JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Differentially Enhanced Insect Resistance, at a Cost, in *Arabidopsis thaliana* Constitutively Expressing a Transcription Factor of Defensive Metabolites

ERIC T. JOHNSON* AND PATRICK F. DOWD

Crop Bio-Protection Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, 1815 N. University St., Peoria, Illinois 61604

A transgenic line of *Arabidopsis thaliana* constitutively expressing a conserved MYB transcription factor of phenylpropanoid biosynthesis resulting in solid-purple leaves had significantly increased resistance to leaf feeding by first instar fall armyworms (*Spodoptera frugiperda*), but no enhanced resistance to cabbage looper (*Trichoplusia ni*) larvae, when compared to wild type plants. However, inflorescence and silique (seed pod) production were significantly reduced by 22 and 52%, respectively, in the transgenic line compared to wild type plants. Reduction in feeding by *S. frugiperda* was significantly positively correlated with reduction in weights of survivors, but both were negatively correlated with the concentration of anthocyanins. These results indicate that a single gene regulator can activate a defensive pathway sufficient to produce increased resistance to insects but that this activation confers a cost in plant productivity.

KEYWORDS: Fall armyworm; *Spodoptera*; cabbage looper; *Trichoplusia*; PAP1; anthocyanin; phenylpropanoid

INTRODUCTION

Insect herbivores not only cause yield losses through physical damage but also can enhance the colonization of plants by fungi that may produce toxins (*1*). Plant resistance to insect feeding is an important strategy of insect management in many crops. Many successful examples of enhanced insect resistance have been accomplished through conventional breeding. Modern molecular biology techniques have also produced plants with increased resistance to insects, including *Bacillus thuringiensis* (*Bt*) corn (*2*) and *Bt* cotton (*3*). Resistance to the European corn borer, *Ostrinia nubilalis* (Hübner), resulted in greatly reduced levels of the fungal toxin fumonisin in maize that expresses high levels of the *Bt* gene compared to wild type plants (*4*−*6*). Identification of additional sources of plant resistance genes may help overcome general concerns of the bacterial origins of the *Bt* gene toxicity and the possible reduction in the efficacy of *Bt* crops through insect resistance. Genomic studies of plants that have advanced the knowledge of resistance mechanisms, coupled with biotechnological methods, may permit introduction or enhancement of plant-derived genes that would be difficult or impossible to achieve by conventional breeding.

Most molecular level investigations of plant-derived resistance traits introduced transgenically have involved proteins that act as direct toxins, such as proteinase inhibitors (*7*), lectins (*8*), and ribosome inactivating proteins (*9*). However, individual resistance proteins are typically considered insufficient to confer robust resistance (*10*). Individual proteins that affect multiple target sites, such as peroxidases, may have more durable resistance, depending on target plant and insect pest (*11*).

Another resistance strategy is the induction of more biochemically costly defensive pathways involving salicylic acid, primarily induced by microbial pathogens, or jasmonic acid, primarily induced by insects (*12*). As part of the induction process, signal molecules can activate gene regulatory proteins which result in the biosynthesis of defensive molecules. For example, methyl jasmonate can activate ORCA3, a transcription factor from *Catharanthus roseus*, which contributes to the production of terpenoid indole alkaloids (*13*). Recent investigations with *Arabidopsis thaliana* identified the gene *PAP1* (Production of Anthocyanin Pigment 1), which is an MYB transcription factor that activates the phenylpropanoid biosynthetic pathway (*14*). PAP1 is highly homologous to other plant MYB-like transcription factors that regulate anthocyanin production and activates pathways responsible for increased biosynthesis of lignins, flavonoids (kaempferol and quercetin derivatives), and anthocyanins (cyanidin derivatives) (*14*). Constitutive overexpression of PAP1 results in purple pigmentation in most parts of the plant (*14*). Because the phenylpropanoid compounds that are increased by PAP1 overexpression are associated with insect resistance under other circumstances (*15*−*18*), we tested the possibility that these transgenic plants were more resistant to some representative insects than wild type plants. Moreover, because of the potential diversion of metabolites and energy away from plant growth and into the production of defensive molecules, plant productivity was also measured.

