

Presence of avidin and aprotinin in the bodies and frass of *Spodoptera litura* larvae feeding on transgenic tobacco plants



J. Todd, L. Malone, J. Christeller, R. Marshall,
E. Burgess, B. Philip

New Zealand

Impacts of Recombinant Proteins

- Recombinant proteins expressed by transgenic plants may have non-target ecological impacts
- For example, proteins aimed at controlling pest insects may have impacts on other invertebrates
- These may include:
 - Non-target herbivores feeding on the plant
 - Predators or parasitoids attacking herbivorous insects that have fed on the plant
 - Decomposers of the plant or insect material





Fate of Recombinant Proteins

- Studies have shown Bt, lectin and protease inhibitor (PI) proteins can be retained in the bodies and frass of insects fed these proteins in diet or transgenic leaf material
- The impacts of these proteins on the next trophic level will depend on the concentration of active protein presented to them, i.e. ;
 - The protein expression level of the plant will determine the level of protein released into the ecosystem
 - Predators and parasitoids may be affected by any active protein retained by herbivorous insects
 - Decomposers will be exposed to active proteins in dead plant material, insect bodies and frass

Assay System

Aim

To determine the concentrations and activity levels of 2 insect control proteins in the bodies and frass of herbivorous insects feeding on transgenic plants

Plants

- Tobacco plants (*Nicotiana tabacum*) were transformed to express either:
 - Aprotinin: an insecticidal protease inhibitor (PI) which binds to trypsin in the insect gut; or
 - Avidin: a biotin-binding protein with broad insecticidal activity
- Untransformed tobacco plants were used as a control

Laboratory Assay

Set-up

- Tobacco leaves were cut from plants of each treatment and the petioles placed in agar to keep fresh
- Each leaf was placed in box on paper towels
- Five boxes of each of the 3 treatments: aprotinin, avidin or control



Laboratory Assay

Insects

- *Spodoptera litura* larvae were used as the target, herbivorous insect
- 120 neonate larvae placed in each box
- Incubated at $24.5 \pm 1^\circ\text{C}$, 65% R.H., 16:8 light:dark
- Leaves replaced as necessary for the first 5 days





Laboratory Assay

Insect Collections

- 20 third instar larvae were collected from each box on the 5th, 6th and 7th Day
- The larvae were weighed (pooled sample of 20 larvae) and frozen for protein analysis

Frass Collections

- On the 5th day all remaining larvae were shifted to a new box containing a fresh leaf
- Larvae were left for 24h
- Frass in each box was collected as a single sample
- Each sample was weighed and frozen for protein analysis
- This was repeated on Day 6 for each box



