

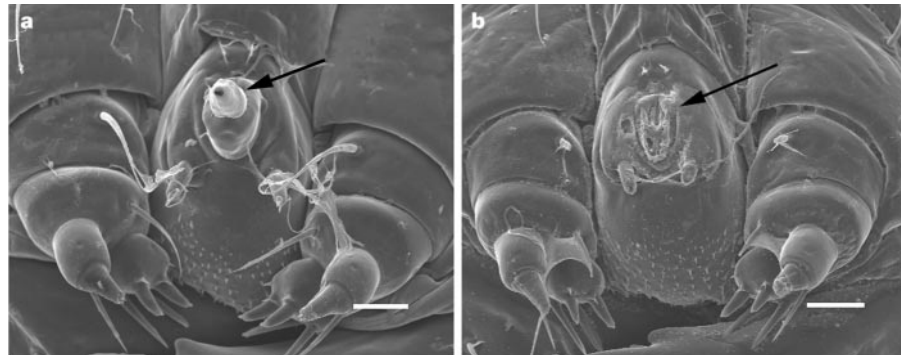
# Caterpillar saliva beats plant defences

A new weapon emerges in the evolutionary arms race between plants and herbivores.

**B**lood-feeding arthropods secrete special salivary proteins that suppress the defensive reaction they induce in their hosts<sup>1,2</sup>. This is in contrast to herbivores, which are thought to be helpless victims of plant defences elicited by their oral secretions<sup>3,4</sup>. On the basis of the finding that caterpillar regurgitant can reduce the amount of toxic nicotine released by the tobacco plant *Nicotiana tabacum*<sup>5</sup>, we investigate here whether specific salivary components from the caterpillar *Helicoverpa zea* might be responsible for this suppression. We find that the enzyme glucose oxidase counteracts the production of nicotine induced by the caterpillar feeding on the plant.

Spinnerets are the principal secretory structures of the labial salivary glands of *H. zea*. To determine whether saliva from this caterpillar affects the induced defences of the tobacco plant *in situ*, we prevented salivation by ablating their spinnerets with a heated probe (Fig. 1a, b). This cauterization inhibits salivation, and hence the secretion of salivary enzymes, as we demonstrated by feeding glucose-soaked fibre discs to these caterpillars and then staining the discs to detect hydrogen peroxide (a product of the action of glucose oxidase)<sup>6</sup>. The ablation procedure prevented the release of glucose oxidase without affecting feeding rate.

Caterpillars with ablated spinnerets and caterpillars with intact spinnerets were indi-



**Figure 1** Ablation of the caterpillar (*Helicoverpa zea*) spinneret to prevent production of saliva. **a,b**, Scanning electron micrographs showing the caterpillar labium (arrows) with the spinneret intact (**a**) and ablated (**b**). Scale bars, 100  $\mu$ m.

vidually caged on the second uppermost fully expanded leaf of an *N. tabacum* plant (one caterpillar per plant) for 24 hours. Three days after feeding, we analysed the damaged leaf for nicotine, an inducible defence compound<sup>7</sup>. Feeding by caterpillars with intact spinnerets reduced foliar nicotine levels by over 26% compared with feeding by caterpillars with ablated spinnerets ( $P < 0.05$ , Tukey–Kramer test; for details of nicotine quantification, see ref. 7).

We removed circular sections from the leaves of the caterpillar-exposed plants and fed them to *H. zea* neonates. Neonates that were fed on leaves that had been ‘treated’ with caterpillars with intact spinnerets showed significantly increased survival and mean body weights relative to neonates that

