

Total Phenolic Content of Asian Elm Leaves and Host Plant Suitability for Gypsy Moth¹

Gretchen Paluch², Fredric Miller³, Junwei Zhu⁴, and Joel Coats⁵

Department of Entomology
Iowa State University, Ames, IA 50011

Abstract

The total phenolic content of foliage from 11 elm species and 2 cultivars was measured, and the relationship to gypsy moth developmental studies was determined. The 13 elms, *Ulmus wilsoniana* (Schneid.), *U. japonica* (Rehd.), *U. glaucescens* (Franch), 'Accolade', 'Triumph', *U. davidiana* (Planch), *U. parvifolia* (Jacq.), *U. szechuanica* (Fang), *U. macrocarpa* (Hance), *U. chenmoui* (Cheng), *U. lamellosa* (C. Wang et S.L. Chang, ex L.K. Fu), *U. castaneifolia* (Hemsl.), and *U. gaussonii* (Cheng), were grown at the Morton Arboretum, Lisle, IL and analyzed at the Pesticide Toxicology Laboratory, Iowa State University, Ames, IA. Significant differences were found in the phenolic content of the 13 elms surveyed. No significant differences were identified between gypsy moth larval longevity, pupal fresh weight, or percentage adult emergence with respect to total leaf phenolic content.

Index words: elm, gypsy moth, phenolics.

Species used in this study: Wilson elm (*Ulmus wilsoniana* Schneid.); Japanese elm (*U. japonica* Rehd.); Glaucous elm (*U. glaucescens* Franch); Accolade (*U. japonica-wilsoniana*); Triumph (*U. japonica-wilsoniana* × *U. japonica-pumilia*); David elm (*U. davidiana* Planch); Chinese elm and Lacebark elm (*U. parvifolia* Jacq.); Szechuan elm (*U. szechuanica* Fang); Bigfruit elm (*U. macrocarpa* Hance); Langya mountain elm (*U. chenmoui* Cheng); Lacebark elm (*U. lamellosa* C. Wang et S.L. Chang, ex L.K. Fu); Multinerved elm (*U. castaneifolia* Hemsl.); Gausson elm (*U. gaussonii* Cheng); gypsy moth (*Lymantria dispar* L.).

Significance to the Nursery Industry

Elm trees (*Ulmaceae*) are one of the most highly valued shade trees in North America. Even after the invasion of Dutch elm disease that first plagued North America in the 1930s, homeowners still take great pride and pleasure in the elm. A survey conducted in an elm leaf beetle-infested neighborhood in Sacramento, CA, found the public reporting major benefits, such as shade and visual aesthetics of the trees, to offset the negative aspects of the insect pests (27). Still, there is intensive effort to develop new hybrids that are not susceptible to Dutch elm disease, elm yellows, verticillium wilt, or insect herbivory. Initial feedback from nursery growers indicate that the new Asian cultivars 'Accolade' and 'Triumph', with good resistance to Dutch elm disease, are performing well and have great potential for use in arboriculture, urban and community forestry, and landscaping. How these hybrids will respond to insect damage is still unclear. There are currently many insects that cause damage to elms, but there is significant concern for controlling the gypsy moth. Research has shown that different elms exhibit various levels of resistance to numerous insect pests (6, 7, 13–19). A basic understanding of the general foliar chemistry and the effects of specific compounds on herbivore resistance will serve a valuable function in breeding programs.

¹Received for publication August 15, 2008; in revised form January 15, 2009. Financial support for this research was provided by the Tree Research and Education Endowment Fund, Champaign, IL, and the Morton Arboretum, Lisle, IL. Portions of this study were also supported by a J. Frank Schmidt Charitable Foundation Grant and **The Horticulture Research Institute, 1000 Vermont Ave., NW, Suite 300, Washington DC 20005.**

²Graduate Student, Department of Entomology, Iowa State University, Ames, IA 50011. gre@iastate.edu

³Professor of Horticulture, Joliet Junior College, 1215 Houbolt Road, Joliet, IL, 60431. fmiller@jjc.edu

⁴Research Entomologist, Department of Entomology, Iowa State University, Ames, Iowa, 50011. jwzhu@iastate.edu

⁵Professor of Entomology and Toxicology, Department of Entomology, Iowa State University, Ames, Iowa, 50011. jcoats@iastate.edu

Introduction

Today, there are many new elm cultivars available in the nursery trade, with European and Asian heritage, offering a variety of natural defenses to disease and insect pests. There have been immense efforts in exploring their natural resistance, and plant breeders are developing new cultivars that are not susceptible to Dutch elm disease, elm yellows, verticillium wilt, or insect herbivory.