* To whom correspondence should be addressed. Tel.: (309) 681-6177. Fax: (309) 681-6693. E-mail johnet@ncaur.usda.gov.

10.1021/jf0308049 This article not subject to U.S. Copyright. Published 2004 by the American Chemical Society
Published on Web 07/16/2004

This study appears to be the first example where this relationship has been investigated in plants with a single gene modification.

MATERIALS AND METHODS

Insects. Fall armyworms (*Spodoptera frugiperda*) were reared at 27 ± 1 °C, 40 ± 10% relative humidity, 14:10 light/dark photoperiod as described previously (*19*). Cabbage loopers (*Trichoplusia ni*) were obtained from Dr. Robert W. Behle (USDA-Peoria) and reared under conditions similar to those used for *S. frugiperda* (*20*). Newly hatched larvae were used in bioassays.

Plants. Wild type *Arabidopsis thaliana* Columbia-O strain seeds were obtained from Lehle Seeds, Round Rock, Texas. Transgenic seeds of Columbia-O strain with the *pap1-D* mutation were obtained from the *Arabidopsis* Biological Resource Center at Ohio State University, Columbus, Ohio. Seed was sown on moistened Metro Mix 350 (Scotts-Sierra Horticultural Products Company, Marysville, OH) and grown under a 14:10 light/dark photoperiod at 25 °C. When plants were ca. 2 weeks old, they were moved to the greenhouse under target temperature conditions of 25 °C and a 14:10 light/dark photoperiod maintained by supplemental lights. The photosynthetic photon flux density measured by a portable spectroradiometer (LI−COR, Inc., Lincoln, NE, model LI-1800) in the greenhouse under full sunlight was 396 μEinstein $m^{-2} \cdot s^{-1}$. Plants were fertilized weekly with Peters Professional 20−20−20 General Purpose fertilizer (Scotts Company, Marysville, OH) at a concentration of 1 g/L. Numbers of inflorescences (flower stalks) and siliques (seed pods) per plant were counted approximately 4 weeks after the initiation of flowering.

Bioassays. Leaves ca. 3−4 cm in length approximately 4 nodes below the apical meristem were removed from at least 25 plants of each type that had initiated flowering for about 1 week. They were placed individually in 5-cm diameter Petri plates with tight fitting lids (Falcon 351006, Becton Dickinson Labware, Franklin Lakes, NJ). Ten larvae were added to each leaf, and the dishes were held in the dark at 27 ± 1 °C and 40 ± 10% relative humidity for 24 (*T. ni*) to 48 (*S. frugiperda*) hours. Leaves were rated for feeding by *S. frugiperda* on day 1 by counting 0.25 mm^2 holes or equivalent area, approximately corresponding to head capsule size (*11*, *21*, *22*). Mortality was also recorded at the same time leaves were rated. On day 1 for *T. ni* and day 2 for *S. frugiperda*, holes of 1 mm^2 were counted. After 48 h, larvae were frozen and subsequently weighed to 0.01 mg, using an analytical balance (Mettler-Toledo AE163 or AX105DR, Columbus, OH).

Chemical Analysis. Prior to the start of the bioassays, a 5-mm diameter leaf disk was cut out of each leaf near the tip and frozen at −20 °C until analysis. Anthocyanins were extracted using a previously published procedure (*23*) by maceration in propanol/HCl/H_2O (18:1:81) and boiling for 3 min. The extracts were incubated overnight at room temperature, and the solid material was pelleted for 5 min at 16000*g* in a microcentrifuge. The anthocyanin level was determined by measuring the optical density of the supernatant at 535 nm minus the background at 650 nm.

Statistical analysis. Statistical differences in feeding rates and weights were determined by ANOVA (Proc GLM) (*24*). Correlation analyses between feeding rates and weights and chemical constituents and feeding rates and weights were determined using ProcReg Option Corr (*24*).

