Ann. appl. Biol. (1991), **119**, 251–256
Printed in Great Britain

The effect of maize leaf damage on the survival and growth rate of *Rhopalosiphum padi*

By S. MORSE[1], S. D. WRATTEN[2], P. J. EDWARDS and H. M. NIEMEYER[3]
Department of Biology, Building 62, The University, Southampton SO9 3TU, UK

(*Accepted 1 July 1991*)

Summary

Two different types of artificial leaf damage, crushing and cutting, were assessed for their effects on the growth and survival of *Rhopalosiphum padi* caged on maize (cv. LG11) leaves. Crushing significantly reduced aphid growth rate by up to 23% and their survival by five to six times, while cutting had no significant effects. Although the area of tissue involved was lower for crushing relative to cutting (*c.* 5% and 10% respectively), the greater level of cellular damage inflicted by crushing may have resulted in more phytochemical changes. These effects suggest that the feeding of one herbivore guild such as leaf chewers may adversely affect the performance of another, such as phloem feeders.

Key words: Maize, damage, *Rhopalosiphum padi*

Introduction

There are many reports of leaf damage adversely influencing the growth of insect herbivores. Many of the examples (Haukioja, 1980; Edwards & Wratten, 1982; Wratten, Edwards & Dunn, 1984; West, 1985) refer to Lepidoptera and to tree species such as birch (*Betula* spp.) and oak (*Quercus* spp.), but the effects have also been observed with herbaceous crop plants such as tomato (Green & Ryan, 1972*a*; Ryan, 1978; Edwards, Wratten & Cox, 1985) and cotton (Karban, 1985; Croxford, Wratten & Edwards, 1989).

Some workers have proposed that the injured plants produce toxic compounds as a form of induced defence to insects, and that such changes are of significance in the population dynamics of the herbivore and the plant (Ryan, 1978; Edwards & Wratten, 1987; Wratten, Edwards & Winder, 1988). Others, however, argue that the herbivores simply respond to changes in food quality brought about in a way unrelated to a direct defensive response to the insects, and that such changes are too small to be of ecological importance (Fowler & Lawton, 1985).

The phytochemical changes which occur after leaf damage have been investigated by some workers. An increase in the concentration of toxins such as phenolics (Baldwin & Schultz, 1983; Wratten *et al.*, 1984; Hartley & Lawton, 1987), alkaloids (Baldwin, 1988), proteinase inhibitors (Ryan, 1978) and hydroxamic acids (Gutiérrez, Castañera & Torres, 1988; Niemeyer *et al.*, 1989), as well as changes in amino acid content (Ciepiela, 1989), have all been reported in damaged plant tissue. In many of these examples artificial damage was also capable of producing the observed responses, but in the case of hydroxamic acids only insect-inflicted damage has been studied.

[1]Present address: School of Development Studies, University of East Anglia, Norwich, Norfolk NR4 7TJ, UK
[2]Author to whom correspondence should be sent
[3]Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

The aim of the current work was to examine the effects of two different types of damage to maize leaves on the growth rate and survival of *Rhopalosiphum padi* L., the bird-cherry aphid, a species which can occur in large numbers on maize in Western Europe and elsewhere (Hand & Carrillo, 1982).

Materials and Methods

Four experiments were carried out, three of which compared two forms of leaf damage (crushing and cutting) on the performance of aphids caged only on the damaged leaves. In the fourth experiment, test aphids were caged on the damaged and undamaged leaves of damaged plants. In each experiment, undamaged controls were included (see Table 1).

The experiments were conducted in a glasshouse kept at approximately 20 °C and a 16 h photoperiod (natural light supplemented with artificial light between 06.00 and 08.00 and 16.00 to 22.00). Four maize seeds (cv. LG11, a temperate hybrid) were planted per pot in John Innes No. 2 compost, and the pots were arranged in a completely randomised design (two treatments, undamaged and damaged, and either 10, 15 or 20 pots as replicates; see Table 1).

At 10 to 13 days after planting (d.a.p.), the plants were thinned to leave one per pot. Care was taken to ensure that all the plants left were of a similar growth stage. The appropriate type of damage (crushing or cutting) was then inflicted upon the third leaves to emerge. The plants in the remaining treatment were left undamaged as controls.

