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Wound-Induced Proteinase Inhibitor in Plant Leaves: A Possible Defense Mechanism against Insects

Abstract. *Wounding of the leaves of potato or tomato plants by adult Colorado potato beetles, or their larvae, induces a rapid accumulation of a proteinase inhibitor throughout the plants' tissues that are exposed to air. This effect of insect damage can be simulated by mechanically wounding the leaves. The transport of a factor out of damaged leaves takes place rapidly after the wound is inflicted and the levels of proteinase inhibitor, in both damaged and adjacent leaves, rises strikingly within a few hours. The rapid accumulation of a powerful inhibitor of major intestinal proteinases of animals in response to wounding of the leaves is probably a defense mechanism.*

The function of naturally occurring proteinase inhibitors in plant tissues has been the subject of speculation (*1*). These inhibitors are usually found in high concentrations in plant storage organs such as seeds or tubers. Some of these proteins have the capacity to inhibit proteolytic enzymes of insect and microbial origins, but rarely proteolytic enzymes of plant origin (*2*). Because of their specificities, they may be protective agents against invading microorganisms and insects (*3*, *4*). The arguments for proteinase inhibitors functioning in seeds as protective agents against insects (*4*) are based on the ability of several of them to inhibit insect digestive proteinases. Our recent discovery of the presence, in potato tubers, of a powerful inhibitor of the two major animal pancreatic exopeptidases (carboxypeptidase A and B) (*5*) further supports the argument for a protective function.

We have been studying the biochemistry, physiology, and function of a well-characterized protein from potato tubers called "inhibitor I," a potent inhibitor of the animal endopeptidases, chymotrypsin and trypsin (*6*). By immunological techniques, we found that this protein was present in potato and tomato leaves as well as in potato tubers. In leaves of both species, the inhibitor was a transient component and was present, in general, at periods preceding and during the breaking of apical dominance. In many instances, its presence in leaves could be correlated with a physiological event. Occasionally, however, differences in amount of inhibitor I in plants of the same age were extraordinary (five- to tenfold variations). Such results led us to suspect that some environmental factor might be responsible for this variability; microorganisms, insects, or physical injury were likely candidates.

Table 1. Colorado potato beetle-induced accumulation of chymotrypsin inhibitor I in tomato plants. Each value is an average obtained from 11 trifoliate leaves (from the second leaf down from apex). Ranges are given in parentheses. Adult beetles were allowed to feed randomly on plants for 24 hours. After an additional 24 hours the tissues were assayed immunologically for inhibitor I (*7*). Experiments were carried out in a greenhouse under natural light. Leaf damage varied from minor damage of a single leaflet to severe damage of all leaflets. The accumulation of inhibitor in leaves varied in proportion to the insect damage inflicted on the plants.

Leaf damage	Average inhibitor I concentration (μg/ml) in:		
	Leaves	Main stem	Roots
Beetle	202 (77–235)	52 (0–73)	<15
No damage	47 (0–120)	<15	<15

The effect of insect damage on inhibitor I concentrations was tested by allowing Colorado potato beetles, common pests of potatoes and tomatoes, to feed on the leaves of young tomato plants. Concentrations of inhibitor I in damaged and undamaged leaves of the plants were subsequently assayed immunologically (*7*). As shown in Table 1, we found that leaves of beetle-infested plants accumulated greater amounts of inhibitor I than did uninfested control plants. Data from individual assays showed that both damaged and apparently undamaged leaves from the beetle-infested plants had accumulated inhibitor I.

We confirmed the observation that damage by beetles to a single leaf could effect the concentration of inhibitor I in undamaged leaves. Adult beetles were then allowed to feed on a single leaf from each of three potato plants without access to the rest of the plants. The beetles fed for 48 hours on the individual leaves and nearly consumed them. At the end of the feeding period, the unwounded leaves of the three plants had an average of 336 μg of inhibitor I per milliliter of leaf juice whereas unwounded control plants had an average of 103 μg of inhibitor I per milliliter.

The accumulation of inhibitor I in leaf tissue far removed from the wounding site suggested that an inducing factor was introduced into the vascular system of the plant when it was wounded. The origin of this factor was undetermined, however, as it could have either entered the leaf cytoplasm from the beetle or originated within the leaf in response to the wound.

The wounding of the leaf appeared to be the primary cause of the induction of inhibitor I accumulation since nearly any type of crushing would cause the same induction. Reproducible results were obtained by using a paper punch

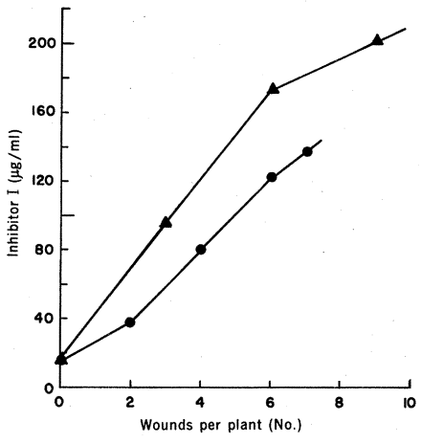

Fig. 1. The accumulation of inhibitor I in leaf juice of the tomato plant as a result of mechanical wounding by crushing leaves between a wooden dowel and file ▲—▲; and by punching holes in leaflets with a paper punch ●—●. The tomato plants were young, 8 to 10 cm in height, with two well-developed leaves, a lower trifoliate and an upper adjacent pentafoliate leaf. The lower leaf was wounded to initiate the experiment. Plants were maintained under midsummer greenhouse conditions for 48 hours. The terminal leaflet of the pentafoliate leaf was assayed immunologically for inhibitor I (*7*).