RESULTS

The *pap1-D* plants grown under our lighting conditions displayed two different pigment patterns on the upper side of the leaf. One group had relatively solid purple leaves, while the other group had primarily purple-veined leaves with a green background. These two groups of *pap1-D* plants were separated and compared to one another or to wild type plants in statistical analyses. Measurements of anthocyanin content in all of the *pap1-D* leaves used in this study found that the solid-purple leaves contained significantly more anthocyanin than the purple-veined leaves. For the bioassays with *S. frugiperda* larvae, the anthocyanin content from solid-purple leaves was 0.38 ± 0.03 (mean ± standard error) absorbance units (AU), while the anthocyanin content from purple-veined leaves was 0.29 ± 0.03 AU (which was significant at $p < 0.05$ by ANOVA). The mean anthocyanin level from representative wild-type leaves (0.023 ± 0.003 AU) was almost 17 times below the mean anthocyanin level from solid- purple *pap1-D* leaves. For the bioassays with *T. ni* larvae, the anthocyanin content from solid-purple leaves was 0.38 ± 0.03 AU, while the anthocyanin content from purple-veined leaves was 0.27 ± 0.03 AU (which was significant at $p < 0.05$ by ANOVA).

Table 1. Effects of Enhanced Phenylpropanoid and Wild Type Leaves of *Arabidopsis* on *S. frugiperda* and *T. ni*[a]

	feeding rating		
	day 1	day 2	weight (mg)
	S. frugiperda		
	(0.25 mm^2)	(1 mm^2)	
pap1-D solid-purple	25.0 ± 1.9a	25.6 ± 1.8a	0.293 ± 0.0093a
pap1-D purple-veined	42.7 ± 4.6b	37.9 ± 2.3b	0.362 ± 0.015 b
pap1-D all	32.9 ± 2.8 z	31.1 ± 1.8 y	0.323 ± 0.0086 z
Wild type	40.4 ± 3.4b z	40.8 ± 2.3bz	0.326 ± 0.0086cz
	T. ni		
	(1 mm^2)		
pap1-D solid-purple	30.1 ± 1.5a	ND	0.306 ± 0.0071a
pap1-D purple-veined	28.8 ± 0.8a	ND	0.311 ± 0.0072a
pap1-D all	29.5 ± 0.9 z	ND	0.308 ± 0.0051 z
wild type	32.5 ± 1.3az	ND	0.306 ± 0.0052az

[a] Values are means ± standard errors. Values followed by different letters are significantly different at $P < 0.05$ by analysis of variance. Letters a–c reflect comparisons between different *pap1-D* types with each other and the wild type, while letters y–z reflect comparisons of both *pap1-D* types combined and wild type. ND = not determined.

The *S. frugiperda* larvae fed the solid-purple *pap1-D* leaves caused significantly less damage than the larvae fed on wild type and *pap1-D* purple-veined leaves on both day 1 and day 2 (**Table 1**). The *S. frugiperda* larvae caused the same amount of damage to wild type and purple-veined *pap1-D* leaves on both days. When *pap1-D* plants were considered together, feeding was significantly less only on day 2 compared to feeding on wild type plants. Less than 5% mortality was noted for larvae fed on either type of leaf. *S. frugiperda* larvae that fed on solid-purple leaves weighed significantly less than larvae that fed on wild type leaves, but larvae that fed on purple-veined leaves weighed significantly more than those that fed on wild-type leaves. When combining both types of *pap1-D* leaves, the mean larval weight was approximately the same as the wild type. Feeding rates were significantly positively correlated with mean weights of larvae that were fed both types of *pap1-D* leaves on day 1 ($R = 0.43$, $P = 0.024$), day 2 ($R = 0.55$, $P = 0.0028$) and wild-type leaves on day 1 ($R = 0.63$, $P = 0.0004$), and day 2 ($R = 0.50$, $P = 0.0092$). The anthocyanin levels of solid-purple *pap1-D* and wild type leaves were significantly negatively correlated with feeding rates on day 1 ($R = -0.46$, $P = 0.0026$), day 2 ($R = -0.61$, $P = <0.0001$). The same trend was apparent between the anthocyanin levels and mean weights of solid-purple *pap1-D* and wild-type leaves, but it was not significant ($R = -0.23$, $P = 0.1514$). These results suggest that the level of upregulated compounds in the solid-purple *pap1-D* leaves reduce consumption and slow the growth of *S. frugiperda* larvae.