Leaf crushing. The maize leaves were crushed using the "rod and file" method outlined by Green & Ryan (1972*b*). A file was placed behind the leaf to be damaged, then the flat end of a wooden rod was pushed down onto the upper leaf surface so as to crush the tissue between the file and the rod. This resulted in an approximately circular area of damage (diameter of about 0.7 cm representing *c*. 5% of the total leaf area), with much of the crushed tissue still in place. Such damage was considered to be closer to that suffered by plants when attacked by insect herbivores, such as newly hatched lepidopteran larvae, than punching leaf holes or cutting the lamina.

Cutting. To simulate damage by larger larvae, leaves were cut with a pair of sharp scissors, making a "V" shape from the edge of the leaf. The tip of the "V" almost reached the central vein. The cut part of the leaf was then removed. This type of damage caused a much greater level of tissue loss (about 10% of total leaf area) relative to the crushing, although fewer damaged cells were left on the plant.

Aphid performance. Immediately after damage, individual aphids (2nd instar nymphs) were weighed and placed in a clip cage attached to the abaxial surface of damaged and undamaged leaves. These aphids were taken directly from a main culture on barley (cv. Golden Promise)

Table 1. *Methods used to study the effects of artificial damage on the growth rate of* Rhopalosiphum padi

Experiment	Form of damage	Number of replicates (plants)	Timings (d.a.p.)	
			Aphid placement	Final weighing
1	Crushing	15	11	13
2	Crushing	20	10	12
3	Cutting	15	13	15
4	Crushing	10	12	14

Experiments 1–3: aphids caged only on the damaged (3rd) leaves. Experiment 4: aphids caged on the damaged (3rd) and undamaged (2nd) leaves.

kept in Perspex ® cabinets in a culture room based on the design of Scopes, Randall & Biggerstaff (1975). One aphid was placed inside each cage, and this was attached about 1.5 to 2.0 cm below the area of damage (i.e. between the damage and the ligule), so the aphid was not in direct contact with the damaged area or in its immediately vicinity.

The aphids were checked for survival 48 h after placement and the surviving aphids were removed and weighed. There was usually some reduction in replicates due to missing aphids. The aphid weights were then used to calculate the mean relative growth rate (M.R.G.R., μg/ μg/day; Adams & van Emden, 1972) for the 48 h period as follows:

$$\text{M.R.G.R.} = \frac{\log_e (\text{final weight}, \mu\text{g}) - \log_e (\text{initial weight}, \mu\text{g})}{2}$$

Aphids on damaged plant caged only on the damaged leaves. In three of the experiments, aphids were caged on the third leaves (damaged and undamaged) of all the maize plants. Thus there were either 15 or 20 undamaged and damaged maize plants each with a single aphid in a clip cage.

Aphids on damaged plant caged on damaged and undamaged leaves. In the fourth experiment, aphids were placed in clip cages attached to the second (undamaged) and third leaves (damaged and undamaged) to emerge of all the plants. There were 10 replicates of each treatment.

Results

Aphids caged only on the damaged leaves. In the first two experiments of this group, when leaves were crushed, there was substantial mortality of the aphids caged on the damaged leaves. The numbers which survived or died over the 48 h of caging are shown in Fig. 1. No aphid mortality was recorded on the undamaged plants of the first experiment. The aphid mortality was significantly higher on the damaged leaves in the second experiment relative to those caged on undamaged leaves (χ^2, with Yates' correction = 7.11; D.F. = 1; $P < 0.01$).

