ELSEVIER

Toxicon 44 (2004) 385–403

TOXICON

www.elsevier.com/locate/toxicon

Review

Antinutritional properties of plant lectins

Ilka M. Vasconcelos*, José Tadeu A. Oliveira

Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará, Caixa Postal 6020, Campus do Pici, CEP 60451-970, Fortaleza, CE, Brazil

Available online 2 June 2004

Abstract

Lectins are carbohydrate binding (glyco)proteins which are ubiquitous in nature. In plants, they are distributed in various families and hence ingested daily in appreciable amounts by both humans and animals. One of the most nutritionally important features of plant lectins is their ability to survive digestion by the gastrointestinal tract of consumers. This allows the lectins to bind to membrane glycosyl groups of the cells lining the digestive tract. As a result of this interaction a series of harmful local and systemic reactions are triggered placing this class of molecules as antinutritive and/or toxic substances. Locally, they can affect the turnover and loss of gut epithelial cells, damage the luminal membranes of the epithelium, interfere with nutrient digestion and absorption, stimulate shifts in the bacterial flora and modulate the immune state of the digestive tract. Systemically, they can disrupt lipid, carbohydrate and protein metabolism, promote enlargement and/or atrophy of key internal organs and tissues and alter the hormonal and immunological status. At high intakes, lectins can seriously threaten the growth and health of consuming animals. They are also detrimental to numerous insect pests of crop plants although less is presently known about their insecticidal mechanisms of action. This current review surveys the recent knowledge on the antinutritional/toxic effects of plant lectins on higher animals and insects.

Keywords: Plant lectins; Antinutritional effects; Higher animals; Insects

Contents

1. Introduction

Plant lectins were originally detected by the medical student Stillmark (1888), in Dorpat/Estonia when he was working on his dissertation thesis on castor beans (*Ricinus communis* L.). At this time he described the presence of a toxic proteinaceous factor in extracts from castor beans which also agglutinated red blood cells. To indicate the source in which it had been found the name ricin was adopted. Thus, historically, this fact is considered the true starting point of the research on plant lectins (Rüdiger, 1998). The term lectin (*legere* = Latin verb for to select) was coined by Boyd many years later to emphasise the ability of some hemagglutinins to discriminate blood cells within the ABO blood group system (Boyd and Reguera, 1949; Boyd and Shapleigh, 1954). Presently, the term lectin is prevalent over that of hemagglutinin and is broadly

* Corresponding author. Fax: +55-85-288-9789.
E-mail address: imvasco@ufc.br (I.M. Vasconcelos).

0041-0101/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2004.05.005

employed to denote 'all plant proteins possessing at least one non-catalytic domain, which binds reversibly to a specific mono- or oligosaccharide' (Peumans and Van Damme, 1995). Alternatively, lectins are also defined as 'proteins or glycoproteins of non-immune origin with one or more binding sites per subunit, which can reversibly bind to specific sugar segments through hydrogen bonds and Van Der Waals interactions' (Lis and Sharon, 1998). Both these definitions are very comprehensive and include non-agglutinating and some enzyme proteins (Van Damme et al., 1998) such as the class I plant chitinase, considered as a lectin because its single carbohydrate-binding domain is structurally distinct of its catalytic site and does not directly participate in the chitinolytic activity of this enzyme (Peumans and Van Damme, 1994). Obviously, chitinases cannot be called as agglutinins or phytohemagglutinins since they do not possess agglutination and/or glycoconjugate precipitation properties, as they are not multivalent carbohydrate-binding proteins. Another class of plant proteins which are also considered as lectins, according to the above definitions, are the type 2 RIPs (ribosome-inactivating proteins). Ricin, abrin and modeccin are well known examples of two-chain RIPs which irreversibly inactivate ribosomes by removing a specific adenine from a highly conserved tetranucleotide 'GA$_{4324}$GA' loop present in 28 S RNA of the large ribosomal subunit (Endo and Tsurugi, 1987; Barbieri et al., 1993). The high toxicity of these proteins to living cells is associated to the A chain which possesses the enzymatic RNA-*N*-glycosidase domain but it is the carbohydrate-binding or lectin domain in the B chain which interacts with cell surface sugar receptors, mediating endocytosis of type 2 RIPs, an essential event that precedes cellular toxicity (Barbieri et al., 1993; Peumans and Van Damme, 1994; Olsnes et al., 1999).

