Structural and Photosynthetic Compensation for Leafminer (Diptera: Agromyzidae) Injury in Lima Beans

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ABSTRACT Palisade mesophyll tissue removed from mature leaves of *Phaseolus lunatus* L. by the leafmining herbivore *Liriomyza trifolii* (Burgess) was replaced with photosynthetically active cells, permitting virtually complete recovery from injury. No significant differences in biomass production or levels of ribulose-1,5-bisphosphate carboxylase were observed between damaged and control plants. Decreases in photosynthesis did not exceed 10% for leaves with approximately one-fourth of the leaf area mined. Development of other, photosynthetically inactive callus cells along vascular bundles and frass deposits served to compartmentalize leafmines, generating a suitable microclimate for regeneration of cells as well as preventing intrusion of disease inoculum and arthropod pests. Such cellular regrowth not only benefits the host, but provides substantial advantages for facultatively cannibalistic larvae that are incapable of relocating to undamaged leaves.

KEY WORDS *Liriomyza trifolii*, photosynthesis, compensation, lima beans

ALTHOUGH MANY studies have documented an increase in plant yield after either actual or simulated damage caused by herbivorous insect populations (Southwood & Norton 1973, Kolodny-Hirsch & Harrison 1982), relatively few studies have demonstrated the basis of plant compensation for such injury. In studies where increased cell division has been demonstrated in response to damage, the potential for compensation by leaves was related to the stage of leaf development at the time of injury; growth from meristematic tissue ceased when immature leaves reached 25–75% of mature size (Bardner & Fletcher 1974). Any additional growth then occurred in response to cell enlargement (Wall & Berberet 1979). We report here an unusual system where rapid structural compensation for leafmining injury in mature foliage leads to the maintenance of high levels of photosynthetic activity and provides considerable advantages to both the host plant and an insect herbivore.

Materials and Methods

The potential for plant compensation for herbivore damage was investigated by allowing adult *Liriomyza trifolii* (Burgess), a polyphagous agromyzid, to oviposit on 'Henderson Bush' lima beans, *Phaseolus lunatus* L. Physical recovery from tissue damage resulting from larval development was then assessed. Experimental plants were germinated in vermiculite, transplanted into U.C. soil mixture (Matkin & Chandler 1957) in pots (12 cm) at 1 wk after germination, and supplied with a slow-release fertilizer. At 10 d after transplanting, 20 plants were selected for experiments requiring destructive sampling. One primary leaf of each was exposed to leafminers for oviposition. Shoots developing during the experiment were removed weekly to maximize the potential for cell elongation or regrowth due to removal of apical dominance (Carmi & Koller 1979, Kolodny-Hirsch & Harrison 1982). Plant material was dried at ca. 70°C for ≥72 h before weighing. Leaf area mined per instar was documented by tracing 44 mines (total area mined from hatching to exit) and determining the area of the tracings using a grid with millimeter increments. Leaves containing mines of selected ages were abscised, sectioned to 1–2 mm on wet blotting paper, and photographed immediately with a photomicroscope (Wild M400). Specimens for thin sectioning were fixed in formaldehyde/alcohol/acetic acid for 24 h, embedded in methacrylate plastic, sectioned with an ultramicrotome at 3–4 μm, and then stained with toluidine blue. Photographs were taken using a photomicroscope (Zeiss Model III).

Photosynthesis rates (milligrams CO_2 assimilated$\cdot s^{-1}\cdot m^{-2}$) of infested and uninfested primary leaves of test plants and uninfested leaves of control plants were measured with a portable photosynthesis system (Li-Cor 6000). Measurements were taken in the greenhouse at leaf temperatures of 28 ± 1.5°C, with sunlight supplemented with artificial light (metal halide) to maintain an average of 840 $\mu E\cdot s^{-1}\cdot m^{-2}$ throughout the sampling period. Photoperiod throughout the experiment was 14:10 (L:D). Data were collected at four intervals: during the first instar, during the second instar, immediately after emergence of third instars from leaves, and 1 wk after larvae had exited the leaves.

