

Addendum

Insect Regurgitant and Wounding Elicit Similar Defense Responses in Poplar Leaves

Not Something to Spit At?

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Addendum to:

Molecular Analysis of Poplar Defense Against Herbivory: Comparison of Wound- and Insect-Elicitor-Induced Gene Expression

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ABSTRACT

How plants perceive insect attacks is an area of active research. Numerous studies have shown that regurgitant from feeding insects elicits a defense response in plants, which is often assumed to be distinct from a wound response. We have characterized the inducible defense response in hybrid poplar and found it to be qualitatively similar between wounding and application of regurgitant from forest tent caterpillar. We suggest that this is likely attributable to our wounding treatment which is much more intense compared to most other studies. These overlapping responses appear to be activated via jasmonic acid signaling, and we speculate that they are both triggered by elicitors of plant origin. Wounding would release such elicitor molecules when leaf cells are disrupted, and regurgitant may contain them in a modified or processed form. This hypothesis could explain why some other necrosis-inducing stresses also induce herbivore defense genes.

Plants have evolved sophisticated adaptive responses to herbivory, including physical and biochemical, constitutive and inducible defense strategies. The induction of defenses implies there are sensitive mechanisms to perceive and transduce herbivory into a coordinated defense response. A variety of evidence suggests that plants interpret diverse stimuli from feeding insects, including physical wounding, insect-derived elicitors in saliva, and metabolites released from damaged cells. In particular, a number of studies have now shown that insect regurgitant can effectively mimic live insects as inducers of plant defense.

We recently used macroarrays to compare transcript profiles in hybrid poplar (*Populus trichocarpa* x *P. deltoides*) leaves elicited by wounding and by regurgitant from forest tent caterpillar, a poplar defoliator (FTC-R; *Malacosoma disstria*). Both treatments upregulate a large number of genes, many of which encode known and suspected anti-herbivore proteins.¹ For the set of genes represented on our array, the effects of these treatments differed only quantitatively (the wound response being stronger), and we detected no significant qualitative differences or insect-specific induction. This finding was somewhat surprising, since recent reports in other systems had demonstrated that insect herbivory can have effects that are quite distinct from wounding.²⁻⁴ However, in comparison to these studies, our wound treatment was much more severe; we wounded plants by crushing leaf margins with pliers rather than the commonly used leaf puncture method. The wounding treatment was applied three times at hourly intervals, which together resulted in the necrosis of approximately 25% of total leaf area. We suggest that both the extent of damage and the repetitive nature of our treatment are responsible for the strong induction of gene expression that we observed, since a recent study using a 'mechanical caterpillar' found that both the spatial and temporal pattern of wounding is key in shaping the defense response.⁵ Therefore, differences in wounding protocols used to simulate insect feeding (i.e., crushing, puncturing, abrading, and tissue removal) can complicate direct comparisons of different studies.

Since our comparisons were carried out at a single time point (24 h) and we used macroarrays with a relatively small number of genes from a wound-induced library, we cannot rule out the possibility that FTC-R induces insect-specific genes that we failed to detect. Nevertheless, our findings do suggest that wound and caterpillar regurgitant responses can be more similar than previously thought. This result may be particularly relevant for long-lived perennials such as poplar, which may deploy a broad response to generalists such as FTC. Interestingly, another tree species, Sitka spruce (*Picea sitchensis*), also showed considerable overlap in its responses to wounding and weevil or budworm herbivory (boring and chewing insects, respectively).⁶

Figure 1. Accumulation of *PtdTI3* (Kunitz trypsin inhibitor 3) mRNA in leaves of hybrid poplar wounded with pliers and treated with (+) or without (-) forest tent caterpillar regurgitant (FTC-R). Leaves 9–11 were wounded with pliers and mock treated with ddH₂O or a 1:5 dilution (v/v with ddH₂O) of FTC-R three times, at 1-h intervals. Leaves 9–11 and 3–5 (local and systemic responses, respectively) were harvested 24-h after start of the treatment and analyzed by northern blot analysis as described¹ (A). The experiment was replicated once with the same results. (B) Quantified transcript abundance from (A). Open bars are mock-treated control plants and hatched bars are FTC-R-treated plants. Percentages above hatched bars show the increase in transcript abundance by FTC-R treatment.

We carried out additional experiments to gain further insights into the overlap between wound- and insect-responses. To determine whether FTC-R could reshape the response induced by intense wounding, we applied FTC-R to plier wounds (Fig. 1). The FTC-R only marginally enhanced the wound response; using *PtdTI3* (a Kunitz trypsin inhibitor) as a marker gene, an increase of approx. 14% in transcript abundance was measured. By contrast, treatment of leaf punctures with FTC-R increases transcripts levels of *PtdTI3* by approx. 300%.¹ This confirms that our wound-induction treatment is intense and suggests it triggers a near-maximal response.