Considering the overall structure of plant lectins they are subdivided into four major classes: Merolectins which are proteins having a single carbohydrate-binding domain; Hololectins, comprising all lectins having di- or multivalent carbohydrate-binding sites; Chimerolectins, proteins consisting of one or more carbohydrate-binding domain(s) plus an additional catalytic or another biological activity dependent on a distinct domain other than the carbohydrate-binding site; and Superlectins which also possess at least two carbohydrate-binding domains but differ from the hololectins because their sites are able to recognise structurally unrelated sugars (Van Damme et al., 1998). Although plant lectins are considered a very complex and heterogeneous group of proteins at the biochemical/physicochemical viewpoint, sequence, structural information and molecular cloning of lectin genes enable subdivision of plant lectins in seven families of structurally and evolutionary related proteins: legume lectins; chitin-binding lectins; type 2 RIP and related lectins; monocot mannose-binding lectins; jacalin-related lectins; amaranthin lectin family and cucurbit phloem lectins (reviewed in Van Damme et al., 1998; Murdock and Shade, 2002). Plant lectins are also subdivided into five groups, according to the monosaccharide for which they exhibit the highest affinity: D-mannose/D-glucose, D-galactose/*N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, L-fucose and *N*-acetylneuraminic acid (Goldstein et al., 1997). Thus depending on the specificity toward a given monosaccharide the lectin will selectively bind to one of these above sugars which are typical constituents of eukaryotic cell surfaces (Lis and Sharon, 1998). In regard to their localisation, plant lectins are generally most abundant in the seeds but they are also found in different vegetative tissues such as in roots, leaves, barks, flowers, bulbs and rhizomes (Broekaert et al., 1987; Peumans et al., 1997; Ratanapo et al., 1998; Van Damme et al., 2000).

The wide distribution of lectins in all tissues of plants and their ubiquitous presence in the plant kingdom suggest important roles for these proteins. One possible physiological function that has emerged is the defensive role of these carbohydrate-binding proteins against phytopathogenic microorganisms, phytophagous insects and plant-eating animals (Chrispeels and Raikhel, 1991; Harper et al., 1995; Gatehouse et al., 1995). Indeed it has been shown that plant lectins possess cytotoxic, fungitoxic, anti-insect and anti-nematode properties either in vitro or in vivo and are toxic to higher animals (Oliveira et al., 1994; Peumans and Van Damme, 1995; Oka et al., 1997; Ripoll et al., 2003). For example, the toxic effects of PHA, the lectin from *Phaseolus vulgaris*, on monogastric animals are well documented (Pusztai, 1991; Pusztai et al., 1995), and the expression in transgenic plants of genes encoding for the lectins GNA (from *Galanthus nivalis*; snowdrop), PSA (*Pisum sativum*; pea), WGA (*Triticum vulgare*; wheatgerm), ConA (*Canavalia ensiformis*; jack bean), AIA (*Artocarpus integrifolia*; jack fruit), OSA (*Oriza sativa*; rice) and UDA (*Urtica dioica*; stinging nettle) resulted in added protection against insect pests and/or phytopathogenic nematodes or fungi (Hilder et al., 1995; Does et al., 1999; Hilder and Boulter, 1999; Powell, 2001; Ripoll et al., 2003).

One of the most important features of plant lectins, compatible with the proposed defensive function, is the remarkably high resistance to proteolysis and stability over a large range of pH, even when they are out of their natural environment.

2. Lectins in plant foods

Plants offer an enormous variety of macro- and micronutrients necessary for heterotrophs such as microbes and plant-eating organisms including nematodes, insects, various other invertebrates and higher animals. Food for human consumption is derived either from plant sources or from animals. Animals themselves are ultimately dependent upon plants for their survival. As lectins are present in the most commonly edible plant foods such as tomato, potato,

beans, peas, carrots, soybeans, cherries, blackberries, wheat germ, rice, corn, garlic, peanuts, mushrooms, avocado, beetroot, leek, cabbage, tea, parsley, oregano, spices and nuts, and also in several non-cultivated plant species (Nachbar and Oppenheim, 1980; Liener, 1986; Gupta and Sandhu, 1998; Oliveira et al., 2000; Leontowicz et al., 2001), the exposure of heterotroph organisms, including human beings, to functionally active lectins is a common event. Actually, the presence of nutritionally significant amounts of active lectins in fresh and processed foods and the lack of public knowledge concerning the deleterious effects of dietary lectins on the gut and health have led to a number of outbreaks of food poisoning. For example, Noah et al. (1980) reported seven incidents involving 43 persons in which the poisonings were attributed to toxins present in uncooked or partially cooked kidney beans (*Phaseolus vulgaris*). In 1981, a television programme by BBC cited a total of 330 outbreaks of kidney bean food poisonings involving 880 people (Bender and Reaidi, 1982). In 1988, a hospital launched a 'healthy eating day' in its staff canteen at lunchtime. One dish contained red kidney beans and 31 portions were served. Between 3 and 7 pm, 11 customers suffered from profuse vomiting and some diarrhoea. No pathogens were isolated from the food, but the beans contained abnormally high concentration of PHA (Gilbert, 1988). These examples all relate to kidney bean. However, it is possible that there have been problems with other lectin-containing foods that have not been reported.