For photosynthesis tests, 10 pairs of plants were evaluated, with one leaf of a plant in each pair infested at the level of one larva per 2-cm^2 leaf

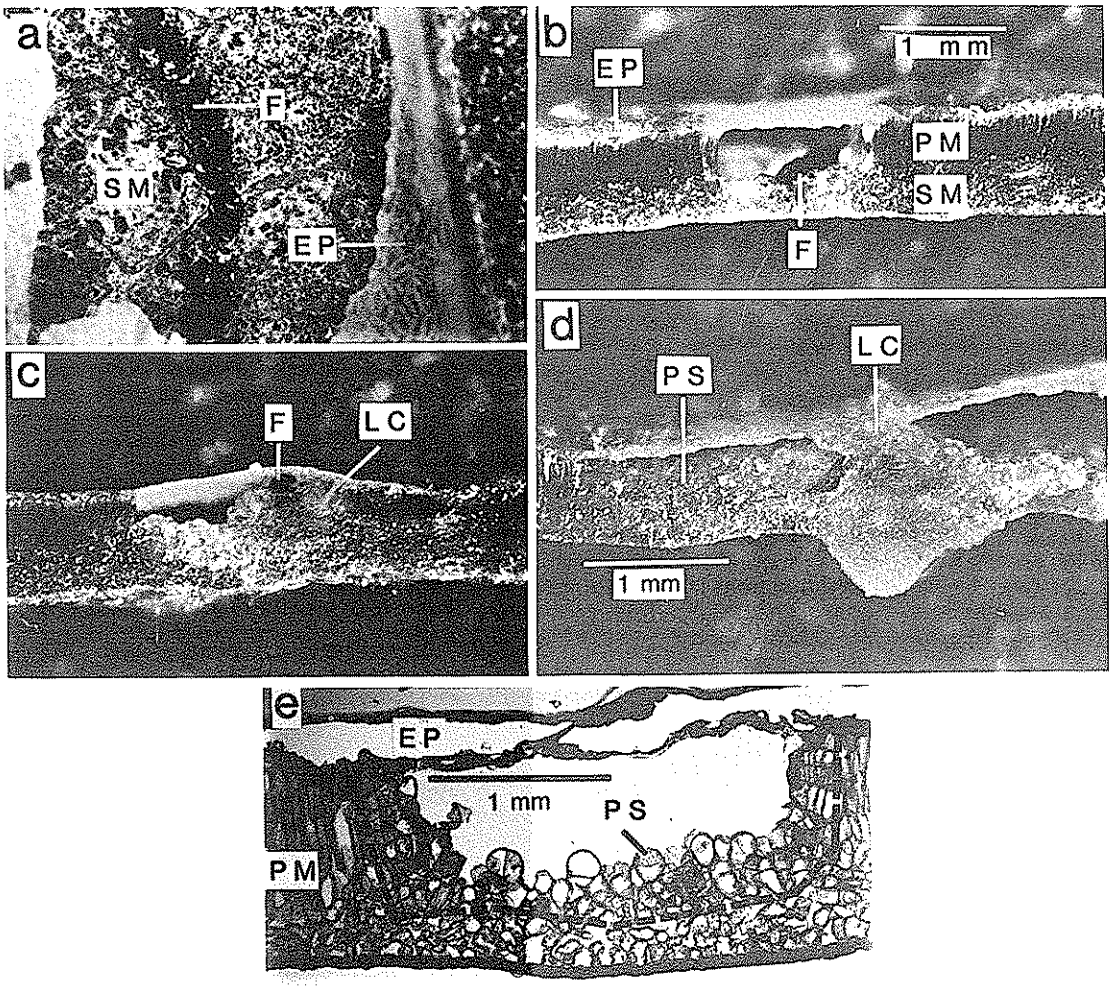

Fig. 1. Growth of restitutive tissue of *P. lunatus* within mines of *L. trifolii*. (a) Surface view of a third-instar mine with dorsal epidermis removed to show frass "band" on the spongy parenchyma; (b) cross section of third-instar mine in palisade parenchyma; (c) cross section of a large, irregular-shaped callus originating from spongy mesophyll beneath the frass band (note that the frass has been lifted as the callus tissue developed); (d) section of a third-instar leafmine compartmentalized by the growth of callus over a vascular bundle (note the closely packed cells filling the left compartment; the right compartment is immediately adjacent to the exit hole cut by the leafminer and is open to the atmosphere); (e) 3-μm section through a third-instar leafmine showing chloroplasts in the new tissue (the area of the original mine is marked with the dotted line). PM, palisade mesophyll; SM, spongy mesophyll; EP, epidermal tissue; F, frass deposit; LC, large callus; PS, photosynthetically active new tissue.

area. The second plant served as a control. Control plants were matched individually with test plants for comparable photosynthetic activity 1 d before infestation. The entire experiment was replicated twice. All new plant growth was harvested and weighed for analysis of biomass production. Comparisons of photosynthetic activity and biomass production between treatments were analyzed statistically by a paired t test. Data were analyzed as a percentage of net photosynthesis rate of damaged plants relative to that of uninfested control plants (100%).