To ascertain the potential signaling role of jasmonates (JAs) in these responses, we compared the FTC-R- and wound-induced gene sets with macroarray data from an analogous methyl-jasmonate (MeJA) induction experiment. A large proportion of the genes (84%) that were induced by both wounding and FTC-R were also MeJA-inducible. Furthermore, the ranked list of the most induced genes is similar for all three treatments; for example seven of the top ten genes for each treatment are common (Table 1). Since JA and its derivatives play key roles in herbivore defense signaling,⁷ this suggests that a common JA signaling pathway activates both FTC-R- and wound-induced responses. The extent of participation of the JA pathway in FTC-R- and wound-induced responses will have to be tested more directly, for example by generating a poplar *coi1* knockout deficient in JA signaling. Analogous experiments in *Arabidopsis* have identified JA-dependent as well as independent pathways involved in insect and wound-induced responses.²

How FTC-R and wounding can lead to a common induction of JA signaling and subsequent downstream responses is not clear. One possibility involves plant-derived elicitor compounds, which are released from damaged plant tissues following wounding and should thus be present in caterpillar regurgitant. We noted that our plier wound treatment leads to large necrotic areas on the damaged leaf, which would be a substantial source of such elicitors. Examples of known plant-derived elicitors include oligogalacturonides, the oligopeptides systemin and inceptin, hydrogen peroxide, and ATP.^{8–10} Furthermore, we found that treatments which cause necrosis via other stresses

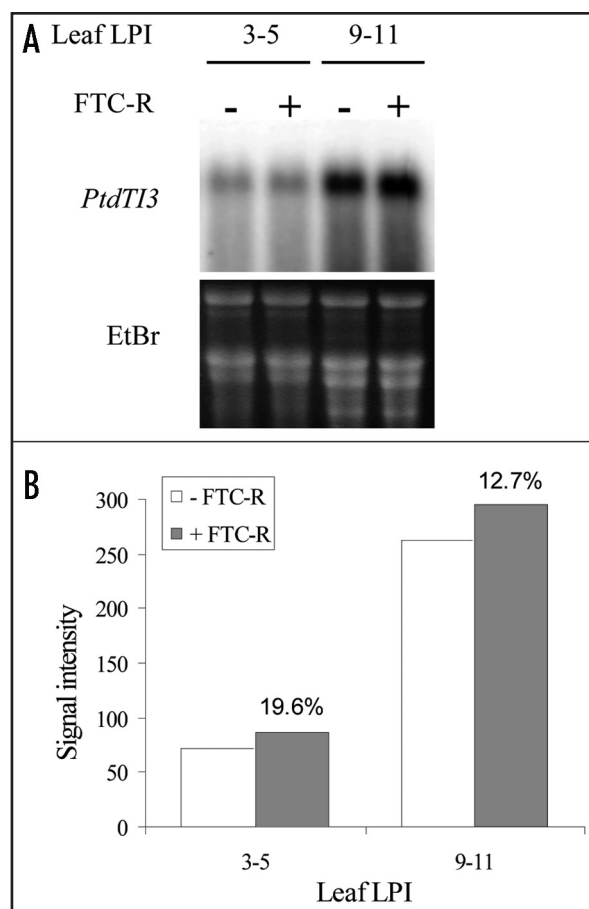


Table 1 Comparison of most strongly FTC-R-, wound- and MeJA-induced genes from macroarray analyses^a

Putative Function	GenBank Accession	FTC-R rank ^b	Wound rank ^b	MeJA rank ^b
Endochitinase <i>win6.2C</i>	CN192741	1	1	2
Lipase, class 3	CN192786	2	5	3
Endochitinase <i>win8</i>	CN192595	6	2	5
Polyphenol oxidase <i>PtdPPO1</i>	CN193334	10	3	4
Apyrase	xxxxxxx	4	6	7
Vegetative storage protein <i>win4.5</i>	CN192930	3	8	8
Unknown	CN192936	5	9	9
Kunitz trypsin inhibitor <i>PtdTI5</i>	CN192805	— ⁸	7	— ⁸
Kunitz trypsin inhibitor <i>PtdTI4</i>	CN193330	— ²⁶	4	1
Kunitz trypsin inhibitor <i>PtdTI3</i>	CN192549	17	11	6
<i>Pop3/SP1</i>	xxxxxxx	11	13	10
Acid phosphatase, class B	CN193016	7	14	15
<i>Pop3/SP1</i> -like	xxxxxxx	9	17	13
Acyl-activating enzyme	CN192663	— ¹⁵	12	25
β-amylase	CN192760	19	15	20

^aMeJA-induced genes (two-fold induction, $p < 0.05$ as measured by a Student's *t*-test) were ranked for fold-induction together with forest tent caterpillar regurgitant (FTC-R) or wounding experiments from our previous study.¹ Rankings for the fifteen most strongly induced genes are shown. MeJA treatment of hybrid poplar was performed as described previously¹⁵, except that MeJA-treated plants were compared to untreated, control plants. Macroarray analysis of MeJA-treatment was performed as described previously.¹ ^bSeveral genes were induced by FTC-R or MeJA treatment, but with a nonsignificant *p*-value. Superscript values denote induction ranking if the *p*-value is ignored.

(e.g., high NaCl) also induce expression of the *PtdTI3* gene (Major IT, Constabel CP, unpublished data). This is unlikely to reflect a general stress response, since *PtdTI3* encodes a functional trypsin inhibitor with anti-herbivore properties. Other studies have also found an overlap in responses to wounding and abiotic stress,^{11,12} and tomato leaves are known to accumulate proteinase inhibitors following salt stress.¹³ We speculate that large necrotic areas can release compounds that effectively elicit defense responses. Potent elicitors identified from insect regurgitant, such as fatty acid-amino acid conjugates (FACs) and the peptide inceptin, are known to originate from plant fatty acids and proteins, respectively. Insects may thus process and perhaps concentrate molecules of plant origin during feeding, thereby increasing their potency as defense elicitors in regurgitant.

We note that our model does not preclude insect-specific responses; insect-specific modification of elicitors may reshape the plant response for any given plant-insect interaction. Likewise, salivary factors such as glucose oxidase¹⁴ may suppress some elements of the general plant response. Nevertheless, our data are consistent with the view that plants have evolved a strategy of defense induction based primarily on the recognition of tissue damage.

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