3. Oral toxicity of plant lectins to higher animals

As already mentioned, the knowledge that some lectins are toxic to animals dates back to 1888, when Stillmark published his work on the deleterious effects of a proteinaceous substance present in castor bean. Soon after similar toxic substances were discovered in the seeds of *Croton triglium* (crotin) and *Abrus precatorius* (abrin) and in the bark of *Robinia pseudoacacia* (robinia) (Van Damme et al., 1998). With the increasing interest on lectins heightened particularly from the discovery of blood group specificity (Renkonnen, 1948; Boyd and Reguera, 1949) and from the realisation that PHA induced mitosis in mature, non-dividing human lymphocytes (Nowell, 1960), many other lectins were identified and isolated. Some of these were found to be toxic or antinutritional for man and animals. In general, nausea, bloating, vomiting and diarrhoea characterize the oral acute toxicity of lectins on humans exposed to them. In experimental animals fed on diets containing plant lectins the evident symptoms are loss of appetite, decreased body weight and eventually death (Liener et al., 1986; Duranti and Gius, 1997; Lajolo and Genovese, 2002). The mechanisms by which lectins mediate toxicity and the characteristic which dictate whether a lectin will be deleterious or not are not completely understood. Nevertheless, from a nutritional viewpoint, it is important to find ways of identifying which lectin is and which is not toxic.

As an attempt to predict the oral toxicity of lectins using small amounts of sample toward minimising costs and reducing the experimental time, many lectins were tested for toxicity by intradermal or intraperitoneal administration in rats and mice (Liener, 1986, 1994; Reynoso-Camacho et al., 2003). From these studies it emerged that whilst most parentally toxic lectins were also orally toxic, a few exhibited no oral toxicity. Contrarily some parenterally non-toxic lectins were highly toxic when orally administered. Therefore, intradermal or intraperitoneal tests designed to evaluate oral toxicity are not a reliable predictor. Furthermore, parenteral administration is not the common route by which animals are exposed to dietary lectins. Data currently available indicate that multiple exposure and long-term studies may be also needed to address lectin toxicity (Grant et al., 2000).

The ability of lectins to selectively bind to different types of blood cells has also been proposed as a means to predict oral toxicity of these proteins. It has been suggested that the hemagglutinating ability could form a basis for in vitro screening of potentially toxic lectins. Thus, lectins which strongly agglutinate a wide range of different red blood cells should generally have high oral toxicity for rats, whereas those agglutinating only rabbit and/or enzyme-treated rat erythrocytes should not (Grant et al., 1983, 1995). However a lack of a significant number of assays to firmly establish such relationship together with exception to this rule found for several lectins such as the *Cratylia argentea* lectin (CAA) which strongly agglutinates a wide range of erythrocytes being essentially non-toxic (Rios et al., 1996), mean that this approach is not a reliable predictor. As with intradermal and intraperitoneal toxicity studies, this procedure cannot be used alone to predict which lectin will or will not have deleterious effects when consumed orally. In fact, the unique reliable way of predicting the oral toxicity of lectins is by feeding the target animal on diets containing the purified lectin. Moreover, it is important to carry out these tests on several different animal species and not just on rats and mice. Accordingly, kidney beans induced pancreas growth in chicks but not in pigs (Huisman et al., 1990a,b). Recently, Douglas et al. (1999) detected that SBA (*Glycine max*; soybean agglutinin) accounted for approximately 15% of the growth depression from raw soybeans in chicks, whereas Liener (1953) estimated that lectins accounted for approximately 50% of the growth-inhibiting effect of raw soybean meal in rats. In addition, it has been considered that the degree to which some lectins affect metabolism is, in part, dependent on dietary history of the animal and the exact composition of the diet (Grant, 1999). However, it is impractical to test all lectins under all circumstances in vivo. To establish a screening system, it is thus important to develop a clear picture of the properties of specific lectins that lead them to be toxic to man and animals.