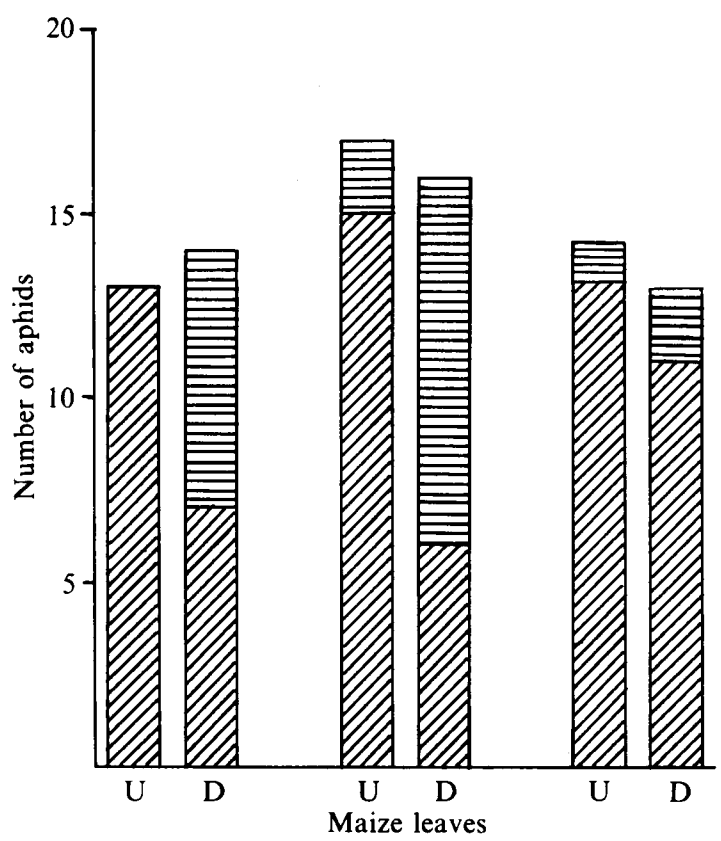

Fig. 1. The survival of *Rhopalosiphum padi* caged on previously damaged maize leaves (Expts 1–3). ▨, live aphids; ▤,dead aphids; U = undamaged; D = damaged.

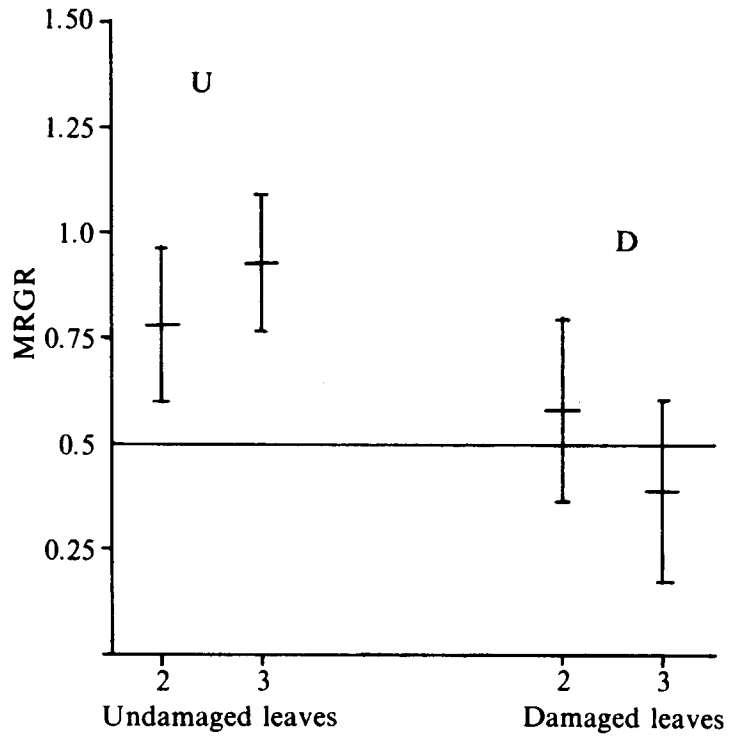

Fig. 2. The mean relative growth rate (MRGR) (μg/μg/day, ±95% confidence intervals) for *Rhopalosiphum padi* caged on undamaged (U) and damaged (D) maize plants (Expt 4). Damage was inflicted on the 3rd leaf. The line at 0.5 signifies zero MRGR because 0.5 was added to the calculated MRGR values before analysis.

If the data from the first and second experiments are pooled, then the mortality of the aphids on the damaged leaves was still significantly higher than for those on the undamaged plants (χ^2, with Yates' correction = 15.1; D.F. = 1; $P < 0.001$). The growth rates of the survivors on the damaged leaves were also lower relative to those on the undamaged leaves.