Some of the most common insect pests on elm include fall cankerworm, *Alsophila pometaria* (Harris), spring cankerworm, *Paleacrita vernata* (Peck), elm leaf beetle, *Pyrrhalta luteola* (Mueller), elm leaf miner, *Fenusa ulmi* (Sundevall), and Japanese beetle, *Popillia japonica* (Newman). More recently, invasive species including generalist feeders like the gypsy moth, *Lymantria dispar* (L.) have raised concern for determining host plant suitability and identifying mechanisms of insect resistance. Elms have various levels of resistance to numerous insect pests (7, 13, 14, 15, 16, 18, 19). More recent work has indicated that plant responses to these insect-herbivore interactions involve plant secondary compounds. There are a wide variety of secondary compounds isolated from plants and include large groups such as alkaloids, glycosides, terpenoids, iridoids, and phenolics.

Phenolic compounds have long been identified as a group of chemicals closely tied to insect-plant interactions (4, 5, 23), but they can also function in protection from UV light, regulation of nutrient cycling within the ecosystem, pollinator attraction, seed dispersal, fungal defense, and allelopathy (30, 12). This large class of compounds can be identified by the presence of a hydroxy-substituted aromatic ring. Some of the more common plant phenolics are grouped as flavonoids, lignins, tannins, and hydroxy-quinones, all of which have been reported in numerous insect-plant relationships. Nishida et al. (21) reported four flavonoids to stimulate oviposition by a citrus-feeding swallowtail, and Harborne (8) noted several flavonoids to be feeding stimulants. For *Boottettix argentatus* (Bruner), a specialist feeder, a surface lignin serves as a feeding stimulant, but it deters other generalist

herbivores (3). Tannins in *Betula resinifera* (Britton) were found to possess high antiherbivore activity (1).

There is a diversity of insect responses to phenolics, and the responses can depend upon the environment, specific concentrations of phenolics that are present, or the physiological state of the receiving insect (26). Past studies have most commonly exhibited a negative feeding response to higher phenolic content (11, 20, 24, 31). However, this is not always the case. Insect attraction to phenolic compounds has also been described (9).

The purpose of this study was to evaluate the host plant suitability of Asian elms for the gypsy moth and compare foliar total phenolic content. Determining total phenolic content is often times one of the preliminary steps in investigating biochemical resistance among other shade trees (2, 10, 28) and could be valuable in comparing the elms. Previous studies have shown that *Ulmus davidiana*, *U. japonica*, *U. wilsoniana*, and *U. propinqua*, elms belonging to the David complex, and the Szechuan elm (*U. szechuanica*) all show moderate to high levels of susceptibility to the generalist insect herbivore, the Japanese beetle (14, 15) suggesting that elm parentage might have an important influence on host plant resistance. The importance of elm parentage is also seen with host plant suitability of the elm leaf beetle, where Asian elms have low levels of suitability for both larval and adult stages (17); however suitability for this insect herbivore is largely influenced by Siberian elm, *U. pumila*, parentage.

Knowledge of certain leaf properties that govern generalist herbivore host plant suitability would be a valuable guide for future elm breeding programs.

Materials and Methods

Measurement of leaf total phenolic content. Thirteen different elms were evaluated in this study: *Ulmus wilsoniana* (Schneid), *U. japonica* (Rehd), *U. glaucescens* (Franch), 'Accolade', 'Triumph', *U. davidiana* (Planch), *U. parvifolia* (Jacq.), *U. szechuanica* (Fang), *U. macrocarpa* (Hance), *U. chenmoui* (Cheng), *U. lamellosa* (C. Wang et S.L. Chang, ex L.K. Fu) (6), *U. castaneifolia* (Hemsl.), and *U. gausseii* (Cheng). Elm leaves from 11 different species and 2 cultivars, with three trees sampled per elm type, were obtained from the Morton Arboretum, Lisle, IL, in July of 2002 and shipped overnight to Iowa State University, Ames, IA, where they were stored at -5°C (23F). Before extraction, the leaves were