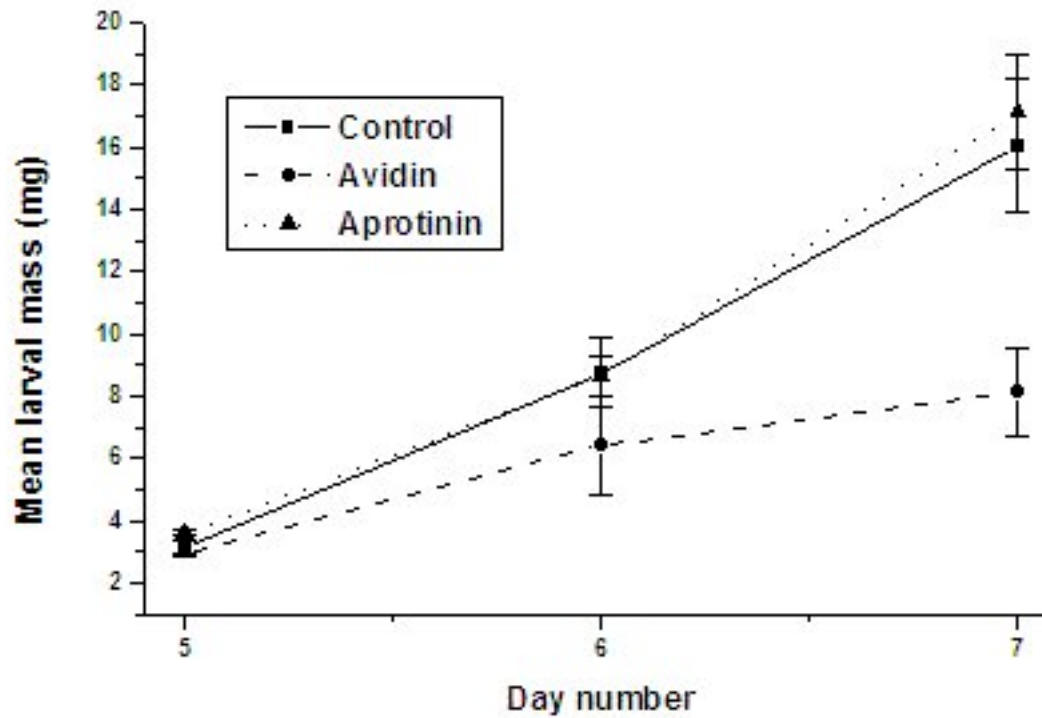
Laboratory Assay

Protein analysis

- Frass and larval samples were analysed for:
 - total protein concentration, and
 - active protein levels
- Aprotinin assessed using 100uL Affigel-10-chymotrypsin column – active aprotinin bound to chymotrypsin – eluted differentially from aprotinin-trypsin
- Avidin assessed using ELISA – active avidin bound to alkaline phosphatase-biotin – subset of total avidin detected using rabbit anti-avidin
- Protein expression levels were also measured in each tobacco plant



Results – larval growth



Growth of larvae feeding on avidin, aprotinin and control tobacco



Results – frass production

- Larvae produced more frass on day 7 than day 6
- This was proportional to the increase in larval size between days 5 and 7
- Avidin-fed larvae produced less frass than aprotinin-fed larvae, in proportion to their smaller size

Frass production (mg frass/mg larva) for each treatment over each 24h period

Day	Control			Avidin			Aprotinin		
	mean	s.e.	n	mean	s.e.	n	mean	s.e.	n
6	0.112	0.016	15	0.128	0.009	15	0.140	0.012	15
7	0.105	0.017	15	0.140	0.035	15	0.114	0.010	15

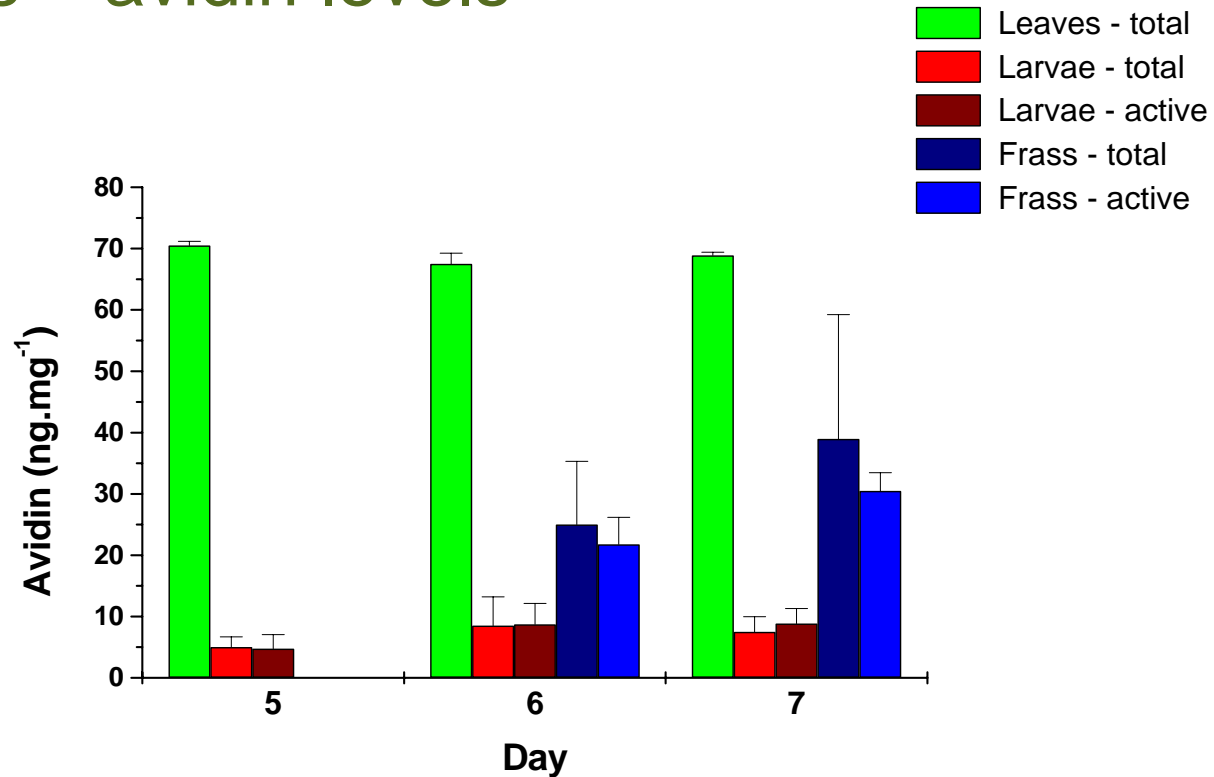


Results – avidin levels

- Avidin was not detected in the control plants, insects or frass
- In avidin-fed larvae, no significant differences were found between the mean concentrations of total and active avidin in either the larvae or their frass on each collection day
 - i.e., all the avidin detected in larvae or their frass was active and still capable of binding biotin
- The avidin concentration in both the larvae and their frass was significantly lower than that of the transgenic leaves on which they had fed