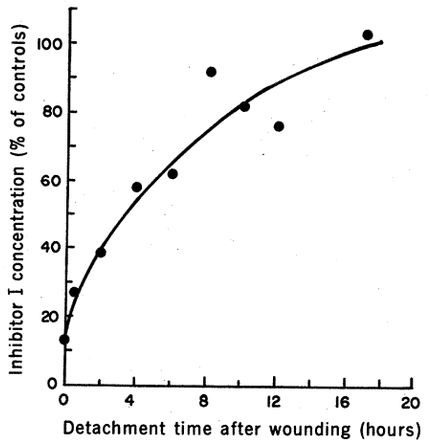

Fig. 2. The effect of detachment of wounded leaves on the accumulation of inhibitor I in tomato plants. Leaves wounded six to seven times by crushing between a wooden dowel and file were detached from the plants at the times indicated. The concentration of inhibitor I in unwounded leaves was determined immunologically (*7*) 48 hours after the initiation of the experiment. Controls were those plants whose wounded leaves were left intact. Experimental methods and conditions were the same as those for Fig. 1.

or by crushing the leaf between the end of a small circular wooden dowel (0.8 cm in diameter) and the rough surface of a flat, bastard file. Figure 1 shows the relation between the number of mechanical wounds and the amount of inhibitor I accumulated in the unwounded terminal leaflet 48 hours after wounding with the paper punch and with the dowel and file. The crushing action of the dowel and file was more effective than the paper punch in inducing inhibitor I and was utilized in subsequent experiments. Scratching the surface of the leaf with a razor blade was effective in inducing inhibitor I, but did not give consistent reproducible results. Pin pricks were less effective. The presence of inhibitor I in the leaflets of young, wounded tomato plants could be detected in leaves within 12 hours. The accumulation of inhibitor I continued for at least 100 hours, often reaching concentrations of more than half a milligram per milliliter of leaf juice.

Although the effect of wounding persisted for a considerable time (in terms of inhibitor I accumulation), the passage of a signal from the wounded leaf to unwounded leaves of the upper petioles was found to be transmitted in an initial pulse. Immediately after being wounded, the leaves were detached from some of the plants by a single cut with a razor blade. Such an operation, with minimum damage to the petiole, does not induce accumulation. Wounded leaves from the remaining plants were detached in the same manner at various intervals. All of the plants were maintained in the greenhouse for 48 hours after being wounded, at which time the terminal leaflet of the pentafoliate was assayed for inhibitor I concentration. Detachment of leaves immediately after being wounded prevented the accumulation of inhibitor I in adjacent leaves (Fig. 2). Detachment of wounded leaves at later intervals was decreasingly effective in preventing the signal from being transmitted, as evidenced by an increased accumulation of inhibitor I in the pentafoliate leaf. The half-time of transmission of the inducing factor out of wounded leaves was about 3½ hours. Detachment of the leaves after 10 hours was ineffective in preventing accumulation of inhibitor I in the pentafoliate leaves.

Our results establish that proteinase inhibitor I can be induced to accumulate in aerial tissues of tomato and potato plants as a direct consequence of insect damage or mechanical wounding of leaves. The chemical signal that initiates inhibitor I accumulation is most likely to be a substance produced or released in the plant, at or near the wound. We have tentatively called this substance the proteinase inhibitor inducing factor (PIIF). Injury near the main vein of leaves is more effective in inducing accumulation of inhibitor I in adjacent leaves than injury to the periphery of the leaves. This suggests that the PIIF gets into the vascular system of the plant through the vascular system of the leaf.

The ability of potato and tomato plant tissues to respond to insect injury by accumulating large quantities of an inhibitor of the animal proteinases chymotrypsin and trypsin, suggests that these inhibitors may be regulated to make the plant less palatable, and perhaps lethal, to invading insects. Digestibility of food is well known to be an important factor in selection for leaf-eating insects (*8*). The effectiveness of proteinase inhibitors as deterrents to insects would depend upon their ability to inhibit the proteinases in the insect digestive tract.

The finding that a proteinase inhibitor accumulates in large quantities in leaves as a result of insect damage demonstrates that insect behavior can rapidly and effectively influence the protein composition of plant leaves. The fact that the accumulated protein is an inhibitor of proteinases and therefore a potential threat to the metabolism of the insect suggests that its function may be that of protection. Such a mechanism of protection could provide another parameter for study in designing new approaches and better methods for biological pest control.

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