Ribulose-1,5-bisphosphate carboxylase–oxygenase (RuBPase) levels in damaged and control leaves were measured using the technique of Lorimer et al. (1977) after emergence of third instars from leaves. Three samples were taken from each of four test plants and four control plants (four replicates with a total of 12 samples per treatment) that were grown and infested as described for the previous test. Data were analyzed using a two-sample t test.

Results and Discussion

Although a variety of physiological mechanisms are likely to play a role in plant compensation for herbivore damage (McNaughton 1979), a conspic-

uous plant response in the leafminer/lima bean system was the formation of restitutive tissue at the wound site. In *P. lunatus*, as in most other hosts, *L. trifolii* feeds primarily in the palisade parenchyma tissue (Spencer & Stegmeier 1973, Parrella et al. 1985), leaving a continous band of frass at the top, sides, or, more commonly, the bottom of the mine (Fig. 1 a and b). Within 7 d after larval mining, the spongy mesophyll on the bottom of the mines developed cell proliferations, and callus formed in abundance where frass was in contact with the cell tissue (Fig. 1c). Callus also was generated rapidly along veins that were crossed by the mines (Fig. 1d). Although vascular cells generally were not directly wounded by larval feeding, the adaxial bundle sheath cells connecting the veins to the palisade parenchyma frequently were disrupted. This disruption was usually associated with rapid callus formation. The close proximity of vascular tissue previously has been reported to enhance the wound response (Lipetz 1970).

Thin sections revealed that callus forming around both frass and vascular bundles consisted of colorless, densely packed cells. These cells did not develop the capacity for photosynthesis within the observation period of 25 d after larvae exited the leaves, but the irregularly shaped cells developing from the spongy parenchyma formed chloroplasts (Fig. 1e). Although these cells did not contain as dense a concentration of chloroplasts as the tissue they replaced (based on a subjective evaluation from all leaf sections made), comparisons of photosynthetic activity between damaged leaves and primary leaves of undamaged plants with comparable preinfestation photosynthesis rates indicated that decreases in photosynthesis were <10% and were not significant ($P > 0.05$, t test) (Fig. 2). In contrast, even a single *L. trifolii* mine per leaf (one larva per 12 cm^2) caused >40% reduction in photosynthetic activity in celery, where leaves did not recover structurally from leafminer injury (Trumble et al. 1985). In tomato foliage, photosynthesis was reduced by >60% even when <20% of the leaf area was mined (Johnson et al. 1983). Simulated insect feeding on other plant species has also produced reductions in photosynthetic activity of remaining tissue of damaged leaves (Hall & Feree 1976).

Because increases in photosynthesis in source leaves (i.e., leaves remaining after partial defoliation) are often associated with an increase in leaf protein content (McNaughton 1979), and RuBPase accounts for at least 50% of leaf protein (Edwards & Walker 1983), we tested the hypothesis that photosynthetic activity in the remaining tissue of damaged leaves could have been elevated because of an increase in RuBPase levels. However, the data showed no significant differences in RuBPase activity between leaves of damaged and undamaged plants (control mean ± SD = 29.8 ± 8.18; treatment mean = 25.15 ± 11.87 μmol $CO_2 \cdot s^{-1} \cdot m^{-2}$). This suggests that RuBPase production was neither stimulated by defoliation nor acting as an important limiting factor, and that other photosynthesis-enhancing mechanisms were operating to maintain equivalent levels of net carbon dioxide assimilation in the damaged leaves.

