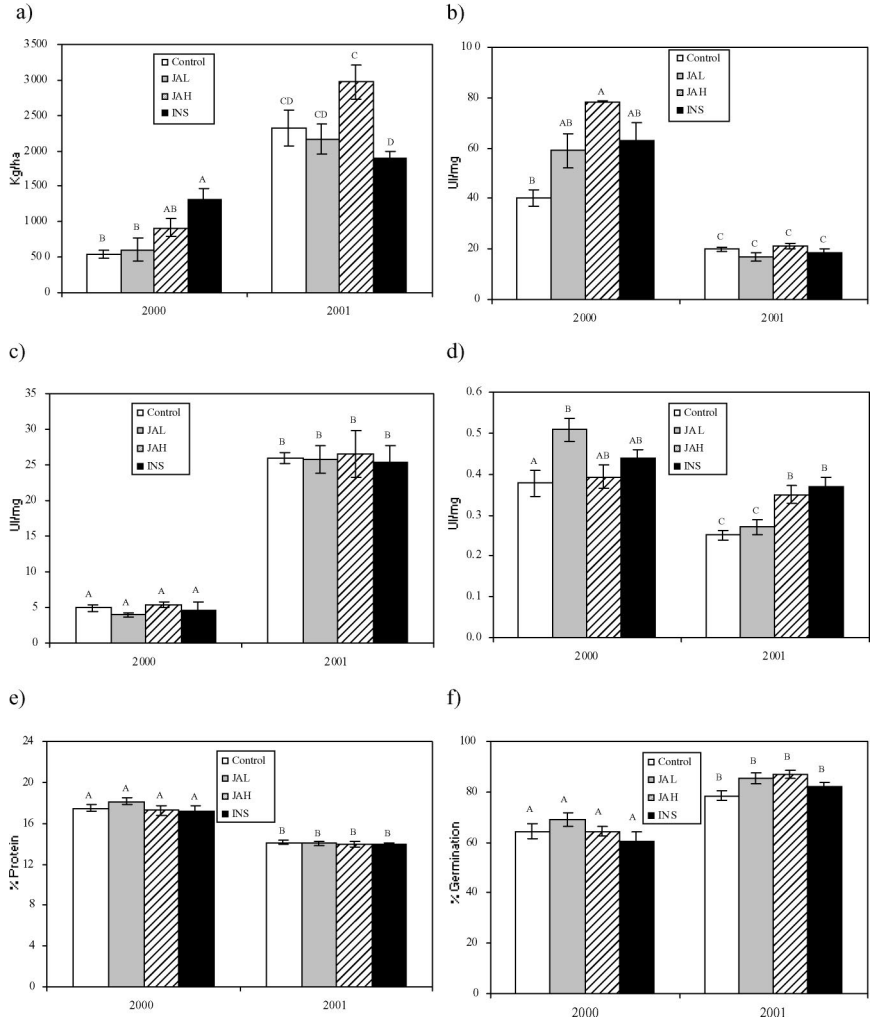


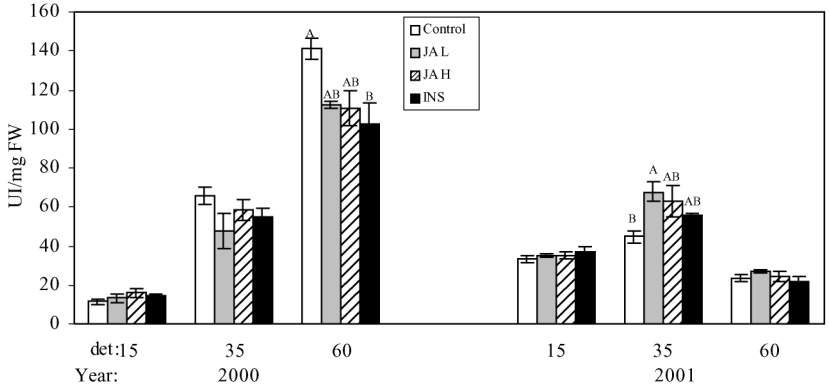
FIG. 3. Mean values (±SE) of grain yield (a), trypsin inhibitor activity levels (b), chymotrypsin inhibitor activity levels (c), α-amylase inhibitor activity levels (d), protein content (e), and percent germination (f), in seed from untreated field grown *A. hypochondriacus* plants (control) or plants treated with a chemical insecticide (INS) or with low (0.5 mM, JAL) or high (1.5 mM, JAH) dosages of jasmonic acid (JA). The results of two independent field experiments performed on consecutive years (2000 and 2001) are shown. Bars with different letters are significantly different at $P < 0.05$ (ANOVA followed by Tukey test).