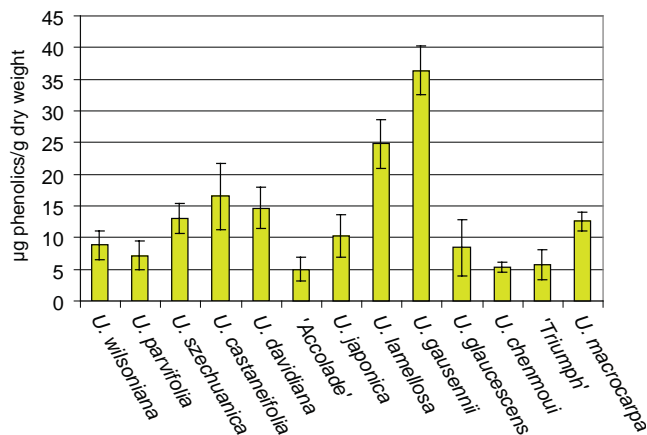


Fig. 1. Total phenolic content of the 11 elm species and 2 cultivars surveyed.

ground to a powder in liquid nitrogen with a chilled mortar and pestle. A 100-mg sample of each was stirred at room temperature for 2 hr in a 20-ml volume of 100% methanol (Fisher Scientific, Pittsburgh, PA). The methanol samples were then filtered and washed with 20 ml of hexane six times before final filtration through a 0.45- μm syringe filter. Each remaining methanol solution was adjusted to a 10-ml volume for total phenolic content measurements by a modified Folin-Ciocalteu procedure described by Torres et al. (29). Samples were analyzed for total phenolic content by mixing 0.1 ml of the methanol sample with 6 ml water. A 0.5-ml volume of Folin-Ciocalteu Reagent (Sigma-Aldrich, St. Louis, MO), was added to the mixture and allowed to stand for at least 1 minute. Next, 1.5 ml of 20% (wt/vol) sodium carbonate solution was added, and the final reaction mixture was brought to a 10-ml final volume with water. Each sample was maintained at 50C (122F) for 2 hours in 25-ml test tubes. The absorbance was measured on a spectrophotometer MV 21 at 765 nm. For comparison, known concentrations of gallic acid (Aldrich) were used to develop a calibration curve.

Leaf toughness and thickness was recorded with the use of a model Digital Force Meter leaf penetrometer (Chatillon, Greensboro, NC). Data were collected on freshly picked leaves from trees at the Morton Arboretum.

Significant differences among the 13 elms were detected by a nested ANOVA using SAS (25). Total phenolic content was analyzed with gypsy moth no-choice growth and developmental studies. A multivariate analysis with PROC GLM (SAS Version 9.1), followed by a univariate analysis was used to search for trends in gypsy moth duration of larval stage (larval longevity), pupal fresh weight, and percentage adult emergence compared with leaf total phenolic content, toughness, and thickness. A correlation matrix was used to equalize the variances between different units of measurement. P-values for variables that showed high levels of correlation were obtained by using PROC CORR, with consideration of parametric and nonparametric methods for estimation (25).

Gypsy moth developmental study. Gypsy moth development on Asian elms (Morton Arboretum, Lisle, IL) was assessed in a laboratory no-choice feeding assay in July of 2001 and previously reported (22). To summarize, individual neonate gypsy moth larvae were placed into a 60-ml (2.5 \times 6.5 cm) plastic tube with a snap cap lid and monitored daily for larval mortality, pupation, and adult emergence. The larvae were provided with fresh leaf material every two days and were maintained in an incubator under a photoperiod of 16:8 (L:D) h at 25C (77F). Foliage was collected from three trees for each of the 13 elm types tested to account for within-species/cultivar variability (30 larvae for each elm). Ten larvae were tested for each of three trees for each of the 13 elms evaluated. Measures of elm suitability for gypsy larvae were defined by larval longevity, pupal fresh weight, and percentage adult emergence. Pupae were weighed within 24 hours of pupation for pupal fresh weight. Natural log transformations were performed on pupal fresh weight and percentage of adult emergence for analysis.

Results and Discussion

Measurement of leaf total phenolic content. Analysis of variance of the total phenolic content of the 13 elms showed significance in the overall model with $P < 0.0001$ (Fig. 1). The

Table 1. Growth and development of gypsy moth on Asian elms in a no-choice laboratory test conducted by Paluch et al. 2008 (22).

