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Wound-induced Proteinase Inhibitor in Tomato Leaves

SOME EFFECTS OF LIGHT AND TEMPERATURE ON THE WOUND RESPONSE[1]

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T. R. GREEN[2] AND C. A. RYAN[3]
Department of Agricultural Chemistry, Washington State University, Pullman, Washington 99163

ABSTRACT

Wounding of single leaflets of young tomato (*Lycopersicum esculentum* var. Bonnie Best) plants causes the release of a proteinase inhibitor inducing factor. This factor is rapidly transported throughout the plant where it causes accumulation of inhibitor I, a potent inhibitor of several serine proteinases from both animals and microorganisms. The wound-induced accumulation of inhibitor I is both light- and temperature-dependent. In total darkness no accumulation results from wounding. The accumulation exhibits a linear dependence upon light up to 300 foot candles. At 600 foot candles and above, the response is maximal. In light the wound response possesses an unusual temperature dependence with an optimum rate of accumulation near 36 C. Below 20 C no accumulation occurs. The over-all process contains two light- and temperature-dependent steps, one involving wounding and transport, the other involving accumulation.

Our search (4) for the role of proteinase inhibitors in vegetative plant tissue has recently resulted in the discovery that an inhibitor of chymotrypsin and trypsin, called inhibitor I, is produced in leaves throughout the plants in large quantities in response to insect or mechanical wounding of single leaves (1). The accumulation of inhibitor is apparently an immune response, directed against insects or microbes, that is mediated by a hormone-like factor released from the wound site called PIIF.[4]

We here report the conditions of light and temperature that contribute to the wound response.

MATERIALS AND METHODS

Young tomato plants (*Lycopersicum esculentum* var. Bonnie Best) were utilized 25 to 30 days after planting. The seeds were germinated in peat pots in a headhouse without regard to lighting. Upon germination they were transferred to a growth chamber and maintained at 1000 ft-c under a 14-hr day and 30 C with a night time temperature of 18 C. Plants were fertilized weekly with approximately one-half gram of fertilizer (18-18-18) per peat pot. The plants under study were from 2 to 3 weeks after emergence, 6 to 10 cm in height, and had three well developing leaves and a small apical leaf.

Plants were wounded by crushing single leaves between a wooden dowel 0.8 cm in diameter and a rat tail file as previously described (1). This gave a uniform size wound that resulted in the production of inhibitor I. For the experiments described herein the lowest leaf was wounded five times near or on the main vein to elicit a maximal response without completely destroying the leaves. After wounding, the plants were incubated in a growth chamber at varying light intensities and temperatures for appropriate intervals as described in the text. For each experimental point shown from six to ten plants were utilized. Light intensities were controlled by varying the distance between plants and fluorescent light fixtures and by suspending successive layers of cheesecloth between the plants and light fixtures to achieve the desired effect. Temperatures within the growth chamber were automatically adjusted and maintained with the aid of a thermostat and heater built into the growth chamber. Temperature readings were read directly off a standard thermometer placed in the vicinity of each set of plants during the course of each experiment. Following incubation the level of inhibitor I was determined in leaves.

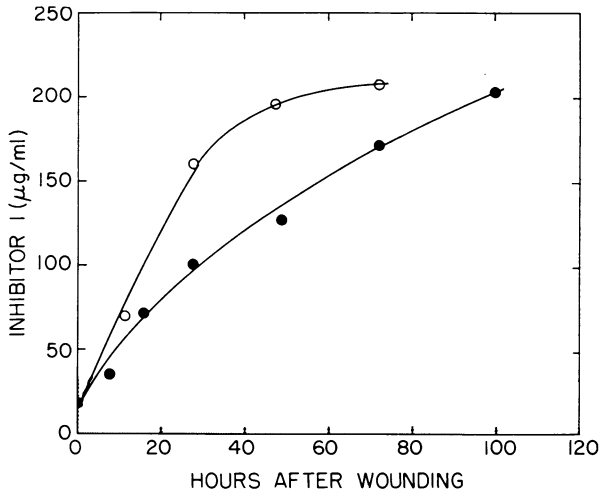

FIG. 1. Time course of accumulation of inhibitor I in young tomato terminal leaflets from leaves adjacent to leaves wounded at zero time. ○: 1000 ft-c, 30 C; ●: greenhouse conditions, late August. The hatched area represents the variability found in control plants maintained in constant darkness. For details see text. Inhibitor I concentration was determined immunologically (3).