For the third experiment, where leaves were cut, there was no significant difference between the two treatments either in terms of aphid mortality or growth rate ($F = 0.232$; D.F. = 1,24; $P > 0.05$) (see Fig. 1).

Aphids caged on damaged and undamaged leaves of the same plant. For the fourth experiment, in which aphids were caged on both the damaged and undamaged leaves of a given plant, the M.R.G.R. values (± 95% confidence intervals) are shown in Fig. 2. Aphids caged on the damaged plants had lower growth rates than those on the undamaged plants ($F = 20.538$; D.F. = 1,26; $P < 0.001$). Aphids on the third and second leaves did not differ significantly in their growth rates ($F = 0.006$; D.F. = 1,26; $P > 0.05$), and neither was there a significant interaction between leaf position and treatment ($F = 3.6$; D.F. = 1,26; $P > 0.05$).

Discussion

In these experiments, the extent of damage to each leaf was relatively small (about 10% of the total leaf area for cutting and 5% for crushing). Leaf crushing had major effects on aphids feeding elsewhere on the tissue. The cause of the effect is not clear, as many changes may occur in maize leaves which are damaged in this way. For example, the damage may have adversely altered the amino acid balance (Ciepiela, 1989) or increased the concentration of toxic compounds such as phenolics and hydroxamic acids (Thackray, Morse & Leech, 1988). The latter response may be defensive in nature, but perhaps aimed at pathogens rather than insect herbivores. Cutting did not have any effect on either aphid survival or growth rate, in spite of the fact that more tissue was removed with this type of damage than with crushing. The greater tissue damage involved with crushing probably resulted in more extensive changes in leaf chemistry than did cutting. The effects of crushing on the aphids were therefore not simply due to leaf tissue loss.

A similar difference between the effects of crushing and cutting was noted by Ryan (1974) in regard to production of Proteinase Inhibitor Inducing Factor (PIIF), a wound "hormone" reported to initiate an increase in the concentration of a proteinase inhibitor in young tomato leaves. Crushing, especially near the main vein, caused a substantial release of PIIF while cutting produced very little.

Although crushing probably more closely resembles damage inflicted by herbivores such as young lepidopteran larvae, there may still be large differences in plant response to artificial and natural damage (Baldwin, 1990). In some cases artificial damage has been found to produce a smaller response relative to natural damage, while in others it apparently produces a much larger response. It should also be noted that in the experiments reported here, the test aphids were confined by the use of cages. In nature, the animals may well move away from the damaged area, and thus avoid contact with any localised changes in phytochemistry. However, this increased movement in itself may have other effects as the insect may be more likely to fall from the plant or become more apparent to some natural enemies (Wratten *et al.*, 1988; Winder, 1990).

These results point to the possibility that in the field, low levels of feeding damage to maize by members of one herbivore guild (e.g. leaf chewers) may affect the performance of another guild (e.g. aphids). This type of interaction has been shown in oak (*Quercus robur*) between leaf chewing and leaf mining Lepidoptera (West, 1985).

Acknowledgements

S. Morse would like to thank the Science and Engineering Research Council for funding.

References

Adams, J. B. & van Emden, H. F. (1972). The biological properties of aphids. In *Aphid Technology*, pp. 88–89. Ed. H. F. van Emden. London: Academic Press.

Baldwin, I. T. (1988). Damage induced alkaloids in tobacco: Pot bound plants are not inducible. *Journal of Chemical Ecology* **14**, 1113–1120.

Baldwin, I. T. (1990). Herbivory simulations in ecological research. *Trends in Ecology and Evolution* **5**, 91–93.

Baldwin, I. T. & Schultz, J. C. (1983). Rapid changes in tree leaf chemistry induced by damage: Evidence for communication between plants. *Science* **221**, 277–279.

Ciepiela, A. (1989). Changes in phenylalanine and tyrosine content and metabolism in ears of susceptible and aphid resistant winter wheat cultivars upon infestation by *Sitobion avenae*. *Entomologia experimentalis et applicata* **51**, 277–281.