4. Resistance of lectins to proteolysis

Plant proteins are generally regarded as more resistant to proteolysis than most proteins of animal origin (Friedman, 1996; Sgarbieri, 1996; El-Adawy, 2002). Indeed, the apparent digestibility of proteins in vivo from leguminous seeds and oilseeds in their raw state varies from 15.6% to about 80% depending upon the origin (Grant et al., 1995; Carbonaro et al., 2000; Preet and Punia, 2000; Cuadrado et al., 2002). In contrast, animal proteins, such as egg albumin or casein, are 85–94% digestible in vivo (Oliveira et al., 1994; Vasconcelos et al., 2001). Many plant lectins in particular have been found to be resistant to degradation by proteases in vitro (Carbonaro et al., 1997) and in the gut in vivo (Pusztai, 1991). This may in fact be a common feature of this class of proteins. PHA (Pusztai et al., 1979; Hara et al., 1984), ConA (Nakata and Kimura, 1985), ConBr (*Canavalia brasiliensis*) (Oliveira et al., 1994), CAA (Oliveira et al., unpublished data), PTA (*Psophocarpus tetragonolobus*; winged bean) (Higuchi et al., 1983), LEA (*Lycopersicon esculentum*; tomato) (Kilpatrick et al., 1985), GNA, SBA, WGA, PSA, SNA-I, SNA-II (*Sambucus nigra*; elderberry), VFL (*Vicia faba*; broad bean) and DGL (*Dioclea grandiflora*; mucuna) (Pusztai, 1991; Bardocz et al., 1995) were all showed to be resistant to in vivo breakdown by proteolytic enzymes. The extent of the in vivo lectin resistance to degradation by the gut enzymes was variable but in some cases it reached very high values. For instance, in one trial with rats fed on known amounts of intragastrically administered pure lectins, the feces were found to contain (by 'rocket immunoelectrophoresis') over 90% of the ingested PHA, ConA and GNA in a form still fully reactive towards rabbit anti-lectin antibodies (Pusztai, 1991). Of a particular interest is the finding that biologically intact WGA was detected in ileostomy effluent and fecal collections from human subjects consuming a diet containing wheat germ (Brady et al., 1978). These observations demonstrated that PHA, ConA, GNA and WGA transverse the rat and human small intestine, respectively, intact. Conceivably orally ingested plant lectins remaining at least partially undigested in the gut may bind to a wide variety of cell membranes and glycoconjugates of the intestinal and colonic mucosa leading to various deleterious effects on the mucosa itself as well as on the intestinal bacterial flora and other inner organs (Rüdiger, 1998).

It is also noteworthy that far less of the lectins survive the in vitro treatment with proteolytic enzymes (Rios et al., 1996). This was the case for ConBr, CAA and DGL, all glucose–mannose specific lectins, which were digested to a far higher extent (52–84%) than in vivo. Thus, it is possible that in the gut the lectins may even be protected from proteolytic degradation during gut passage perhaps as a result of binding to epithelial or lumenal gut components. The slight protection from proteolytic degradation in vitro conveyed by adding glucose, Ca^{++} and Mn^{++} to the reaction mixture is consistent with this possibility. Nevertheless, as a result of high resistance to proteolytic degradation in vivo nutritionally significant amounts of certain dietary lectins will survive in an intact and highly reactive form within the gut lumen. Obviously this is crucial for them to exhibit the full spectrum of their biological activities on animals (Fig. 1).