Results – avidin levels



Mean concentrations (\pm SEM) of total and active avidin in tobacco leaves, larvae and their frass

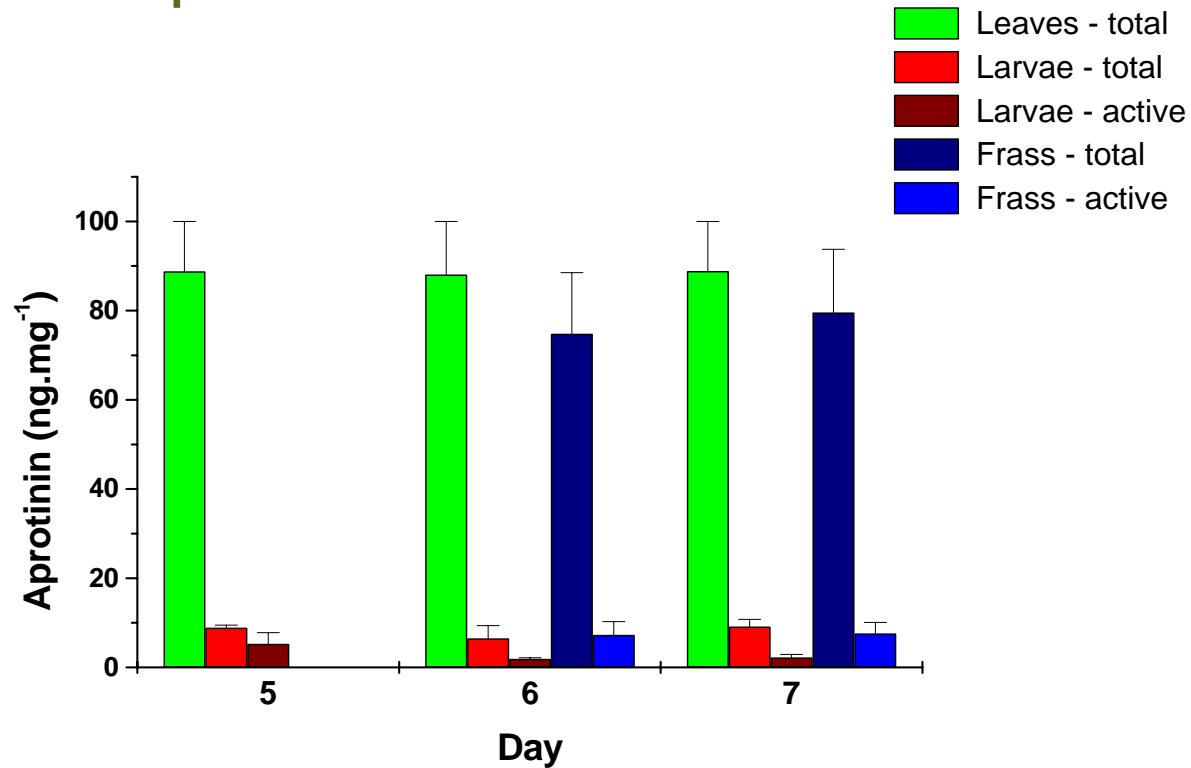


Results – aprotinin levels

- Aprotinin was not detected in the control plants, insects or frass
- In aprotinin-fed larvae, active aprotinin was detected at a significantly lower concentration than total aprotinin on each collection day
 - i.e., most of the aprotinin had bound to trypsin in the insect gut and been inactivated
- The level of aprotinin in larvae was significantly less than in the leaves on which they had fed.
- Aprotinin levels in the frass of these insects was similar to that in the leaves but only 1/10 remained active



Results – aprotinin levels



Mean concentrations (\pm SEM) of total and active aprotinin in tobacco leaves, larvae and their frass

Conclusions



- Both avidin and aprotinin were present and retained some of their biological activity in larvae that ate the leaves
- The level was about 1/10 that in the leaves suggesting the protein was present in gut lumen only
- Predators that eat prey whole may be affected
- Both proteins also present in the frass
- Levels similar to the original leaves
- Avidin's activity retained but 90% of aprotinin's activity lost
- Impacts of frass will be similar to or less than those from falling leaves
- Field studies required to confirm results
- Keep plant expression levels low