

on woody plant foliage have higher gut pH than those feeding on herbaceous plants. Condensed tannins are characteristic of trees, and high pH may be advantageous in reducing the stability of tannin–protein complexes. The association between phylogeny, diet, and midgut pH was considered further by Clark (1999). Exopterygote insects have near-neutral midguts, and physicochemical conditions in grasshopper guts are apparently not influenced by patterns of host plant use (Appel and Joern 1998), although Frazier *et al.* (2000) suggest that some grasshopper diets pose significant acid–base challenges. In an excellent review, Appel (1994) has stressed the importance of the gut lumen of insect herbivores as the site of interaction of nutrients (often refractory), insect and plant enzymes, allelochemicals, and pathogens. These interactions are affected by widely differing pH, redox conditions, and antioxidant activities. Although alkaline pH weakens protein–tannin binding, this mode of action is now considered less likely than oxidation of phenols to reactive quinones (Appel 1994). Oxidation of allelochemicals to toxic metabolites is, in fact, favoured by high pH. However, low oxygen levels in the gut lumens of herbivores reduce the rates of oxidation of allelochemicals (Johnson and Rabosky 2000). Ascorbate, an essential nutrient for many insects, is an antioxidant that maintains phenols in a reduced state in the gut lumen, minimizing their negative effects, and the recycling of ingested ascorbate may be the biochemical basis of differing tolerances to tannins among caterpillar species (Barbehenn *et al.* 2001).

Antinutrient proteins

Research on plant allelochemicals has shifted primarily to antinutrient proteins, because of their enormous potential in plant biotechnology (Lawrence and Koundal 2002). Development of transgenic crops expressing genes for insect resistance was first based on expression of Bt toxins, but transgenic plants equipped with genes for proteinase inhibitors and lectins are providing interesting opportunities for collaboration between chemists, physiologists, and applied ecologists. Proteinase inhibitors are inducible plant defences that are synthesized in leaves as a direct response to

feeding, not only at the site of attack, but also throughout the plant, although the response declines with plant age. They are also constitutively produced in seeds and storage organs of many staple crops (Jongsma and Bolter 1997). The signalling cascade that is initiated by feeding damage and leads to proteinase inhibitor gene expression is described by Koiwa *et al.* (1997). Proteinase inhibitors work by binding directly to the active sites of the enzymes to form complexes, mimicking the normal substrates but effectively blocking the active sites. Digestion of plant protein is inhibited and the insects are effectively starved of amino acids and prone to amino acid deficiencies.

Soybean trypsin inhibitor was the first proteinase inhibitor shown to be toxic to insects, and the trypsin inhibitors are particularly well known, partly because trypsin is commonly used in screening procedures for proteinase inhibitors (Lawrence and Koundal 2002). Based on primary sequence data, there are at least eight families of serine proteinase inhibitors in plants (Koiwa *et al.* 1997). Cysteine proteinase inhibitors (cystatins) are best studied in rice and are effective against some Coleoptera, whereas serine proteinase inhibitors are most effective against Lepidoptera. Effects on performance are commonly evaluated in insects feeding either on artificial diets containing the proteins or on the transgenic crops (this provides an opportunity to evaluate the effect of plant allelochemicals in natural diets, by comparing herbivore performance with that on unmodified crops). Jongsma and Bolter (1997) present data from numerous studies investigating the effects of proteinase inhibitors on various fitness parameters of insects. Frequently, the results of feeding experiments have been disappointing in comparison to those from *in vitro* experiments with gut extracts and proteinase inhibitors. Moreover, inhibitory effects may be surprisingly poor in transgenic plants, as shown by tomato moth larvae, *Lacanobia oleracea* (Noctuidae) subjected to a soybean inhibitor in artificial diets and in transgenic tomato plants (Fig. 2.13) (Gatehouse *et al.* 1999). There are many factors that may be responsible for such discrepancies, such as expression levels in the plant tissue, inhibitor–enzyme affinity, diet quality,

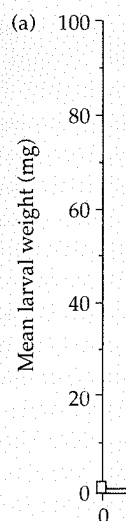


Figure 2.13 Effect of a feeding on (a) artificial d
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