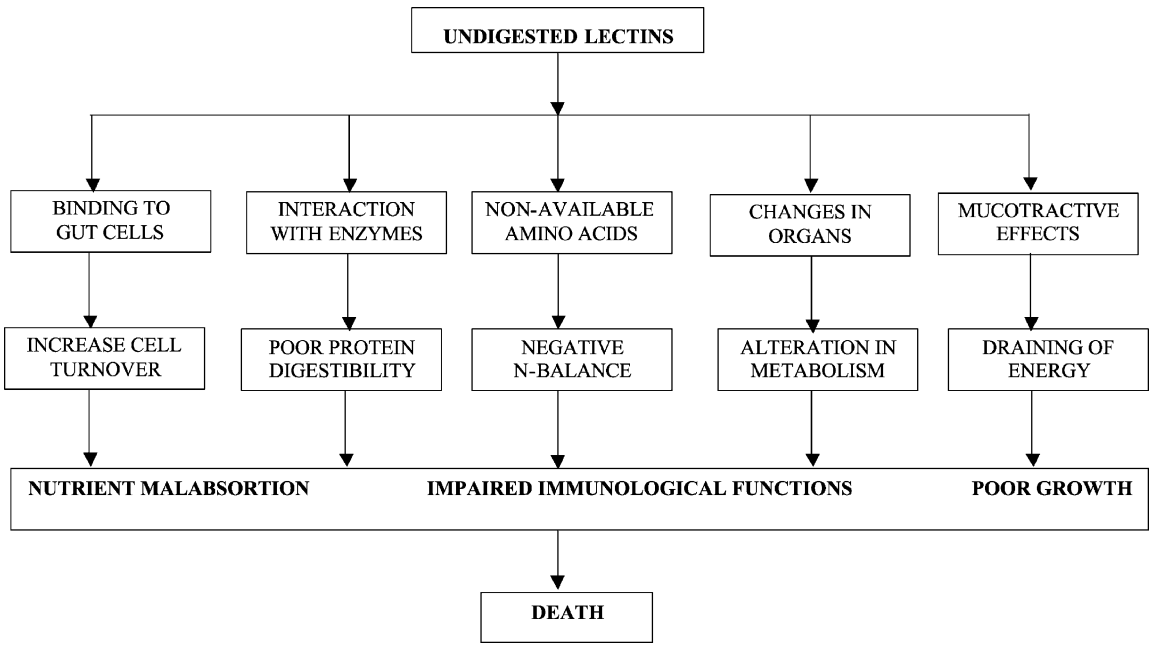

Fig. 1. Spectrum of biological activities of plant lectins on higher animals.

Thus the deleterious effects of lectins depend definitively on the degree of lectin resistance to proteolytic degradation (Caygill, 1999).

5. Effects of lectins on the digestive tract

As most lectins are not degraded during their passage through the digestive tract they are able to bind the epithelial cells which express carbohydrate moieties recognised by them. This event is undoubtedly the second one in importance for determining the toxicity of orally fed lectins. Indeed, lectins which are not bound by the mucosa usually induce little or no harmful antinutritive effect for the consumers (Pusztai and Bardocz, 1996). Once bound to the digestive tract, the lectin can cause dramatic changes in the cellular morphology and metabolism of the stomach and/or small intestine and activate a cascade of signals which alters the intermediary metabolism. Thus, lectins may induce changes in some, or all, of the digestive, absorptive, protective or secretory functions of the whole digestive system and affect cellular proliferation and turnover. In 1960, Jaffé suggested that the toxic effects of ingested lectins were due to their ability to combine with specific receptor sites of the cells lining the small intestine and to cause a non-specific interference with absorption and nutrient utilisation (Jaffé, 1960). Many observations either in vivo or in vitro support Jaffé's hypothesis (Oliveira et al., 1988; Hajos et al., 1995). PHA was also able to attach to gastric mucosal and parietal cells inhibiting the gastric acid secretion in conscious rats (Kordás et al., 2000, 2001). Additionally, PHA-treated pigs showed an increase in stomach weights and mucosa thickness (Radberg et al., 2001). In fact, if appropriate carbohydrate moieties are expressed on the surface of any structure or organ, lectins will be able to bind to them (Pusztai, 1991; Jordinson et al., 1997; Otte et al., 2001; Bryk and Gheri, 2002). Moreover, in rats some lectins appeared to progressively mediate changes in the glycosylation of the gut leading to an increase in the binding sites to which they could attach (Pusztai et al., 1995). As the glycosylation state of the gut among higher animals shows many similarities it is plausible to suppose that the adverse effects of dietary lectins observed on experimental animals will be comparable in human beings. Obviously the severity of these adverse effects will depend on the gut region to which the lectin will bind (Baintner et al., 2000).