Inhibitor I concentrations in leaves were determined im-

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[2] Present address: Department of Chemistry, University of California, Los Angeles, Calif. 90024.

[3] Career Development Awardee of the United States Public Health Service; to whom reprint requests should be made.

[4] Abbreviation: PIIF: proteinase inhibitor-inducing factor.

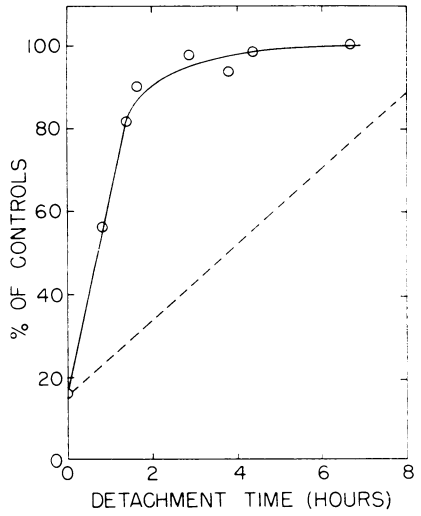

FIG. 2. The 48-hr accumulation of inhibitor I in young tomato terminal leaflets from leaves adjacent to leaves wounded at zero time. The wounded leaves were detached from the plant at the times shown in an effort to prevent transport of PIIF. Details are given in the text. ○: 1000 ft-c, 30 C; – – –: greenhouse conditions from the data in reference 1. Inhibitor I concentration was determined immunologically (3).

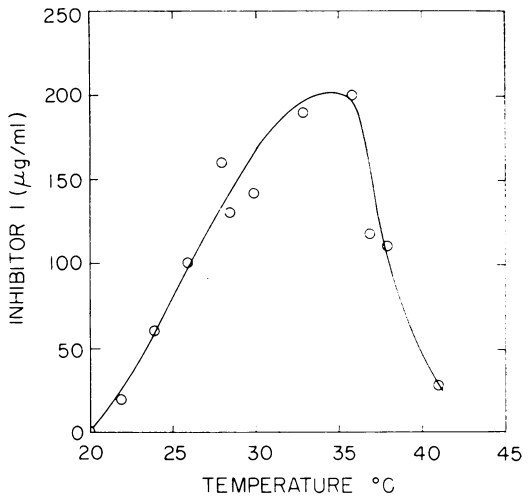

FIG. 3. The temperature dependence of inhibitor I accumulation in young tomato leaves adjacent to wounded leaves. Plants were incubated at 1000 ft-c for 24 hr at the temperatures shown. Inhibitor I concentration was determined immunologically (3).

munologically by the radial diffusion method employing agar gels containing rabbit antiinhibitor I serum as described previously (3). In this method tissue is macerated in a small mortar and pestle, the juice squeezed out of the macerate and placed directly into and just filling, wells previously punched in the agar. The radial diffusion of inhibitor I into the agar results in a precipitin ring whose diameter is a function of the inhibitor I concentration of the juice. Purified inhibitor I (2) from potato tubers was used as the standard.

RESULTS

Mechanically wounding single leaves of tomato plants results in the accumulation of inhibitor I in leaves of the entire plants within a few hours. The time course of accumulation was followed for over 100 hr after wounding the lowest leaves of young tomato plants. Immediately after wounding the plants were placed under various light conditions: (*a*) in a greenhouse under natural light (late August) with daylight temperatures of about 30 C maximum and night temperatures of about 18 C minimum; (*b*) in total darkness at 30 C; or (*c*) in a growth chamber under constant light of 100 ft-c at 30 C. Terminal leaves on the petiole above and adjacent to the wounded leaves were assayed for inhibitor I. Figure 1 shows that the plants maintained in the greenhouse under day-night conditions accumulated inhibitor I at a lesser rate than those maintained in the growth chamber under constant light and temperature. The plants held in constant darkness did not accumulate inhibitor I.