TABLE 9. ANOVA OF THE EFFECT OF TREATMENT IN DIFFERENT DEVELOPMENTAL STAGES PER YEAR ON CHEMICAL AND PLANT PRODUCTIVITY VARIABLES IN *A. Hypochondriacus* PLANTS SUBJECTED TO FOUR DIFFERENT TREATMENTS (0.5 mM JA, 1.5 mM JA, INSECTICIDE, AND UNTREATED

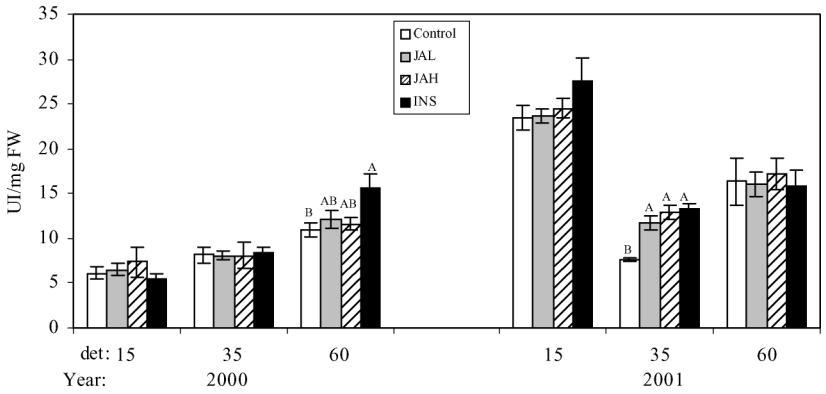
		Year 2000												Year 2001											
		YL			DL			ML			S			YL			DL			ML			S		
Variable	Factor	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Yield	Treatment		n.d.			n.d.			n.d.		3	6.61	0.007		n.d.			n.d.			n.d.		3	4.66	0.022
	Residuals										12												12		
TIA	Treatment	3	n.s.		3	n.s.		3	4.34	0.030	3	5.92	0.014	3	n.s.		3	3.90	0.037	3	n.s.		3	n.s.	
	Residuals	11			12			11			10			12			12			12			12		
CTIA	Treatment	3	n.s.		3	n.s.		3	3.76	0.041	3	n.s.		3	n.s.		3	16.78	<0.001	3	n.s.		3	n.s.	
	Residuals	4			12			12			11			12			12			12			12		
AAIA	Treatment	3	n.s.		3	4.31	0.028	3	n.s.		3	4.28	0.029	3	4.59	0.023	3	n.s.		3	5.86	0.011	3	9.80	<0.001
	Residuals	12			12			12			12			12			12			12			12		
LAPA	Treatment	3	n.s.		3	n.s.		3	n.s.			n.d.		3	n.s.		3	n.s.		3	n.s.			n.d.	
	Residuals	11			12			12						12			12			12					
PGA	Treatment	3	n.s.		3	6.24	0.038	3	n.s.			n.d.		3	n.s.		3	n.s.		3	n.s.			n.d.	
	Residuals	3			5			12						12			11			12					
BGA	Treatment	3	n.s.		3	4.62	0.025	3	n.s.			n.d.		3	8.96	0.002	3	n.s.		3	19.76	<0.001		n.d.	
	Residuals	11			11			12						12			8			9					
CHIA	Treatment	3	n.s.		3	n.s.		3	25.18	<0.001		n.d.		3	19.68	<0.001	3	n.s.		3	n.s.			n.d.	
	Residuals	8			7			12						12			12			12					

Note. YL = young leaf; DL = developing leaf; ML = mature leaf; S = seed; TIA = trypsin inhibitor activity; CTIA = chymotrypsin inhibitor activity; AAIA = α-amylase inhibitor activity; LAPA = leucine aminopeptidase activity; PGA = polygalacturonase activity; BGA = β-1,3-glucanase activity; CHIA = chitinase activity; n.d. = not determined; n.s. = not significant at $P < 0.05$.