The rate of leaf consumption by *T. ni* larvae was much more rapid than that for *S. frugiperda* larvae (note area of feeding rating in **Table 1**). Preliminary tests indicated that *T. ni* larvae would often consume all of the wild type and *pap1-D* leaves in less than 2 days; therefore, feeding ratings were only assayed

Table 2. Inflorescence and Silique Production by Different *A. thaliana* Plant Types Approximately 4 Weeks after Flower Initiation[a]

plant type	inflorescence	siliques (N)
pap1-D solid-purple	5.2 ± 0.3a	257.5 ± 19.9a (15)
pap1-D purple-veined	6.5 ± 0.4b	326.9 ± 24.5b (12)
pap1-D all	5.7 ± 0.3 y	288.3 ± 16.7 y (27)
Wild type	6.7 ± 0.3bz	533.9 ± 25.3cz (26)

[a] Values are means ± standard errors. Values followed by different letters are significantly different at $P < 0.05$ by analysis of variance. Letters a–c reflect comparisons between different *pap1-D* types with each other and the wild type, while letters y–z reflect comparisons of both *pap1-D* types combined and wild type.

after 1 day. In contrast to the results with *S. frugiperda*, there were no significant differences in feeding rating or weights of *T. ni* larvae on the three leaf types after 1 day. Less than 5% mortality was noted for any larvae fed either type of leaf. These results suggest *T. ni* feeding is not inhibited by the elevated levels of phenylpropanoid molecules in the *pap1-D* mutant.

Inflorescence production from individual plants was measured to estimate the reproductive costs of the *pap1-D* mutation (**Table 2**). Wild type plants produced significantly more flower stalks and siliques (∼1.9 times higher) than all of the *pap1-D* plants considered together. Flower stalk and silique production by the solid-purple *pap1-D* plants was significantly lower than that of the purple-veined *pap1-D* plants. These results suggest that the energy invested in high phenylpropanoid biosynthetic products lowers the available resources for generation of reproductive organs.

DISCUSSION

Differential sensitivity was noted when *S. frugiperda* and *T. ni* were fed the leaves of the different *Arabidopsis* lines. Overall feeding ratings were much lower for *S. frugiperda* than *T. ni* on all of the *Arabidopsis* leaf types. Members of the Brassicaceae, which include *Arabidopsis*, are attractive hosts for *T. ni* (*25*, *26*). While the Brassicaceae are not a preferred host, they can still be attacked by *S. frugiperda* larvae (*26*). The *S. frugiperda* larvae fed the solid-purple *pap1-D* leaves ate significantly less and weighed significantly less that those fed on the purple-veined *pap1-D* and wild-type leaves. It is not clear why the *S. frugiperda* larvae fed the purple-veined leaves weighed more than larvae fed on wild type leaves since these values were still negatively correlated with anthocyanin levels. We did not observe the *S. frugiperda* larvae avoiding the pigmented areas of the purple-veined leaves. It may be possible that the defensive molecules of the *pap1-D* mutant enhance growth up to a certain concentration (e.g., in the purple-veined leaves) but then are toxic at higher concentrations (e.g., in the solid-purple leaves). Some phenylpropanoid compounds (e.g., the flavonoids rutin and quercitrin) either increase feeding rates or are toxic to the same insect species depending on the concentration (*27*).

At least three general classes of compounds are expressed at higher levels in *pap1-D* leaves: flavonol derivatives, anthocyanin derivatives, and lignin (*14*). Each of these groups of compounds has been demonstrated to provide protection from a variety of stress conditions (*28*) and all may be involved in insect resistance (*15*−*18*). Because of this, it is difficult to speculate which molecule (or combination of molecules) is responsible for the enhanced resistance to *S. frugiperda* in the *pap1-D* mutant. It may be argued that the *pap1-D* mutant simply produces more glucosinolates (*29*). *T. ni* herbivory of *A. thaliana* is lowest in ecotypes with high glucosinolate levels (*30*). However, glucosinolates are not likely enhanced in *pap1-D* leaves because *T. ni* feeding ratings and weights were not significantly different for the solid-purple and wild-type leaves (**Table 1**). Further analysis of *pap1-D* leaves is required to identify which molecule(s) inhibit feeding of *S. frugiperda* but not *T. ni*.