Elm	Larval longevity ^{yx}	Pupal fresh weight ^{yx} (g)	Percentage adult emergence ^{yx}
<i>U. castanifolia</i>	6a	0a	0a
<i>U. davidiana</i> ^z	5a	0a	0a
<i>U. chennouii</i>	4a	0a	0a
'Triumph' ^{yz}	7a	0.4a	3a
<i>U. wilsoniana</i> ^z	5a	0a	0a
'Accolade' ^{yz}	6a	0.5ab	3a
<i>U. japonica</i> ^z	4a	0a	0a
<i>U. macrocarpa</i>	4a	0a	0a
<i>U. szechuanica</i> ^z	10ab	0.5ab	3a
<i>U. lamellosa</i>	4a	0.3a	3a
<i>U. glaucescens</i>	4a	0a	0a
<i>U. gausseii</i>	4a	0.2a	13ab
<i>U. parvifolia</i>	5a	0a	0a
Preferred hosts			
<i>Malus spp.</i>	16b	0.6b	93c
<i>Quercus palustris</i>	10ab	0.8b	70bc

^zElms belonging to, or closely associated with the David complex.

^yAverages from 10 replicates per elm, 3 trees per species/cultivar (30 larvae total).

^xValues within columns followed by the same letter are not significantly different. (*, P < 0.05; Student-Newman-Keuls multiple comparison test).

Gausseii elm (*U. gausseii*) had the highest phenolic content and 'Accolade' (*U. japonica-wilsoniana*) was the lowest.

Gypsy moth developmental study. Table 1 reports the summary of gypsy moth growth and development from a 2008 paper. Larvae were only able to complete development on 5 of the 13 elms tested, including *U. szechuanica*, *U. gausseii*, *U. lamellosa*, 'Accolade', and 'Triumph'. However, there were no significant differences between elms in larval longevity, pupal fresh weight, or percentage adult emergence (Table 1).

The 11 elm species and 2 cultivars surveyed are significantly different in total phenolic content. Two of the elms sampled had foliage containing the highest amount of total

Table 2. Leaf thickness and toughness of Asian elms.

Elm	Thickness ^y (cm)	Toughness ^y (g)	
		Inner	Outer
<i>U. castanifolia</i>	0.041c	31.00c	28.60d
<i>U. davidiana</i> ^z	0.027b	31.69d	28.15d
<i>U. chennouii</i>	0.037bc	28.19c	24.06c
'Triumph' ^{yz}	0.031b	47.67f	49.03g
<i>U. wilsoniana</i> ^z	0.030b	33.46d	30.01e
'Accolade' ^{yz}	0.021a	33.23d	34.91f
<i>U. japonica</i> ^z	0.018a	12.94a	10.58a
<i>U. macrocarpa</i>	0.037bc	37.86e	44.76g
<i>U. szechuanica</i> ^z	0.022a	23.70b	24.65c
<i>U. lamellosa</i>	0.038bc	59.02g	59.93h
<i>U. glaucescens</i>	0.021a	22.61b	19.20b
<i>U. gausseii</i>	0.017a	12.80a	12.62a
<i>U. parvifolia</i>	0.027b	38.68e	30.37e

^zElms belonging to, or closely associated with the David complex.

^yValues within columns followed by the same letter are not significantly different. (*, P < 0.05; Student-Newman-Keuls multiple comparison test).

phenolic content, *U. szechuanica* and *U. gausseii*, were not found significantly suitable for gypsy moth growth and development among the Asian elms sampled in this study. However, these data are representative of leaf samples collected from trees at two time points in 2001 and 2002, and thus, the results do not account for variability between years. Neither of the remaining leaf variables tested (i.e. leaf toughness and thickness) (Table 2) were significantly related to elm host plant susceptibility to the gypsy moth. Other chemical or physical factors may be responsible for mediating generalist insect feeding behavior (22).

