Substantial hydraulic signals are triggered by leaf-biting insects in tomato

J-J. Alarcon and M. Malone

Horticulture Research International, Wellesbourne, Warwicks. CV35 9EF, UK

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Abstract

In various plants, defence responses can be induced throughout the shoot by localized damage or insect attack. Activation of such systemic defence responses must involve a rapid long-distance signal of wounding. There is firm evidence that, in the case of localized heat wounds, systemic signalling occurs by hydraulic dispersal of chemical elicitors. However, more natural wounds (such as those imposed by leaf-biting insects) may trigger only small hydraulic events, and it is not clear whether hydraulic dispersal could account for wound signalling in these cases.

It is shown here that partial defoliation offers a method for amplifying wound-induced hydraulic events in tomato. Using this amplification, it is demonstrated that brief feeding by individual leaf-eating insects triggers substantial hydraulic events. The mass flows associated with these events are shown to be sufficient to drive hydraulic dispersal of elicitors through the tomato plant. It is concluded that hydraulic dispersal could be of major importance for wound signalling in plants in the natural environment.

Key words: Lycopersicon esculentum, Spodoptera littoralis, wound signalling, systemic defence responses, hydraulic signals.

Introduction

There is considerable interest in inducible defence responses in plants. Such responses can involve specific wound-induced genes (Ryan, 1992) and they offer novel possibilities for pest control in agriculture (Ryan, 1989; Scheel and Parker, 1990; Karban, 1991). In the shoots of tomato and some other plants, localized attack by insects or localized mechanical damage, triggers the systemic accumulation of proteinase inhibitors (PI) (Ryan, 1992; Bradshaw et al., 1989). These PI are toxic to insects and are thought to play a defensive role (Orozco-Cardenas et al., 1993). In potato, the products of wound-inducible genes can become apparent in remote leaves within 20 min of a localized wound (Peña-Cortes et al., 1988). Clearly, the systemic induction of PI must involve a signal which travels rapidly through the plant from the wound site. Recent evidence indicates that, in tomato, long-distance wound signalling occurs by hydraulic dispersal of chemical elicitors in the xylem-borne mass flows associated with wound-induced hydraulic events (Malone et al., 1994a). Consistent with this mechanism are observations that: (1) chemical elicitors are present in the sap released from damaged cells and these elicitors can induce PI in healthy tissue (Ryan, 1992); (2) hydraulic events are triggered at wound sites in various plants, including tomato (Boari and Malone, 1993), and the mass flow which accompanies these 'hydraulic signals' can carry chemical elicitors rapidly over considerable distances through the plant (Malone et al., 1994a). Alternative mechanisms of wound signalling have been considered, including cell-to-cell electrical transmission (Pickard, 1973; Wildon et al., 1992) and phloem transmission (Pearce et al., 1991). Various arguments against these latter possibilities have been discussed (Malone et al., 1994a). In particular, recent findings that wound signals pass rapidly through heat-killed zones of the tomato petiole (Malone and Alarcon, unpublished) appear to eliminate both phloem-, and electrical-wound signalling, since each of these requires the participation of living tissue. Only xylem-borne signals, such as hydraulic dispersal, remain feasible.

For practical reasons, almost all experimental work on hydraulic signals and hydraulic dispersal has considered localized wounding by heat. The mechanism and kinetics of these signals have been discussed previously (Malone...
Tomato plants were grown as previously described (Malone, 1992, 1993). Heat wounds release large amounts of water (cell sap) from the damaged region and they therefore trigger relatively large basipetal mass flows. In the natural environment, however, wounds (principally from insects) are generally smaller or more progressive, and the resulting mass flows are probably much smaller. In view of this, it is not clear whether hydraulic dispersal could be significant for wound-signalling in the natural environment. Here, we address this question by measuring hydraulic events triggered by individual leaf-biting insects.

Materials and methods

Tomato plants were grown as previously described (Malone et al., 1994b) and used in experiments when 6-weeks old. Pupae of Spodoptera littoralis (Boisd.) were a gift from Dr F. Kimmins, NRI, Chatham, Kent. These lepidopteran insects were reared on an artificial diet based on wheatgerm supplemented with vitamins, and the caterpillars were used when in the final instar, about 2.5 cm in length. Only a single larva was applied to each plant or (in experiments with individual excised leaflets) to each leaflet. Leaf thickness was measured with displacement transducers as previously described (Malone, 1992). In all cases, the transducer was placed towards the distal end of the lamina of the terminal leaflet of leaf three. The lamina of this leaflet was about 7 cm in length and its fresh weight, when excised with about 5 mm of petiole attached, was approximately 0.45 g.