It is not known whether PAP1 regulates phenylpropanoid biosynthesis in *Arabidopsis* as part of a developmental pathway or stress response. Under continuous white light, the hypocotyl and cotyledon edges of *Arabidopsis* seedlings accumulate anthocyanins, peaking at 4 days after germination and then dissipating; lignin measurements were not performed (*31*). It is possible that this short accumulation of anthocyanins reflects activity of PAP1 in protecting the young seedling from insects, pathogens and changing light quality. Immature plant tissues are also reported to have higher levels of defensive compounds active against insects (*32*). A variety of light, pathogen, and hormonal stresses can trigger anthocyanin production in mature *Arabidopsis* plants but no responses described to date involve PAP1 (*31*, *33*, *34*). More experiments are needed to delineate the involvement of PAP1 during development and stress conditions.

The creation of the *pap1-D* mutant is a unique example of changing a constitutively expressed plant defense pathway. Constitutive plant defense programs are potentially costly in terms of overall productivity but invaluable in cases where an induced response to an herbivore is simply not rapid enough (*35*). In the present study, we noted significantly lower inflorescence and silique production by the solid-purple plants compared to the purple-veined *pap1-D* and wild type plants. The silique production by the solid-purple and purple-veined leafed *pap1-D* plants was about half that of the wild type plants. This lower productivity is indicative of the metabolic cost incurred for constitutive expression of phenylpropanoid biosynthesis.

Manipulating induced defensive pathways has been suggested as a valuable means for increasing host plant resistance. It is uncertain whether resistance pathways can be turned on rapidly enough and comprehensively enough to provide resistance to multiple species and ages of insect pests (*35*). Our study shows that constitutive expression of the phenylpropanoid pathway can enhance resistance to one pest, but not another and also lowers the productivity of plants. This information suggests that comprehensive expression of defensive pathways must be weighed against other multigenic strategies for pest resistance such as specific expression of gene products that affect multiple target sites (*10*) or are multi-functional (*36*), which are not as likely to reduce productivity because of the smaller number of gene products involved.

ABBREVIATIONS USED

PAP1, production of anthocyanin pigment 1; *pap1-D*, production of anthocyanin pigment 1-Dominant; *Bt*, *Bacillus thuringiensis*; AU, absorbance units.

ACKNOWLEDGMENT

We thank D.A. Lee and E.K. Bell for technical assistance and R.W. Behle for providing *T. ni* larvae. We are grateful to the *Arabidopsis* Biological Resource Center for providing the *pap1-D* seed. We thank Fernando Vega, Michael Muhitch and Pat Slininger for comments on prior versions of this manuscript.

LITERATURE CITED

(1) Dowd, P. F. The involvement of arthropods in the establishment of mycotoxigenic fungi under field conditions. In *Mycotoxins in Agriculture and Food Safety*; Sinha, K. K., Bhatnagar, D., Eds.; Marcel Dekker: New York, 1998; pp 307−350.

(2) Koziel, M. G.; Beland, G. L.; Bowman, C.; Carozzi, N. B.; Crenshaw, R.; Crossland, L.; Dawson, J.; Desai, N.; Hill, M.; Kadwell, S.; Launis, K.; Lewis, J.; Maddox, D.; McPherson, K.; Meghji, M. R.; Merlin, E.; Rhodes, R.; Warren, G. W.; Wright, M.; Evola, S. V. Field performance of Elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. *Biotechnology* **1993**, *11*, 194−200.

(3) Perlak, F. J.; Deaton, R. W.; Armstrong, T. A.; Fuchs, R. L.; Sims, S. R.; Greenplate, J. T.; Fischhoff, D. A. Insect resistant cotton plants. *Biotechnology* **1990**, *8*, 939−943.

(4) Munkvold, G. P.; Hellmich, R. L.; Rice, L. G. Comparison of fumonisin concentrations in kernels of transgenic Bt maize hybrids and nontransgenic hybrids. *Plant Dis.* **1999**, *83*, 130−138.