Qualitative and quantitative data on elm foliar phenolics would be beneficial to identify differential activities among the common phenolic groups including flavonoids, lignins, tannins, phenolic glycosides, etc. as they relate to gypsy moth growth and development on elm. Other studies have reported an inverse relationship between phenolic glycoside content and gypsy moth larval development (32, 33). Feeding trials with tremulacin, which significantly reduced gypsy moth feeding rates and efficiency with ingested food (34) as well as the flavonoid quercetin (35), highlight the negative impacts of specific phenolics on gypsy moth larval development. However, there are also accounts of positive associations between levels of condensed tannins and gypsy moth performance (34, 36). These data provide examples of how foliar phenolics can impact the gypsy moth larval stage in different systems. Further work comparing the phenolic composition of elm leaves with respect to parentage and susceptibility to the gypsy moth would aid in identifying key chemical factors. Gypsy moths feed on numerous other types of plants, and there are most likely different biological, chemical, and physical variables for each host that determine the insects' preferences. Our current study highlights differences in generalist insect feeding behaviors, which will lead to a more effective pest management strategies incorporating host-plant resistance.

Literature Cited

1. Ayres, M.P., T.P. Clausen, S.F. MacLean, Jr., A.M. Redman, and P.B. Reichardt. 1997. Diversity of structure and antiherbivore activity in condensed tannins. *Ecology* 78:1696–1712.
2. Bergelson, J., S. Fowler, and S. Hartley. 1986. The effects of foliage damage on casebearing moth larvae, *Coleophora serratella*, feeding on birch. *Ecol. Entomol.* 11:241–250.
3. Chapman, R.F., E.A. Bernays, and T. Wyatt. 1988. Chemical aspects of host-plant specificity in three *Larrea*-feeding grasshoppers. *J. Chem. Ecol.* 14:561–579.
4. Coley, P.D., J.P. Bryant, and F.S. Chapin. 1985. Resource availability and plant antiherbivore defense. *Science* 230:895–899.
5. Feeny, P.P. 1976. Plant apparency and chemical defense. *Recent Adv. Phytochem.* 10:1–40.
6. Fu, Li-Guo. 1980. Studies in the genus *Ulmus* in China. Northeastern Forest Institute, Harbin, Heilongjiang. 40 pp.
7. Hall, R.W., A.M. Townsend, and J.H. Barger. 1987. Suitability of thirteen different host species for elm leaf beetle, *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae). *J. Environ. Hort.* 5:143–145.
8. Harborne, J.B. 1988. *Introduction to Ecological Biochemistry*, 3rd ed. Academic Press, London. 356 pp.
9. Hartley, S.E. and J.L. Lawton. 1987. Effects of different types of damage on the chemistry of birch foliage, and the responses of birch feeding insects. *Oecologia* 74:432–437.
10. Hartley, S.E. and R.D. Firn. 1989. Phenolic biosynthesis, leaf damage, and insect herbivory in birch (*Betula pendula*). *J. Chem. Ecol.* 15:275–283.