Many lectins either directly or indirectly cause profound morphological and physiological modifications in the small intestine. Such alterations characteristically lead to increased shedding of brush border membranes, accelerated cell-loss and shortened, sparse and irregular enterocyte microvilli (Pusztai, 1991; Bardocz et al., 1995; Herzig et al., 1997). In fact, binding of lectins to the gut epithelium is frequently accompanied by disruption of the brush borders and disorganisation of the main absorptive cells, which cause reduction in the absorptive surface area and absorption of nutrients. One result of this is cellular hyperplasia and increased endogenous secretion leading to large increases in the small intestinal weight of rats fed lectins (Otte et al., 2001; Sasaki et al., 2002). Actually some lectins have been shown to trigger notable trophic effects on the intestine. PHA and PNA consistently reversed the fall in gastrointestinal and pancreatic growth associated with total parenteral nutrition by stimulating the release of specific hormones, such as gastrin, cholecystokinin (CCK) and enteroglucagon (Jordinson et al., 1999). Parenteral administration of ConA significantly elevated epithelial cell proliferation in the rat intestine (Fitzgerald et al., 2001). Regardless of the precise nature of the initial response by the small intestinal cells to the growth signal, it is clear that there is an obligatory accumulation of polyamines, such as putrescine, spermidine and spermine, in small crypts (Pusztai, 1991; Bardocz et al., 1996). This is followed by secondary responses, which include increased DNA, RNA, protein and carbohydrate tissue contents (Pusztai et al., 1988; Bardocz et al., 1995). Increased rat small intestinal weight has also been related to SBA, ConA, ConBr, WGA and PNA (peanut agglutinin) (Pusztai, 1991; Grant, 1991, 1999). A dramatic dose-dependent hyperplastic growth of the small intestine was also observed by oral administration of ML-1 (*Viscum album*; mistletoe lectin), probably due to the avid binding and endocytosis of this lectin by gut epithelial cells (Pusztai et al., 1998). In contrast, no weight effect was found for the small intestine in the PHA-treated pigs. However, morphometric analyses of the small intestine of these animals showed a decrease in villus heights, an increase in crypt depths and crypt cell mitotic indices, fewer vacuolated enterocytes per villus and reduced vacuole size. Additionally, a decrease in the absorption of different-sized marker molecules after gavage feeding and a decrease in intestinal marker permeability were observed (Radberg et al., 2001). PHA also induces growth of the large intestine (Bardocz et al., 1995). Nevertheless, in this digestive tract section the changes were much less marked than those observed in the small intestine (Grant, 1999).

In addition to the disruptive effects on cell membrane, lectins have been shown to inhibit various intestinal and brush border enzymes. A non-competitive inhibition of enterokinase from rat duodenal brush borders was observed in vitro in the presence of ConA, PHA or SBA, PHA being the most potent inhibitor (Rouanet et al., 1983). PHA also interacted and inhibited brush border dipeptidase in vitro (Erickson et al., 1985). Sucrose, maltase, alkaline phosphatase, leucine aminopeptidase and γ-glutamyltransferase, all suffered a significant decrease in activities by inclusion of PTA in a basal diet offered to rats (Higuchi et al., 1984). RCA (*R. communis*), PHA and UEA interacted with various human small intestine brush-border hydrolases such as sucrase, isomaltase, maltase glucoamylase, lactase, neutral and acid aminopeptidases and dipeptidyl peptidase IV (Triadou and Audran, 1983). PHA decreased the hydrolysis

of casein, bovine serum albumin and heat-treated bean extract by pepsin and pancreatin (Thompson et al., 1986). The insecticidal GNA given to pathogen-free rats increased the activities of the brush border enzymes alkaline phosphatase and aminopeptidase whereas sucrase–isomaltase activity was nearly halved (Pusztai et al., 1996). ASA, the lectin from edible garlic (*Allium sativum*), induced increased activities of disaccharidases and acid phosphatase in the rat jejunum whereas the activities of alkaline phosphatase, lactate dehydrogenase and adenosine triphosphatase were lower (Gupta and Sandhu, 1998). Thus, the well established lectin-induced disruption of intestinal microvilli combined with the in vivo inhibitory effects on gut enzymes suggest that the lectins interfere, either directly or indirectly, not only with the utilisation of dietary protein and carbohydrate but also with the initial and final stages of protein/carbohydrate digestion and transport.

An additional secondary toxic effect of undigested lectin, particularly PHA, in the small intestine which may further reduce the efficiency of digestion and absorption of food is a dramatic overgrowth of coliform bacteria (Wilson et al., 1980; Pusztai et al., 1993a; Bardocz et al., 1996). The mechanism by which lectins promote proliferation of coliforms, mainly *Escherichia coli*, is not fully understood. However, it has been established that in relation to PHA, the bacterial proliferation arises primarily as a result of its effects on epithelial cell metabolism (Pusztai, 1991; Pusztai et al., 1995). Indeed PHA-mediated mucus secretion, epithelial cell loss, serum protein leakage and reduced digestion of dietary protein possibly further aid bacterial proliferation by providing a good source of nutrients (Grant, 1999). In consequence, the increase in bacterial numbers in the small intestine may lead to overproduction of bacterial toxins which also contributes to the worsening of animal health.