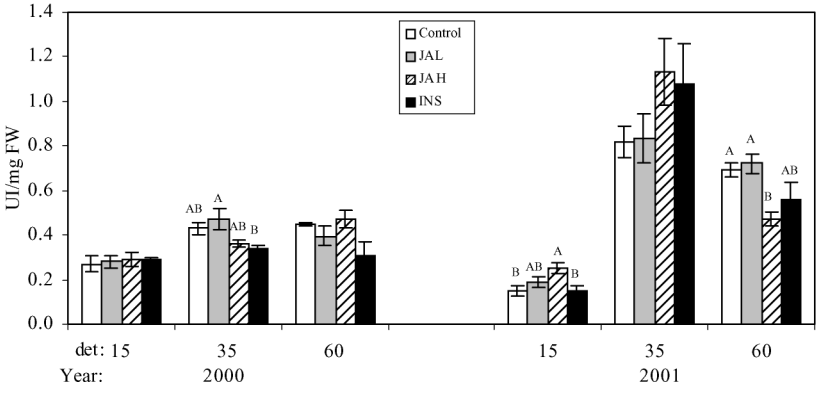
a)


Control
JA L
JA H
INS
UI/mg FW
det:15
35
60
15
35
60
Year:
2000
2001

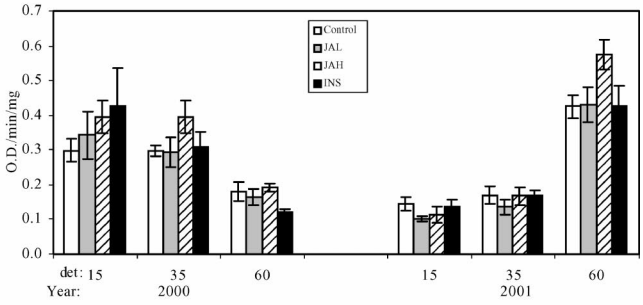
b)


Control
JAL
JAH
INS
UI/mg FW
det: 15
35
60
15
35
60
Year:
2000
2001

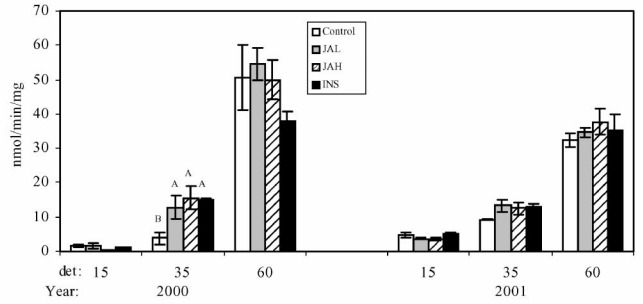
c)


Control
JAL
JAH
INS
UI/mg FW
det: 15
35
60
15
35
60
Year:
2000
2001

d)


Control
JAL
JAH
INS
O.D./min/mg
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
det: 15 35 60 15 35 60
Year: 2000 2001

e)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JAH
INS
nmol/min/mg
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30
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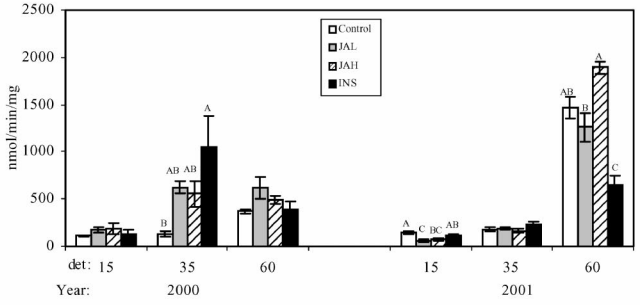
det: 15 35 60 15 35 60
Year: 2000 2001

f)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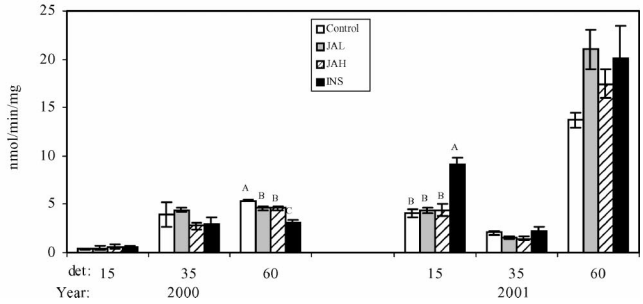
Year: 2000 2001

g)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det: 15 35 60 15 35 60
Year: 2000 2001