11. Haukioja, E., P. Niemelä, and S. Sirén. 1985. Foliage phenols and nitrogen in relation to growth, insect damage, and ability to recover the defoliation, in the mountain birch, *Betula pubescens* spp. *tortuosa*. *Oecologia* 65:214–222.
12. Lois, R. 1994. Accumulation of UV-absorbing flavonoids induced by UV-radiation in *Arabidopsis thaliana* L. *Planta* 194:498–503.
13. Miller, F. and G. Ware. 1994. Preference for and suitability of Asian elm species and hybrids for the adult elm leaf beetle (Coleoptera: Chrysomelidae). *J. Environ. Hort.* 12:231–235.
14. Miller, F., G. Ware, and S. Jerdan. 1999. Feeding preference of adult Japanese beetles (Coleoptera: Scarabaeidae) for Asian elms species and their hybrids. *J. Econ. Entomol.* 92:421–426.
15. Miller, F. 2000. Insect resistance of elm genotypes, pp. 137–54. *In: Dunn, C. P. (ed), The Elms: Breeding, Conservation, and Disease Management.* Kluwer Academic Publishers, Boston.
16. Miller, F., G. Ware, and J. Jackson. 2001. Preference of temperate Chinese elms (*Ulmus* spp.) for the adult Japanese beetle (Coleoptera: Scarabaeidae). *J. Econ. Entomol.* 94:445–448.
17. Miller, F. and G. Ware. 2001. Host suitability of Asiatic elm species and hybrids for larvae and adults of the elm leaf beetle (Coleoptera: Chrysomelidae). *J. Arboriculture* 27:118–125.
18. Miller, F., K. Malmquist, and G. Ware. 2001. Evaluation of Asian, European, and North American elm (*Ulmus* spp.) biotypes to feeding by spring and fall cankerworms. *J. Environ. Hort.* 19:216–221.
19. Miller, F., K. Malmquist, and G. Ware. 2003. Resistance of Ulmaceae to feeding by the adult elm leaf beetle (Coleoptera: Chrysomelidae). *J. Arboriculture* 29:98–103.
20. Niemela, P., E.M. Aro, and E. Haukioja. 1979. Birch leaves as a resource for herbivores. Damage-induced increase in leaf phenols with trypsin-inhibiting effects. *Rep. Kevo Subartic Res. Stat.* 15:37–40.
21. Nishida, R., T. Ohsugi, S. Kokubo, and H. Fukami. 1987. Oviposition stimulants of a citrus-feeding swallowtail butterfly, *Papilio xuthus*. *Experientia* 43:342–344.
22. Paluch, G., F. Miller, J. Zhu, and J. Coats. 2008. Influence of elm foliar chemistry for the host suitability of the Japanese beetle, *Popillia japonica*, and the gypsy moth, *Lymantria dispar*. *J. Agric. Urban Entomol.* 23:209–223.
23. Rhoades, D. F. and R.G. Cates. 1976. Toward a general theory of plant antiherbivore chemistry. *Recent Adv. Phytochem.* 10:168–213.
24. Rossiter M.C., J.C. Schultz, and I.T. Baldwin. 1988. Relationships among defoliation, red oak phenolics and gypsy moth growth and reproduction. *Ecology* 69:267–277.
25. SAS Institute. SAS system for Windows version 9.1. 2003. SAS Institute Cary, NC.
26. Scriber, J.M. and F. Slansky, Jr. 1981. The nutritional ecology of immature insects. *Annu. Rev. Entomol.* 26:183–211.
27. Sommer, R.O. and J. Summit. 2000. Public involvement with elms and other species. pp. 318–330. *In: Dunn C.P. (Ed). The Elms: Breeding, Conservation, and Disease Management.* Kluwer Academic Publishers, Boston, MA. 384 pp.
28. Suomela, J., V. Ossipov, and E. Haukioja. 1995. Variation among and within mountain birch trees in foliage phenols, carbohydrates, and amino acids, and in growth of *Epirrita autumnata* larvae. 1995. *J. Chem. Ecol.* 21:1421–1446.
29. Torres, A.M., T. Mau-Lastovicka, and R. Rezaaiyan. 1987. Total phenolic and high-performance liquid-chromatography of phenolic acids of avocado. *J. Agric. Food Chem.* 35:921–925.
30. Waterman, P.G. 1992. Roles for secondary metabolites in plants. pp. 255–275. *In: Chadwick D.J. and J. Whelan (Eds). Secondary metabolites: Their Function and Evolution.* Wiley, Chichester, 318 pp.
31. Wratten, S.D., P.J. Edwards, and I. Dunn. 1984. Wound-induced changes in the palatability of *Betula pubescens* and *Betula pendula*. *Oecologia* 61:372–375.
32. Lindroth, R.L. and J.D.C. Hemming. 1990. Responses of gypsy moth (Lepidoptera: Lymantriidae) to tremulacin, an aspen phenolic glycoside. *Environ. Entomol.* 19:842–847.
33. Lindroth, R.L. and A.V. Weisbrod. 1991. Genetic variation in response of the gypsy moth to aspen phenolic glycosides. *Biochem. Syst. Ecol.* 19: 97–103.
34. Hemming, J.D.C. and R.L. Lindroth. 1995. Intraspecific variation in aspen phytochemistry: effects on performance of gypsy moths and forest tent caterpillars. *Oecologia* 103:79–88.
35. Beninger, C.W. and M.M. Abou-Zaid. 1997. Flavonol glycosides from four pine species that inhibit early instar gypsy moth (Lepidoptera: Lymantriidae) development. *Biochem. Sys. Ecol.* 25:505–512.
36. Kleiner, K.W. and M.E. Montgomery. 1994. Forest stand susceptibility to the gypsy moth (Lepidoptera: Lymantriidae): species and site effects on foliage quality to larvae. *Environ. Entomol.* 23:699–711.