Croxford, A., Wratten, S. D. & Edwards, P. J. (1989). Temporal and spatial variation in palatability of soybean and cotton leaves following wounding. *Oecologia* **79**, 520–525.

Edwards, P. J. & Wratten, S. D. (1982). Wound induced changes in palatability in birch (*Betula pubescens* Ehrh. ssp. *pubescens*). *The American Naturalist* **120**, 816–818.

Edwards, P. J. & Wratten, S. D. (1987). Ecological significance of wound-induced changes in plant chemistry. In *Proceedings of the 6th International Symposium on Insect-Plant Relationships*, pp. 213–218. Eds V. Labeyrie, G. Fabres and D. Lachaise. Dordrecht.

Edwards, P. J., Wratten, S. D. & Cox, H. (1985). Wound-induced changes in the acceptability of tomato to larvae of *Spodoptera littoralis*: a laboratory bioassay. *Ecological Entomology* **10**, 155–158.

Fowler, S. V. & Lawton, J. H. (1985). Rapidly induced defenses and talking trees: The Devils advocate position. *The American Naturalist* **126**, 181–195.

Green, T. R. & Ryan, C. A. (1972*a*). Wound-induced proteinase inhibitor in tomato leaves: a possible defense mechanism against insects. *Science* **175**, 776–777.

Green, T. R. & Ryan, C. A. (1972*b*). Wound-induced proteinase inhibitor in tomato leaves: some effect of light and temperature on the wound response. *Plant Physiology* **51**, 19–21.

Gutiérrez, C., Castañera, P. & Torres, V. (1988). Wound-induced changes in DIMBOA (2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one) concentration in maize plants caused by *Sesamia nonagrioides* (Lepidoptera: Noctuidae). *Annals of Applied Biology* **113**, 447–454.

Hand, S. C. & Carrillo, J. R. (1982). Cereal aphids on maize in southern England. *Annals of Applied Biology* **100**, 39–47.

Hartley, S. E. & Lawton, J. H. (1987). Effects of different types of damage on the chemistry of birch foliage, and the responses of birch feeding insects. *Oecologia* **74**, 432–437.

Haukioja, E. (1980). On the role of plant defences in the fluctuation of herbivore populations. *Oikos* **35**, 202–213.

Karban, R. (1985). Resistance against spider mites in cotton induced by mechanical abrasion. *Entomologia experimentalis et applicata* **37**, 137–141.

Niemeyer, H. M., Pesel, E., Copaja, S. V., Bravo, H. R., Franke, S. & Francke, W. (1989). Changes in hydroxamic acid levels of wheat plants induced by aphid feeding. *Phytochemistry* **28**, 447–449.

Ryan, C. A. (1974). Assay and biochemical properties of the proteinase inhibitor inducing factor, a wound hormone. *Plant Physiology* **54**, 328–332.

Ryan, C. A. (1978). Proteinase inhibitors in plant leaves: A biochemical model for pest-induced natural plant protection. *Trends in Biochemical Science* **5**, 148–150.

Scopes, N. E. A., Randall, R. E. & Biggerstaff, R. H. (1975). Constant temperature, ventilated perspex cage for rearing phytophagous insects. *Laboratory Practice*, January, 33–34.

Thackray, D. J., Morse, S. & Leech, C. (1988). The role of hydroxamic acids in wheat and maize in conferring resistance to aphids. *Aspects of Applied Biology* **17**, *Part 2, Environmental aspects of applied biology*, pp. 225–227.

West, C. (1985). Factors underlying the late seasonal appearance of the lepidopterous leaf-mining guild on oak. *Ecological Entomology* **10**, 111–120.

Winder, L. (1990). Predation of the cereal aphid *Sitobion avenae* by polyphagous predators on the ground. *Ecological Entomology* **15**, 105–110.

Wratten, S. D., Edwards, P. J. & Dunn, I. (1984). Wound induced changes in the palatability of *Betula pubescens* and *B. pendula*. *Oecologia* (*Berlin*) **61**, 372–375.

Wratten, S. D., Edwards, P. J. & Winder, L. (1988). Insect herbivory in relation to dynamic changes in host plant quality. *Biological Journal of the Linnean Society* **35**, 339–350.

(*Received 21 December 1990*)