(5) Dowd, P. F. Indirect reduction of ear molds and associated mycotoxins in *Bacillus thuringiensis* corn under controlled and open field conditions: Utility and limitations. *J. Econ. Entomol.* **2000**, *93*, 1669−1679.

(6) Bakan, B.; Melcion, D.; Richard-Molard, D.; Cahagnier, B. Fungal growth and fusarium mycotoxin content in isogenic traditional maize and genetically modified maize grown in France and Spain. *J. Agric. Food Chem.* **2002**, *50*, 728−731.

(7) Broadway, R. M.; Duffey, S. S.; Pearce, G.; Ryan, C. A. Proteinase inhibitors: a defense agains herbivorous insects. *Entomol. Exp. Appl.* **1986**, *41*, 33−38.

(8) Down, R. E.; Gatehouse, A. M. R.; Hamilton, W. D. O.; Gatehouse, J. A. Snowdrop lectin inhibits development and decreases fecundity of the glasshouse potato aphid (*Aulancorthum solani*) when administered in vitro and via transgenic plants both in laboratory and glasshouse trials. *J. Insect Physiol.* **1996**, *42*, 1035−1045.

(9) Dowd, P. F.; Zuo, W. N.; Gillikin, J. W.; Johnson, E. T.; Boston, R. S. Enhanced resistance to *Helicoverpa zea* in tobacco expressing an activated form of maize ribosome-inactivating protein. *J. Agric. Food Chem.* **2003**, *51*, 3568−3574.

(10) Gould, F. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annu. Rev. Entomol.* **1998**, *43*, 701−726.

(11) Dowd, P. F.; Lagrimini, L. M.; Nelsen, T. C. Relative resistance of transgenic tomato tissues expressing high levels of tobacco anionic peroxidase to different insect species. *Nat. Toxins* **1998**, *6*, 241−249.

(12) Maleck, K.; Dietrich, R. A. Defense on multiple fronts: how do plants cope with diverse enemies? *Trends Plant Sci.* **1999**, *4*, 215−219.

(13) van der Fits, L.; Memelink, J. ORCA3, a jasmonate-responsive transcriptional regulator of plant primary and secondary metabolism. *Science* **2000**, *289*, 295−297.

(14) Borevitz, J. O.; Xia, Y.; Blount, J.; Dixon, R. A.; Lamb, C. Activation tagging identifies a conserved MYB regulator of phenylpropanoid biosynthesis. *Plant Cell* **2000**, *12*, 2383−2394.

(15) Hedin, P. A.; Jenkins, J. N.; Collum, D. H.; White, W. H.; Parrott, W. L.; MacGown, M. W. Cyanidin-3-β-glucoside, a newly recognized basis for resistance in cotton to the tobacco budworm *Heliothis virescens* (Fab.)(Lepidoptera: Noctuidae). *Experientia* **1983**, *39*, 799−801.

(16) Jenkins, J. N.; Hedin, P. A.; Parrott, W. L.; McCarty, J. C., Jr.; White, W. H. Cotton allelochemics and growth of tobacco budworm larvae. *Crop Sci.* **1983**, *23*, 1195−1198.

(17) Lattanzio, V.; Arpaia, S.; Cardinali, A.; Di Venere, D.; Linsalata, V. Role of endogenous flavonoids in resistance mechanism of *Vigna* to aphids. *J. Agric. Food Chem.* **2000**, *48*, 5316−5320.

(18) Tilquin, M.; Meyran, J. C.; Marigo, G. Dietary toxicity of decomposed arborescent leaf litter against larval mosquito: involvement of a lignin−polypeptidic complex. *J. Agric. Food Chem.* **2002**, *50*, 6378−6382.

(19) Dowd, P. F. Toxicological and biochemical interactions of the fungal metabolites fusaric acid and kojic acid with xenobiotics in *Heliothis zea* (F.) and *Spodoptera frugiperda* (J. E. Smith). *Pestic. Biochem. Physiol.* **1988**, *32*, 123−134.