In addition to treatment, effects of year on the seed head insect community were detected (Table 5). As mentioned, JA treatments had a negative effect on the abundance of all three components of the insect community, particularly PF insects. Drought probably reduced the attractiveness of JA-treated plants to insects by some undetermined mechanism. Previous studies indicate that drought (in tomato) or changes in irrigation scheduling (in cotton) affected defense-related plant chemistry and the performance or abundance of insect pests and predators on field grown plants (Flint et al., 1994, and references therein; English-Loeb et al., 1997). The results also suggest that any possible beneficial effect produced by a reduction in PF pests observed in JA-treated *A. hypochondriacus* plants in 2000 could have been counterbalanced by the negative effect that (low) JA treatments had on the PP insect community in seed heads. This effect on PP insects coincides, to some extent, with data that found no significant increase in the abundance of predaceous insects in JA-treated tomato plants (Thaler et al., 2001).

Aphids are a common insect pest of grain amaranths (Aragón-García and López-Olguín, 2001). In 2002, aphid numbers (*Macrosiphum euphorbiae* Thomas) in foliage of high JA-treated *A. hypochondriacus* plants were monitored throughout an 8-wk period. Aphid numbers were affected by T, S, and its interaction, and was consistent with the wide fluctuation in aphid numbers observed (Figure 1). Hence, high JA treatment reduced aphid numbers only in the youngest plants, whereas numbers in insecticide-treated plants were significantly higher on the last and next to last sampling dates (Table 3, Figure 1). The results from JA-treated plants were not in accordance, except on the first sampling date, with previous reports that showed that dosages of JA identical to those employed in this study decreased the preference, performance, and abundance of herbivores, including aphids, in tomato (Thaler, 1999a,b; Thaler et al., 2001). However, the fluctuating aphid numbers observed in JA-treated amaranth plants may reflect the lack of

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FIG. 4. Mean activities (±SE) of trypsin inhibitors (a), chymotrypsin inhibitors (b), α-amylase inhibitors (c), leucine aminopeptidases (d), polygalacturonases (e), β-1,3-glucanases (f), and chitinases (g), in leaves of untreated field grown *A. hypochondriacus* plants (control) or plants treated with a chemical insecticide (INS) or with low (0.5 mM, JAL) or high (1.5 mM, JAH) dosages of jasmonic acid (JA). Four leaves per plant were sampled from the upper third segment of five plants per experimental plot, 48 hr after each treatment. Treatments were applied on young plants (15 d after emergence and seedling thinning, "det"), developing plants (35 det), and mature plants (60 det). The results of two independent field experiments performed on consecutive years (2000 and 2001) are shown. Significant differences (at $P < 0.05$) between treatments within each developmental stage within each year are indicated by different letters above bars (ANOVA followed by Tukey test).

consistent responses to induced resistance usually shown by these insects. This behavior has been reported by other researchers in other plant species and appears to depend on the aphid species involved, its biotype within a species, and the plant development stage (Thaler, 1999b, and references therein). Conversely, the abrupt increase in aphid numbers observed in insecticide-treated plants on late sampling dates could be associated with a reduction in the natural abundance of predators (such as *O. insidiosus*; not shown) caused by insecticide treatments. This was inferred from the susceptibility to insecticide shown by this component of the insect community in seed heads of *A. hypochondriacus*, particularly in 2002 (Table 6, Figure 2c).