Although almost all the theory about the general deleterious effects of dietary lectins on animals was built from various comprehensive studies carried out with PHA, other lectins showed some similar effects. We showed that ConBr, a lectin from *Canavalia brasiliensis*, which shares 90% amino acid sequence similarity with ConA (Grangeiro et al., 1997), inhibited rat growth probably through interference in the metabolism as elevated losses of nitrogen and dry matter in the feces and lower retention of ingested nitrogen were observed (Oliveira et al., 1994). Recently, we observed that another ConA related lectin, CAA (*Cratylia argentea* agglutinin), interacted with the epithelium of duodenum, jejunum and ileum of rats fed on diets containing the *C. argentea* seed meal or CAA at 2% protein level. CAA-binding to the cells lining the gut lumen (Fig. 2) may have significantly impaired nutrient absorption which led to a reduced body weight gain in such animals, higher fecal and nitrogen outputs and lower nutritional parameters.

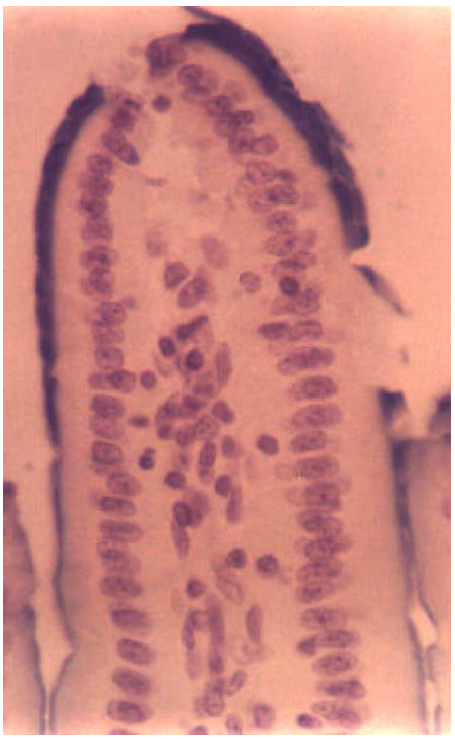

Fig. 2. Light micrograph (600 ×) of a duodenum villus of a rat fed for 10 days on a diet containing 2% *Cratylia argentea* seed lectin. Incubation of a duodenum transverse section with rabbit anti-lectin immunoglobulins and alkaline phosphatase-conjugated goat anti-rabbit IgG produced a dark staining on the villus surface representing the immunolabelling lectin bound to a mature enterocyte. No enterocyte staining was observed in control rats fed on an egg white based diet.

6. Systemic effects of dietary lectins

High rates of internalisation of dietary lectins by enterocytes after binding to the small intestine cells appear to be a common event (Pusztai, 1991; Bardocz et al., 1995). Lectins, which bind to epithelial cells, may be taken up into cells by endocytosis, be released by exocytosis into the intracellular space from where they are subsequently transported throughout the body. As a result, reactive forms of the lectins are distributed in the circulation and internal tissues and may lead to deleterious systemic effects. For instance, the *N*-acetylglucosamine-specific agglutinins, WGA, UDA and DSA (*Datura stramonium*; thorn apple), when offered to rats at the level of 7 g/kg diet, bound to and were endocytosed by the epithelial cells of the small intestine (Pusztai et al., 1993b). An appreciable portion of the endocytosed WGA, the most damaging amongst the three lectins tested, was transported across the gut wall into the systemic circulation where it was deposited in the walls of the blood and lymphatic vessels (Pusztai et al., 1993b).

PHA was found to induce a powerful, selective humoral response of the IgG-type when raw beans were fed to rats

(Grant et al., 1985), growing steers (Williams et al., 1984) and pigs (Begbie and King, 1985), indicating its absorption through the gut wall. However, development of circulating antibodies to the lectin was unable to prevent either bacterial proliferation or the uptake of the dietary lectin into the systemic circulation (Pusztai, 1989). For instance, the presence of antibodies from human blood against the banana lectin (BanLec-1) was detected in a screening for reactivity to foodstuff components (Koshte et al., 1990, 1992). Circulating antibodies to three structurally related legume lectins, ECorL (*Erythrina corallodendron*), PNA and SBA, and to WGA were purified by lectin-affinity chromatography from human sera indicating that antibodies to dietary lectins commonly present in human diets exist in the sera of healthy humans (Tchernychev and Wilchek, 1996). The authors argued that the significant amounts of circulating antibodies which reacted with EcorL, as it is not a dietary protein, is because legume lectins share considerable structural similarities (Van Damme et al., 1998). Recently, the mucosal immunogenicity of a number of plant lectins, with different sugar specificities, was investigated in mice (Lavelle et al., 2000). Accordingly both oral and intranasal delivery of five plant lectins, LEA, ML-1, PHA, WGA and UEA-1, stimulated the production of specific serum IgG and IgA antibodies after three intranasal or oral doses.