The maximum potential size of any hydraulic signal is determined by the prevailing leaf water status. This is because no further swelling can occur after the relative water content (RWC) reaches 100% and thus, the lower the starting water status, the larger the maximum possible effect. Aside from this, wound-induced hydraulic signals seem little affected by water status over the physiological range (Malone, 1992). Nevertheless, where possible, differences in water potential at treatment were minimized by allowing leaf thickness (and thus leaf water potential) to return to approximately the same value between successive treatments (Fig. 2). In addition, although excised leaves were initially brought to 100% RWC (by application of droplets of water to the petiole until no further swelling occurred), they were allowed to lose some water (to c. 90% RWC) before the onset of treatments so that wound-induced effects would be revealed in full, without the problem of truncation at 100% RWC. Excised leaves were partially enclosed in polythene bags to reduce water loss during experiments. Nonetheless, gradual loss of water by transpiration introduces a falling baseline into data from these leaves (Figs 2, 4).

In some experiments, change in leaf weight was measured simultaneously with thickness. An unenclosed excised leaflet was placed on the pan of a balance (Sartorius R200D, reading to 0.01 mg). A 30 g brass weight was suspended by thread from a lever attached to an LVDT displacement transducer. The weight (counterbalanced to 10 g) rested directly on the leaflet, towards the distal end of the lamina, as in other transducer experiments. Balance and transducer outputs were logged continuously on a computer. These outputs were averaged every 20 s, each average comprising about 75 individual readings from each of the two devices. The force (weight) applied to the balance by the transducer assembly was verified to be constant. These experiments involved the same range of RWC as other excised-leaf experiments presented here; they began with the leaflet near 100% RWC and continued until RWC fell to about 90%.

Heat-wounds were applied using large brass bolts of known head area. The bolts were heated for 5 min in boiling water, dried briefly, and placed on a leaflet, near the base of the lamina, for 30 s. To avoid thermal expansion artefacts, the hot metal bolts were always isolated from the transducer frame. In any case, such artefacts would have been revealed by the blank transducers.

All experiments were repeated at least three times.

Results and discussion

The larvae of Spodoptera littoralis are common leaf-eating pests of tomato in some regions (Broadway et al., 1986). Localized feeding by these and other insects has been shown to induce systemic PI in tomato (Broadway et al., 1986; Wolfson and Murdock, 1990; Alarcon and Malone, unpublished).

Hydraulic events induced by wounding can be monitored by their effect on the thickness of remote leaves (Malone, 1993). However, when a Spodoptera larva fed on one leaflet of an intact tomato plant, no significant changes could be detected in the thickness of remote leaves on the same plant (not shown). This was true even where relatively large areas of leaflet (up to 4 cm²) were consumed by the insect. In contrast, heat-wounds applied to comparable areas of leaf induced marked and systemic changes in leaf thickness in intact plants (examples in Fig. 1). These findings indicate that the hydraulic events generated by insect feeding are small.

Hydraulic events are amplified in defoliated plants

The transient mass flow which emanates from a wound site is shared between all the remaining tissue on the leaflet. Heat wounds of the areas shown. Time and vertical scales are indicated on the markers.
plant. Therefore, the swelling observed at a remote site should depend not only on the size of the wound, but also on the total amount (or, more precisely, the accessible hydraulic capacity) of healthy 'receiver' tissue present on the plant (Malone, 1993). Wound-induced swelling should thus be larger after partial defoliation of the plant. This was found to be the case. In the extreme situation, when the 'plant' was reduced to a single leaflet, heat wounding of small areas of leaflet produced large effects on thickness (Fig. 2). Measurements were also made during successive defoliation of a set of plants: the effect of heat-wounding a particular area increased as more of the plant was removed (Fig. 3). The transducers remained undisturbed throughout this experiment and the results for successive defoliations are therefore directly comparable. From the gradients of the curves in Fig. 3 it is evident that heat-wounding of a particular area induces some 20-fold more swelling in individual excised leaflets than in the intact parent plant. Clearly, the reduced 'plant' offers a more sensitive detection system for wound-induced hydraulic events.