The application of exogenous JA did not have a negative impact on productivity measured as grain yield, seed germination percentage, and seed protein content (Figure 3a, e, and f). Seed vigor was not altered (results not shown). Even the low yields obtained from *A. hypochondriacus* plants treated with low JA in 2000 (807 kg/ha; Figure 3a) compare favorably with those reported by other workers (Aragón-García and López-Olguín, 2001), but are still lower than the 2–3 metric tons/ha reported for selected, high yielding, *A. hypochondriacus* lines in Mexico (Maldonado and Estrada, 1986). The production of reasonable yields, even under drought stress, was not surprising, considering that this is a defining characteristic of the *A. hypochondriacus* variety employed in this study (A. Borodanenko, personal communication). On the other hand, the higher yields produced by high-JA-treated plants in 2001 may reflect a higher level of tolerance to herbivory in plants not subjected to drought stress. Tolerance to herbivory is dependent, in addition to plant genotype, on environmental conditions, being favored when photosynthetic rates and water and nutrient uptakes are high (Agrawal, 2000). Thus, insect-derived damage could have affected yield, even in damage-tolerant *A. hypochondriacus* plants, under conditions in which they were unable to compensate adequately for herbivory losses due to drought stress.

The results shown in Tables 7 and 8 indicate that Y, DS, and its interaction were the factors that more significantly influenced chemical and plant productivity. The strong effect of Y suggests that the basal metabolism of *A. hypochondriacus* plants and, possibly, their response to JA (see below) are influenced by changes in environmental conditions. For instance, the higher levels of TIA detected in seed and leaves, in 2000, could be explained by the proposed protective role that has been assigned to the accumulation of trypsin inhibitors in a number of plant species undergoing desiccation (Reviron et al., 1992; Lopez et al., 1994; Welham et al., 1998; Lam et al., 1999). This possibility is supported by the observed accumulation of trypsin inhibitors in foliage of *A. hypochondriacus* plants subjected artificially to drought and salt stress (J. Délano-Frier and S. Valdés-Rodríguez, unpublished data). On the other hand, the physiological relevance of the changes in activity levels detected between years in other proteins, particularly in seed and foliar AAIA and CTIA levels, has yet to be determined. Lower germination efficiency in 2000 was probably also caused by drought stress, since seed dormancy is favored under

adverse environmental conditions (Bewley, 1997; Holdsworth et al., 1999). The difference in protein content in seeds is difficult to explain, but could have been caused similarly by the differential accumulation of stress-protective proteins (e.g., dehydrins and LEA proteins) (Xu et al., 1996). This possibility is under investigation.

The analysis of foliar chemistry showed that the levels of activity were influenced also by the development stage of the plant (Table 9, Figure 4). The relatively high levels of activity detected in some stages, particularly of protease and amylase inhibitors, coupled with the rather modest and sporadic increases in activity produced by exogenous JA, indicate that, in addition to their possible stress-protective role, these proteins might be part of a constitutive and developmentally regulated defense mechanism. In other plant species (e.g., potato, *Nicotiana tabacum* and *N. attenuata*), toxic proteins produced in induced defense responses are also known to accumulate as constitutive defenses (Gatehouse, 2002). Moreover, in plant systems subjected to heavy herbivore pressure, as appears to be the case of amaranth, inducible defenses are advantageous, and constitutive mechanisms are favored (Wolson and Murdock, 1990; Wittstock and Gershenzon, 2002).

Our results are in agreement with well-documented studies in other plant species that report that exposure to abiotic stress or changes occurring during development modify the activity and/or the JA-inducibility of several putative defense proteins (Vera et al., 1988; Cordero et al., 1994; Alarcon and Malone, 1995; Cipollini, 1997; English-Loeb et al., 1997; Cipollini and Redman, 1999; van Dam et al., 2001). Abiotic stress in plants can change plant chemistry and influence herbivore performance, both positively and negatively (Cipollini, 1997; English-Loeb et al., 1997); and increased resistance against insect herbivores can still be obtained in JA-treated plants even when simultaneous environmental stimuli reduce the activity of defense-related proteins (Cipollini and Redman, 1999).

It was difficult to identify in this study to what extent the changes detected in *A. hypochondriacus* plants were a consequence of exogenous JA. In general, the effects observed in JA-treated plants were rather modest and in agreement with the poor performance that chemical elicitors of defense responses in plants frequently have in field conditions (Lyon and Newton, 1999). Nevertheless, a number of findings from this study merit further research. First, JA did not have an adverse effect on productivity, and when the high dosage was applied in suitable growing conditions, it actually enhanced grain yield. Second, the application of JA had a significant effect on the insect community in foliage and seed heads. Third, drought stress had a powerful influence on all the variables. This implies that some of the chemical responses examined (e.g., TIA) could have a role in protection against adverse environmental conditions in addition to their suggested defensive role. The mechanism(s) responsible for the JA- and/or drought-induced changes remain to be determined. However, similar to the induction of jasmonate-induced proteins in barley (Lehman et al., 1995), osmotin in tobacco (Xu et al., 1994), and proteinase inhibitors in tomato (Dombrowski, 2003), a stress-induced activation of JA signalling and/or increase in endogenous JA levels, could be involved.