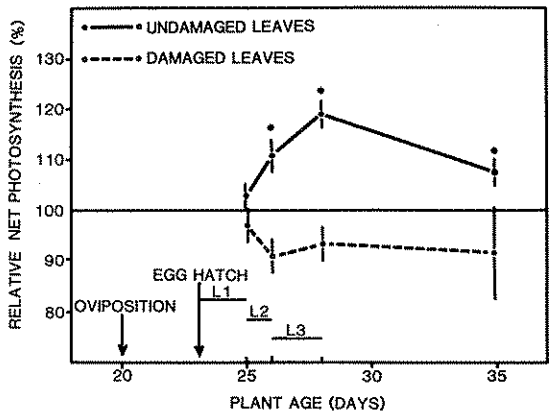

Fig. 2. Relative net photosynthesis rates of primary leaves of *P. lunatus* damaged by *L. trifolii* mining opposite, undamaged leaves from the same plants. Data are expressed as a percentage of net photosynthesis rate (milligrams CO_2 assimilated$\cdot s^{-1} \cdot m^{-2}$) of damaged plants relative to uninfested control plants (100%) selected for equivalent photosynthetic activity when plants were 19 d old. Asterisks above individual points signify a significant difference ($P < 0.05$, t test) from control leaves; damaged leaves were not significantly different from control leaves at any time. Vertical lines indicate standard errors. L1–L3 refer to the occurrence of the three instars of *L. trifolii* within the test leaves.

In contrast, net photosynthesis rates were significantly increased in opposite, undamaged leaves of the infested plants as compared with the corresponding control plants (Fig. 2). This increase in photosynthetic activity in uninfested leaves of the damaged plants was consistent with previous reports, which suggested that partial defoliation could enhance photosynthesis by increasing chlorophyll content (Satoh et al. 1977), enhancing cytokinin levels (Wareing et al. 1968), or causing changes in mesophyll resistance (von Caemmerer & Farquhar 1984). In damaged plants, which produced new tissues in amounts not significantly different from those of control plants ($P > 0.05$, t test; range of controls, 0.73–0.87 g dry weight; range of damaged plants, 0.65–0.78 g dry weight), the increased assimilate demand on remaining leaf area also may have enhanced photosynthetic activity (Gifford & Marshall 1973). In the alfalfa canopy, such compensatory responses maintain net photosynthesis in plants heavily damaged by the alfalfa blotch leafminer, *Agromyza frontella* (Rondani), at levels equivalent to those in undamaged plants (P. Daley, Lawrence Livermore National Laboratory, personal communication).

The overall benefit to the lima bean plant in forming restitutive tissue encompassses aspects of

disease and pest avoidance as well as direct compensation for leafminer injury. Callus ridges developing over vascular bundles and frass served to compartmentalize the mines (Fig. 1d). The process started before larvae left the leaves and helped to minimize the surface area accessible to disease inoculum, as well as reducing potential microhabitats for thrips, which are known to feed in abandoned mines (Hering 1951). Because the epidermal cells and cuticle were usually not damaged, closed and humid chambers were created that were instrumental in providing the microclimate necessary for a more restitutive wound response than simple lignification of cell walls typical of wound-closing mechanisms in most leaves (Kahl 1982). As early as 1902, similar callus formation was noted in mines within young (immature) leaves caused by other leafmining insects, and the regrowth was ascribed to both higher temperatures and increased relative humidity in the leafmines (Hering 1951).

Only small, restrictive areas of leafmines in our study were subject to complete desiccation and subsequent loss from the functional portions of the leaves. This occurred when larvae substantially wounded the spongy parenchyma or breached the epidermis. Exit holes at the terminal ends of the mines also became desiccated, but the total leaf area permanently lost was generally <10% of any given mine (based on visual evaluation of mines and leaf sections where average individual mine size [$\bar{x} \pm$ SD] = 0.43 $\pm$ 0.08 cm^2; n = 44) due, in part, to the aforementioned plant compensatory response.

The advantages of feeding in a host capable of such a response are considerable for leafminer larvae. The potential for cannibalistic behavior of larvae (Parrella 1983) is reduced substantially when mines are compartmentalized because the opportunity for larvae to enter neighboring mines and effectively locate the occupant or another transient larva is reduced. Reductions in larval interactions would be particularly advantageous for early instars, where the mines fill rapidly (2–4 d) with regenerated tissue. Even if the restitutive tissue in the old mines proved nutritionally or physically unsuitable for leafminer larvae, the regenerated host leaf is less succeptible to desiccation and would, therefore, remain capable of supporting leafminer development for an extended period. Thus, this novel herbivore/plant system will be of interest to scientists investigating the ecological implications of plant/insect interactions, as well as the source-sink relationships in assimilate demand of plant structures.

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References Cited

Bardner, R. & K. E. Fletcher. 1974. Insect infestations and their effects on the growth and yield of field crops: a review. Bull. Entomol. Res. 64: 141–160.