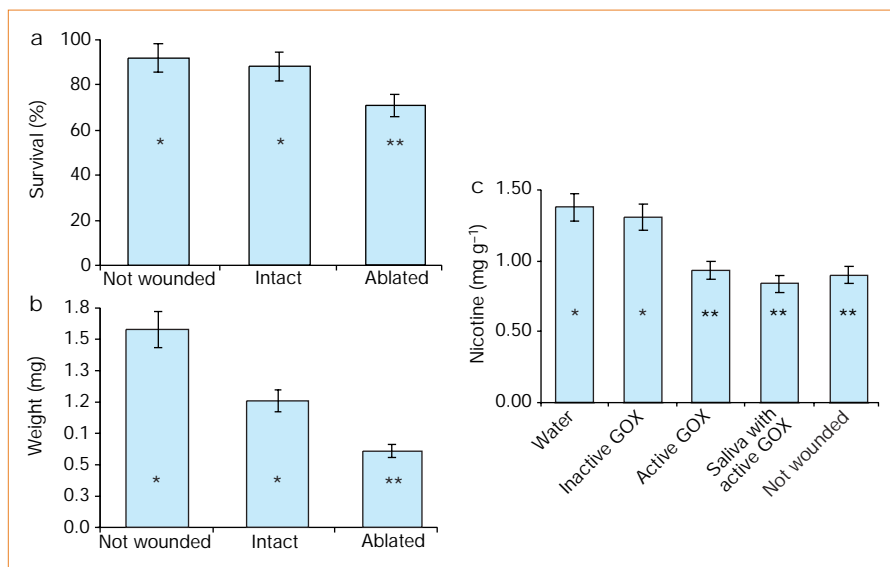
were fed on leaves exposed to caterpillars without spinnerets (Mann–Whitney *U*-test; Fig. 2a, b).

Glucose oxidase is the principal salivary enzyme in *H. zea*, and converts D-glucose and molecular oxygen to D-gluconic acid and hydrogen peroxide<sup>1,6</sup>. To determine whether this protein could be the salivary component that is responsible for suppressing the plant’s herbivore-induced resistance, we cut one attached leaf per plant with a 1.5-cm-diameter cork borer in order to simulate insect damage. Six holes were uniformly distributed on the second-uppermost expanded leaf.

We then treated individual leaves with one of four preparations: purified, active glucose oxidase (prepared as in ref. 6); unpurified salivary-gland extract; inactivated (autoclaved) purified glucose oxidase; or water. Leaves treated with salivary extract received 20  $\mu$ g protein in total; each wound received about 10  $\mu$ l water. Three days later, leaves treated with enzyme or salivary extract contained significantly less nicotine than plants that were treated with water or inactive enzyme (Tukey–Kramer test; Fig. 2c).

We also analysed the survival and growth of second-instar caterpillars on plants treated with either purified glucose oxidase or water. Survival and mean larval weights were significantly greater for caterpillars fed on enzyme-treated leaves than for caterpillars fed on leaves treated with water ( $P < 0.05$ ; Tukey–Kramer test). We also applied the individual reaction products  $H_2O_2$  and gluconic acid to leaf wounds (10  $\mu$ l 40 mM solution) and found that these products reduced nicotine levels in the leaf relative to a water control by 43.6% and 29.3%, respectively (Tukey–Kramer test; results not shown).

The saliva of herbivorous insects has been overlooked as a factor in overcoming



**Figure 2** Effect of caterpillar saliva on induced resistance in tobacco plants (*Nicotiana tabacum*). **a**, Proportion of neonates surviving after feeding on leaves previously damaged by sixth-instar caterpillars. **b**, Weights of surviving neonates fed on leaves damaged by sixth-instar caterpillars. **c**, Glucose oxidase (GOX) and salivary-gland extracts suppress nicotine production in leaves that have been wounded to mimic insect damage. Wounded leaves were treated with water, inactive GOX, active GOX, or salivary-gland extracts containing active GOX; the nicotine content of treated leaves was analysed by high-performance liquid chromatography<sup>7</sup>. Asterisks represent significant differences ( $P < 0.05$ ) between treatments; error bars represent mean  $\pm$  s.e.

host defences<sup>1,2</sup>. Our results show that glucose oxidase, one of the principal components of *H. zea* saliva, is responsible for suppressing induced resistance in *N. tabacum*. This enzyme may prevent the induction of nicotine by directly inhibiting the wound-signalling molecule jasmonic acid and/or by antagonizing its interaction with other signalling pathways. As glucose oxidase is produced by a wide variety of caterpillar species<sup>1,6</sup>, we may have discovered a new feature of the evolutionary arms race between plants and herbivores.