APPENDIX

METEOROLOGICAL CONDITIONS RECORDED IN THE EXPERIMENTAL FIELDS OF THE INSTITUTE OF AGRICULTURAL SCIENCES (UNIVERSITY OF GUANAJUATO)

Month	Temperature (°C)			Relative humidity (%)			Total rainfall (mm)	Mean evaporation (mm)	Mean isolation (h)
	Maximum	Mean	Minimum	Maximum	Mean	Minimum			
2000									
June	26.7	20.2	13.7	98.6	68.9	39.3	119.4	5.7	7.5
July	27.1	19.6	12.2	98.9	66.7	34.5	172.4	6.7	9.3
August	26.6	19.2	11.9	98.0	65.8	33.7	64.5	5.6	8.3
September	27.6	19.1	10.6	96.7	62.9	29.2	40.7	5.7	9.0
October	27.3	18.2	9.1	96.4	61.7	27.1	15.4	4.8	8.5
November	26.2	16.7	7.2	95.4	60.7	26.1	0.0	4.9	8.8
December	23.6	14.1	4.3	93.2	60.5	27.7	9.2	3.5	8.1
2001									
June	27.5	20.5	13.5	95.9	64.7	33.6	205.0	6.60	8.7
July	26.5	19.6	12.8	97.5	65.2	33.0	217.8	6.40	9.2
August	26.6	19.5	12.5	97.1	65.1	33.1	260.9	5.96	8.2
September	26.4	19.2	12.0	97.1	64.3	31.5	128.7	5.10	8.0
October	26.0	16.9	7.9	94.8	62.0	29.2	36.0	4.50	9.4
November	25.2	14.9	4.6	96.9	58.9	20.9	3.5	4.00	9.6
December	24.0	14.6	5.2	94.7	61.0	27.4	1.4	3.39	7.5

APPENDIX CONTINUED

Month	Temperature (°C)			Relative humidity (%)			Total rainfall (mm)	Mean evaporation (mm)	Mean isolation (h)
	Maximum	Mean	Minimum	Maximum	Mean	Minimum			
2002									
June	30.0	21.8	13.6	93.4	61.7	30.0	71.8	7.37	8.8
July	26.2	19.6	13.0	98.0	67.5	36.9	237.8	6.41	6.9
August	26.6	19.4	12.3	97.1	64.7	32.3	201.0	7.08	9.3
September	26.0	19.6	13.3	97.4	67.6	37.8	129.6	4.82	6.5
October	27.3	19.1	10.9	97.7	63.9	30.1	53.4	4.25	8.0
November	24.4	15.3	6.2	96.1	58.2	20.6	50.9	3.46	7.1
December	23.9	13.8	3.8	96.2	59.6	23.0	0.3	3.00	7.5
1979–2002									
June	28.4	21.3	14.3	84.7	58.4	32.0	117.4	7.79	8.4
July	26.1	20.0	13.7	90.0	63.2	36.6	159.8	6.43	7.7
August	25.9	19.5	13.2	89.6	63.3	36.3	168.5	6.41	8.0
September	25.5	18.9	12.4	90.5	62.5	34.8	107.4	5.62	7.4
October	25.5	17.5	9.5	86.3	57.1	29.3	43.4	5.10	8.2
November	24.8	15.7	6.6	86.1	55.7	26.1	10.1	4.40	8.5
December	23.2	14.3	5.3	84.3	55.4	26.5	8.8	3.80	7.9

Acknowledgments—This work was supported by two grants (00-03-201-062 and 01-03-201-141) to JDF by The Council for Science and Technology of the State of Guanajuato (Concyteg). We thank Dr Manuel Vázquez-Arista for help and guidance during insect sampling, and Veronica Ramírez-Solorzano and Lucrecia Contreras-Rivera for their patience, tenacity, and discipline displayed while counting and classifying insects.

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