The increased rate of inhibitor I accumulation under constant light and temperature could be due to either an increased rate of response to the PIIF released from the wound site to the rest of the plant or to an increased rate of its transport,

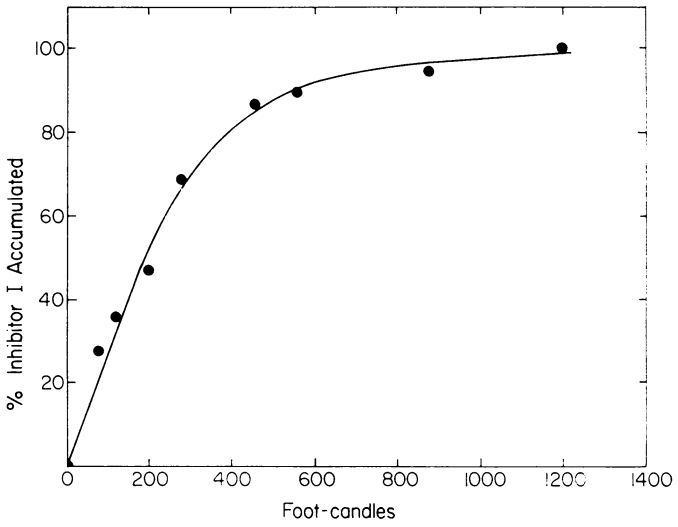

FIG. 4. The light dependence of inhibitor I accumulation in young tomato leaves adjacent to wounded leaves. Plants were incubated at 30 C for 24 hr at the light intensities shown. Inhibitor I concentration was determined immunologically (3).

Table I. *Temperature and Light Dependence of the Wound-induced Accumulation of Inhibitor I in Young Tomato Plants*

Young tomato plants were wounded five times on the lowest leaf with a dowel and file (1) and incubated 4 hr under conditions described below. After 4 hr of incubation the wounded leaf was detached cleanly with a razor blade. Plants were incubated an additional 20 hr as described below and the adjacent terminal leaflet was assayed for inhibitor I.

Experiment	Wounding and Transport of PIIF 4 hr Incubation		Accumulation of Inhibitor I 20-hr Incubation		Final Inhibitor I Accumulation[1]
	Light	Temperature	Light	Temperature	
		C		C	μg/ml
1	1000 ft-c	28	1000 ft-c	28	206
2	darkness	28	1000 ft-c	28	41
3	1000 ft-c	21	1000 ft-c	28	92
4	1000 ft-c	28	1000 ft-c	21	39
5	1000 ft-c	28	darkness	28	31

[1] Average concentration from seven plants per experiment. Inhibitor I concentration was determined immunologically (3).

or to both. In our earlier report (1) we demonstrated that the effect of PIIF was to cause accumulation of inhibitor I and that its effect could be prevented by detaching the injured leaf with a single clean slice with a razor blade before PIIF was transported out of the leaf. After detaching injured leaves at various times following wounding, the plants were incubated under greenhouse conditions for 48 hr. The levels of inhibitor I in leaves above and adjacent to the detached wounded leaf were assayed. The half time of a full response was about 3.5 hr. We have performed similar experiments under constant light and temperature to determine if the wound response was accelerated over greenhouse conditions. Figure 2 shows that under constant lighting of 1000 ft-c at 30 C the $t_{1/2}$ for a full response was less than 1 hr. In Figure 2 these data are compared to the similar data under the greenhouse conditions previously reported (1).

It was apparent from the foregoing data that either light or temperature or both were important to achieving a full wound response. A series of experiments to determine the temperature effect on the wound response was performed in a temperature regulated growth chamber under constant lighting of 1000 ft-c. Young tomato plants were wounded and incubated for 24 hr at temperatures between 20 and 41.5 C. The terminal leaflet of the petiole above and adjacent to the wounded leaf was assayed for inhibitor I concentration. Figure 3 shows the temperature effect on the accumulation of inhibitor I due to wounding under constant lighting of 1000 ft-c.