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COMMUNICATIONS ARISING

Biodiversity

### Suspect evidence of transgenic contamination

Quist and Chapela<sup>1</sup> claim that transgenic DNA constructs have been introgressed into a traditional maize variety in Mexico, and furthermore suggest that these constructs have been reassorted and introduced into different genomic backgrounds. However, we show here that their evidence for such introgression is based on the artefactual results of a flawed assay; in addition, the authors misinterpret a key reference<sup>2</sup> to explain their results,

concluding that reassortment of integrated transgenic DNA occurs during transformation or recombination.

The discovery of transgenes fragmenting and promiscuously scattering throughout genomes would be unprecedented and is not supported by Quist and Chapela's data<sup>1</sup> — the incorrectly cited work<sup>2</sup> merely claims that multiple transgenes or transgene fragments can integrate into genetically linked regions of the genome during transformation, and not that they can move around the genome by recombination after integration (W. Pawlowski, personal communication).

The discovery of cauliflower mosaic virus promoter sequences, an element of

transgenic constructs, in the authors' samples is more consistent with F<sub>1</sub> hybridization than introgression. In introgression, a small, polymorphic genomic region is bred into a given variety through repeated backcrossing, so all progeny (or kernels) from an individual in which a (trans)gene has been introgressed will possess that gene. Quist and Chapela report, however, that on the basis of "low amplification" by the polymerase chain reaction (PCR), the transgene is evident only in a small percentage of kernels in each cob, citing a press release that reported a 3–10% abundance of transgenes in similar samples to support their claim.

The authors interpret their inverse PCR (i-PCR) results as evidence of a high frequency of transgene insertion into a range of genomic contexts, inferring from this that introgression events are relatively common and well maintained. However, their i-PCR products all seem to be artefacts of the methodology used. We examined the sequences of the reported i-PCR products (Fig. 1) and found that none contains a reasonable number of the features that would be expected in a legitimate product of amplified genomic DNA flanking the anchor sequence. An i-PCR product derived from the circularization of a single piece of DNA should contain the sequence

Editorial note

In our 29 November issue, we published the paper "Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico" by David Quist and Ignacio Chapela. Subsequently, we received several criticisms of the paper, to which we obtained responses from the authors and consulted referees over the exchanges. In the meantime, the authors agreed to obtain further data, on a timetable agreed with us, that might prove beyond reasonable doubt that transgenes have indeed become integrated into the maize genome. The authors have now obtained some additional data, but there is disagreement between them and a referee as to whether these results significantly bolster their argument.

In light of these discussions and the diverse advice received, *Nature* has concluded that the evidence available is not sufficient to justify the publication of the original paper. As the authors nevertheless wish to stand by the available evidence for their conclusions, we feel it best simply to make these circumstances clear, to publish the criticisms, the authors' response and new data, and to allow our readers to judge the science for themselves.  
 Editor, *Nature*



**Figure 1** Alignment of primers with cauliflower mosaic virus 35S promoter sequences and the ends of inverse polymerase chain reaction (i-PCR) products. The upstream and downstream orientations with respect to the 35S promoter were confused in the original publication<sup>1</sup> and are corrected here. In parentheses: primer name, or GenBank accession number and fragment size. Shaded regions represent the maize genomic sequences with the highest homology (BLAST e-value shown) to the preceding i-PCR fragment. The reverse complements of even-numbered primers are shown for alignment. The footprint of the *EcoRV* restriction enzyme is shown in bold and italicized if in alignment with the site on the 35S promoter. Capitals denote bases that match 35S sequences and are underlined in the regions of the primers; a dashed underline denotes these respective sequences in cases in which they appear at the wrong end and in the wrong orientation on the product. For the hypothetical legitimate i-PCR products, plus signs indicate areas where further transgene DNA would be found.