Hydraulic events induced by insect feeding

The amplified detection system (above) was used to search for hydraulic events during insect feeding. When a larva fed at the base of an excised leaflet, marked changes in thickness were observed (Fig. 4). The larvae tended to feed in bouts of various durations. During the longer feeding bouts, greater leaf area was consumed, and larger remote swellings were usually induced (Fig. 4). These results demonstrate that feeding by individual leaf-biting insects transmits hydraulic signals in tomato.

Consumption of a particular area by a larva induced...
only about 15% as much remote swelling as did heat-wounding of the same area (cf. Figs 2 and 4). This indicates that only about 15% of the available water in the tissue is released to the plant during insect feeding, the remainder being consumed by the larva. This estimate appears consistent with the dimensions of the larva’s mandibles and of its bite (not shown): the bite radius is about 250 \( \mu \text{m} \), and each bite leaves a perimeter some 50 \( \mu \text{m} \) wide, of visibly damaged tissue. It can be calculated that, if half the water in this peripheral damaged region is consumed by the insect (together with all the water in the excised portion), then 18% of the available water in the tissue will be released to the plant during feeding. This corresponds closely to the 15% estimated above. The kinetics of the insect-induced effect on leaf thickness are slower than those associated with heat wounds (cf. Figs 2 and 4). This is because, whereas heat wounds release the tissue water in a single burst, insect feeding induces a succession of tiny spurts of water, one for each bite (bites occur at a frequency of perhaps 3 Hz). The overall pattern with insects is the cumulative hydraulic effect of the train of bite-wounds which comprise a feeding bout.

**Spodoptera feeding will drive substantial hydraulic dispersal**

To establish whether the insect-induced hydraulic events are significant for wound signalling, we need to consider whether the volumes of water involved are sufficient to fuel appreciable hydraulic dispersal of elicitors. Calibration of changes in leaf thickness in terms of changes in leaf water-volume was done by monitoring simultaneously the weight and thickness of individual excised leaflets, as water was lost by transpiration. An approximately linear relationship was found (example in Fig. 5), and the slope of the relationship between weight and thickness was reasonably constant at 2.2 \( \pm 0.7 \mu \text{m} \text{mg}^{-1} \) ( \( \bar{x} \pm \text{sd}, n = 8 \)), between leaflets from different plants (all were terminal leaflets from leaf three). Using this relationship, the volume of fluid released for mass flow during insect feeding can be estimated: insect-feeding bouts caused increases of 2–20 \( \mu \text{m} \) in the thickness of individual excised leaflets (Fig. 4). From the calibration above (Fig. 5) such changes in thickness would be associated with changes in weight of about 1–10 mg, respectively. Thus, during each feeding bout, approximately 1–10 \( \mu \text{l} \) of fluid must have been released to the leaf by the feeding action of the insect. This fluid is available to fuel mass flow and drive hydraulic dispersal. In the intact plant, wound-induced mass flow will be mainly basipetal, and will occur through the xylem vessels of the petiole of the damaged leaf. From a comparison of the sizes of all the vessels present, it can be estimated that the largest vessel, typically about 14 \( \mu \text{m} \) in radius, will carry some 13\% of any flow (Malone, 1993). 13\% of the 1–10 \( \mu \text{l} \) of water released by insect feeding will displace the contents of this largest xylem vessel through a distance of 0.27 to 2.7 m, respectively. Therefore, in the intact plant, even the smaller feeding bouts will induce mass flows which are sufficient to drive considerable hydraulic dispersal of the elicitors present in cell sap. Moreover, if basipetal flow from the wound site proceeds only as far as the junction with the stem (typically <0.1 m in these plants), the elicitors will then be further transported with the transpiration stream, at least in the acropetal direction.

In conclusion, hydraulic dispersal is likely to be an important mechanism of signalling of biting-insect damage in the tomato, and possibly in other plants. Hydraulic factors should be considered more carefully in future research. For example, Hartley and Lawton (1991) attempted to mimic insect damage in poplars by defoliation using scissors. However, cutting with scissors will not liberate as much water (per area excised) as does insect feeding and, therefore, it will not mimic insect damage in hydraulic terms.

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**References**