Carmi, A. & D. Koller. 1979. Regulation of photosynthetic activity in the primary leaves of bean (*Phaseolus vulgaris* L.) by materials moving in the water-conducting system. Plant Physiol. 64: 285–288.

Edwards, G. & D. A. Walker. 1983. C3, C4: mechanisms, and cellular and environmental regulation, of photosynthesis. Univ. of California, Berkeley.

Gifford, R. M. & C. Marshall. 1973. Photosynthesis and assimilate distribution in *Lolium multiflorum* Lam. following differential tiller defoliation. Aust. J. Biol. Sci. 26: 517–526.

Hall, F. R. & D. C. Feree. 1976. Effects of insect injury simulation on photosynthesis of apple leaves. J. Econ. Entomol. 69: 245–248.

Hering, E. M. 1951. Biology of the leafminers. Junk, The Hague.

Johnson, M. W., S. C. Welter, N. C. Toscano, I. P. Ting & J. T. Trumble. 1983. Reduction in tomato leaflet photosynthesis rates by mining activity of *Liriomyza sativae* (Diptera: Agromyzidae). J. Econ. Entomol. 76: 1061–1063.

Kahl, G. 1982. Molecular biology of wound healing: the conditioning phenomenon, pp. 211–267. *In* G. Kahl [ed.], Molecular biology of plant tumors. Academic, New York.

Kolodny-Hirsch, D. M. & F. P. Harrison. 1982. Comparative damage and leaf area consumption by the tobacco budworm and corn earworm on Maryland tobacco. J. Econ. Entomol. 75: 168–172.

Lipetz, J. 1970. Wound-healing in higher plants. Int. Rev. Cytol. 27: 1–28.

Lorimer, G. H., M. R. Badger & T. J. Andrews. 1977. D-ribulose-1,5-bisphosphate carboxylase–oxygenase: improved methods for the activation and assay of catalytic activities. Anal. Biochem. 78: 66–75.

Matkin, O. A. & P. A. Chandler. 1957. The U.C.-type soil mixes, pp. 68–85. *In* K. Baker [ed.], The U.C. system for producing healthy container-grown plants. Calif. Agric. Exp. Stn. Man. 23: 1–23.

McNaughton, S. J. 1979. Grazing as an optimization process: grass–ungulate relationships in the Serengeti. Am. Nat. 113: 691–703.

Parrella, M. P. 1983. Intraspecific competition among larvae of *Liriomyza trifolii* (Diptera: Agromyzidae): effects on colony production. Environ. Entomol. 12: 1412–1414.

Parrella, M. P., V. P. Jones, R. R. Youngman & L. M. LeBeck. 1985. Effect of leaf mining and leaf stippling of *Liriomyza* species on photosynthetic rates of chrysanthemum. Ann. Entomol. Soc. Am. 78: 90–93.

Satoh, M., P. E. Kriedeman & B. R. Loveys. 1977. Changes in photosynthetic activity and related processes following decapitations in mulberry trees. Physiol. Plant. 41: 203–210.

Southwood, T. R. E. & G. A. Norton. 1973. Economic aspects of pest management strategies and deci-

sions, pp. 153–184. *In* P. Geier, L. Clark, D. Anderson & H. Nix [eds.], Insects: studies in population management. Ecological Society of Australia Memoirs, Sidney.

Spencer, K. A. & C. E. Stegmeier. 1973. Agromyzidae of Florida. Florida Department of Agriculture & Consumer Services, Gainesville.

Trumble, J. T., I. P. Ting & L. Bates. 1985. Analysis of physiological, growth, and yield responses of celery to *Liriomyza trifolii*. Entomol. Exp. Appl. 38: 15–21.

von Caemmerer, S. & G. D. Farquhar. 1984. Effects of partial defoliation, changes of irradiance during growth, short-term water stress and growth at enhanced $p(CO_2)$ on the photosynthetic capacity of leaves of *Phaseolus vulgaris* L. Planta 160: 320–329.

Wall, R. G. & R. C. Berberet. 1979. Reduction in leaf area of Spanish peanuts by the rednecked peanutworm. J. Econ. Entomol. 72: 671–673.

Wareing, P. F., M. M. Khalifa & K. J. Treharne. 1968. Rate-limiting processes in photosynthesis at saturating light intensities. Nature (London) 220: 453–457.

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