(20) Behle, R. W.; Tamez-Guerra, P.; McGuire, M. R. Field activity and storage stability of *Anagrapha falcifera* nucleopolyhedrovirus (*Af*MNPV) in spray-dried lignin-based formulations. *J. Econ. Entomol.* **2003**, *96*, 1066−1075.

(21) Dowd, P. F.; Lagrimini, L. M. Examination of different tobacco (Nicotiana spp.) types under- and overproducing tobacco anionic peroxidase for their leaf resistance to *Helicoverpa zea*. *J. Chem. Ecol.* **1997**, *23*, 2357−2370.

(22) Dowd, P. F.; Lagrimini, L. M. Examination of different tobacco (*Nicotiana* spp.) types under- and overproducing tobacco anionic peroxidase for their leaf resistance to *Helicoverpa zea*. *J. Chem. Ecol.* **1997**, *23*, 2357−2370.

(23) Nagata, T.; Todoriki, S.; Masumizu, T.; Suda, I.; Furuta, S.; Du, Z.; Kikuchi, S. Levels of active oxygen species are controlled by ascorbic acid and anthocyanin in *Arabidopsis*. *J. Agric. Food Chem.* **2003**, *51*, 2992−2999.

(24) *SAS−STAT Guide v. 8.0*, SAS Institute: Cary, N.C., 2000.

(25) Jander, G.; Cui, J.; Nhan, B.; Pierce, N. E.; Ausubel, F. M. The TASTY locus on chromosome 1 of *Arabidopsis* affects feeding of the insect herbivore *Trichoplusia ni*. *Plant Physiol.* **2001**, *126*, 890−898.

(26) Metcalf, R. L.; Metcalf, R. A. *Destructive and Useful Insects*, Fifth ed.; McGraw-Hill: New York, 1993.

(27) Schoonhoven, L. M. Secondary plant substances and insects. *Rec. Adv. Phytochem.* **1972**, *5*, 197−224.

(28) Dixon, R. A.; Paiva, N. L. Stress-Induced Phenylpropanoid Metabolism. *Plant Cell* **1995**, *7*, 1085−1097.

(29) Wittstock, U.; Halkier, B. A. Glucosinolate research in the *Arabidopsis* era. *Trends Plant Sci.* **2002**, *7*, 263−270.

(30) Kliebenstein, D.; Pedersen, D.; Barker, B.; Mitchell-Olds, T. Comparative analysis of quantitative trait loci controlling glucosinolates, myrosinase, and insect resistance in *Arabidopsis thaliana*. *Genetics* **2002**, *161*, 325−332.

(31) Kubasek, W. L.; Shirley, B. W.; McKillop, A.; Goodman, H. M.; Briggs, W.; Ausubel, F. M. Regulation of flavonoid biosynthetic genes in germinating *Arabidopsis* seedlings. *Plant Cell* **1992**, *4*, 1229−1236.

(32) Morse, S.; Wratten, S. D.; Edwards, P. J.; Niemeyer, H. M. Changes in the hydroxamic acid content of maize leaves with time and after artificial damage; implications for insect attack. *Ann. Appl. Biol.* **1991**, *119*, 239−249.

(33) Veronese, P.; Narasimhan, M. L.; Stevenson, R. A.; Zhu, J. K.; Weller, S. C.; Subbarao, K. V.; Bressan, R. A. Identification of a locus controlling *Verticillium* disease symptom response in *Arabidopsis thaliana*. *Plant J.* **2003**, *35*, 574−587.

(34) Deikman, J.; Hammer, P. E. Induction of anthocyanin accumulation by cytokinins in *Arabidopsis thaliana*. *Plant Physiol.* **1995**, *108*, 47−57.

(35) Wittstock, U.; Gershenzon, J. Constitutive plant toxins and their role in defense against herbivores and pathogens. *Curr. Opin. Plant Biol.* **2002**, *5*, 300−307.

(36) Dowd, P. F. A functional approach to more effective multigenic host plant pest resistance. *Mycopathologia* **2002**, *155*, 46.

Received for review December 9, 2003. Revised manuscript received May 17, 2004. Accepted May 17, 2004. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

JF0308049