The effect of light on the wound response was investigated in an analogous manner by varying the light intensity while maintaining a constant temperature. Young tomato plants were wounded in the usual manner and incubated for 24 hr at 30 C under light intensities of 80 ft-c to 1200 ft-c and in darkness. Temperatures near 30 C were selected for study in these and subsequent experiments because of the necrotic effects observed at temperatures near and above 36 C. The terminal leaflet of the petiole above and adjacent to the wounded leaf was assayed for inhibitor I concentration as before. Figure 4 shows the effect of light intensity on the accumulation of inhibitor I while maintaining a constant temperature.

The wound response is the result of an interlocking series of events culminating in the accumulation of inhibitor I throughout the aerial tissue of the plant. Therefore, the effect of both light and temperature on (*a*) the release and transport of PIIF as well as (*b*) the subsequent accumulation of inhibitor I were studied independently of one another with the hope of locating light- and temperature-dependent steps in the wound response. Wounding and subsequent transport of PIIF was considered as the causal phase of the response, and the accumulation of inhibitor I was treated as the resultant phase. Figure 2 shows that under growth chamber conditions of 1000 ft-c and 30 C PIIF is exported from wounded leaves with a $t_{1/2}$ of about 40 min. This indicates a transport rate of about 3 to 5 cm/hr. At this rate, in 4 hr PIIF should have reached the upper adjacent terminal leaflet. Within the next 20 hr the level of inhibitor I rises dramatically in response to PIIF. The causal and resultant phases of the wound response are therefore, at least in part, subject to independent study. By varying light and temperature in one phase, while maintaining optimum lighting and temperature in the other, light- and temperature-dependent steps in each phase were investigated in the following experiments.

Young tomato plants were wounded on the lowest leaf and the plants were immediately placed in either total darkness or in 1000 ft-c of light at low (20–21 C) or high (28 C) temperature for 4 hr. The wounded leaf was then detached, and the plants were incubated for 20 hr under the various conditions of light and temperature just described. At the termination of each experiment the terminal leaflet of the petiole adjacent to and above the wounded leaf was assayed for inhibitor I concentration. These experiments were designed in this manner to show (*a*) which conditions are necessary for PIIF release and (*b*) which conditions are necessary for accumulation after PIIF has been released. In the first three experiments light and temperature variations on the causal phase were investigated while maintaining inducing conditions for a wound response in the resultant phase. In the fourth and fifth experiments conditions were maintained at 1000 ft-c and 28 C during the first 4 hr after wounding (causal phase) but were varied for the latter 20 hr of each experiment (resultant phase). These results are shown in Table I. The first experiment shows the over-all wound response at 1000 ft-c and 28 C. The second and third experiments show that light and temperature are required during the causal phase whereas experiments 4 and 5 demonstrate that temperature and light are required for the resultant phase.

DISCUSSION

The wound response in young tomato leaves, resulting in the release and transport of PIIF and subsequent accumulation of inhibitor I, is both light- and temperature-dependent. Optimal conditions for accumulation after wounding were found to be above 500 ft-c at 36 C.

At least two steps in the over-all wound response were found to be dependent upon both light and temperature, *i.e.*, the release of PIIF upon wounding and the subsequent process of accumulation of inhibitor I in distal leaves. When either process occurs in unfavorable conditions, *i.e.*, either temperatures below 22 C or in darkness, the response is severely curtailed. The lower temperature is apparently affecting the accumulation process. Table I shows that 21 C inhibited the causal phase of the response only about 45%, whereas 20 C virtually stopped the resultant accumulation of inhibitor I when PIIF was present. Thus, the accumulation process or resultant phase is mainly responsible for the unusual temperature sensitivity. It is evident that a series of biochemical and physiological events take place before inhibitor I begins to accumulate. These events include (*a*) the production of PIIF by wounding, (*b*) its transport to other plant cells, and (*c*) its initiation of the accumulation process. We are unable at this time to determine whether the light- and temperature-sensitive steps in the causal phase of the wound response are effecting only PIIF transport from the wound site to the remainder of the plant or if, in addition, they influence PIIF production.

The results presented here show that light and temperature are important parameters of both the causal and resultant phases of the wound response that occurs in young tomato plants, resulting in an accumulation of inhibitor I. We have chosen a constant lighting of 1000 ft-c at 30 C as our conditions for our continuing studies of this wound response.

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