The ability of most plant lectins to transverse the gut barrier is not limited to antibody production but can also trigger histamine release from basophils (Haas et al., 1999). Accordingly 16 common lectins, particularly ConA, PHA, PSA, SNA and LCA (*Lens culinaris*; lentil) were able to induce human basophils to secrete interleukin-4 (IL-4) and IL-13, the key promoters of Th2 responses and IgE synthesis. Since lectins can enter the circulation after oral uptake, they might play a role in inducing the so-called early IL-4 required to switch the immune response towards a Th2 response and type I allergy.

In a recent review, Cordain et al. (2000) stated that the interaction of dietary lectins with enterocytes and lymphocytes might facilitate the translocation of both dietary and gut-derived pathogenic antigens to peripheral tissues causing a persistent peripheral antigenic stimulation in genetically susceptible individuals. This might ultimately result in the expression of overt rheumatoid arthritis (RA).

A striking anatomical change evident in rats fed on lectins is the severe atrophy of the thymus. This occurs to such a degree that in some cases the organ nearly disappeared after feeding PHA for 10 days (Oliveira et al., 1988, 1994). Although the precise significance of this involution is not clear, it is quite likely that it may affect cell-mediated immune responses having serious consequences for the animal. Thus, the proliferation of normally innocuous intestinal bacteria and even the absorption of an antigenic form of lectin which has been shown to gain entry to the systemic circulation are possibly related to the incapacity of these animals to develop an adequate humoral and cell-mediated immune response.

Usually animals fed on legume diets develop pancreas hypertrophy. Formerly this phenomenon had been exclusively associated to dietary protease inhibitors. Studies by Oliveira et al. (1988) and Pusztai et al. (1988) unequivocally showed that the purified PHA included into diets offered to rats promoted hypertrophy of the pancreas in a dose response fashion. This was in fact the first time that pancreatic hypertrophy was shown to be due to diets which contained purified lectins free from trypsin inhibitors. Since then, this effect has consistently been observed in rats fed on different diets containing purified lectins from other legumes (Oliveira et al., 1994; Vasconcelos et al., 2001). Recently Kelsall et al. (2002) demonstrated in long-term feeding trials that even low doses (40 μg/rat/day) of PNA given to rats could significantly influence pancreatic growth. They suggested that this trophic action might have potential adverse implications for the development of pancreatic cancer in humans. Accumulation of polyamines has been shown to precede organ hypertrophy (Pusztai et al., 1988; Bardocz et al., 1996). Further PHA, SBA, PNA and WGA-induced pancreatic growth of rats, in vivo, are related to increasing CCK plasma levels with corresponding increase in pancreatic protein output suggesting that the ingestion of lectins might have major CCK-mediated effects on gastrointestinal function and pancreas growth (Herzig et al., 1997; Jordinson et al., 1997). CCK is a peptide hormone released from the I-cells of the upper small intestine which is a potent stimulator of various physiological functions such as pancreas enzyme secretion, reduction of food intake, inhibition of gastric emptying and stimulation of gall bladder contraction (Sayegh and Ritter, 2003). Grant et al. (1999) suggested that secretion of pancreatic digestive enzymes induced in rats by first-time oral exposure to PHA (E_2L_2 isolectin) was mediated only in part by CCK. They suggested that additional mechanisms or hormones, such as secretin, might play a role in modulating later exocrine pancreas responses to PHA. PHA was also able to stimulate pancreatic amylase secretion in rats which was blocked by devazepide, a CCK-A receptor antagonist (Kordás et al., 2000). Radberg et al. (2001) observed that the size of the pancreatic acini was greater in the PHA-treated pigs, but no increases in enzyme content or pancreatic weight could be determined. However, the blood plasma levels of CCK were higher in the PHA-treated than in the control pigs.

Disturbances of the hormonal homeostasis of animals were also observed upon feeding lectins. PHA reduced the circulating levels of insulin initially by interfering with its secretion from the pancreas but later insulin synthesis was also impaired. Surprisingly, normal blood glucose concentrations were maintained in rats fed with PHA despite the low circulating insulin levels (Pusztai, 1991; Bardocz et al., 1996). The exact mechanism by which lectins cause changes in insulin production remains unclear. However, it is

possible that through its binding to neuroendocrine cells in the gut or to other non-pancreatic endocrine organs, PHA may trigger the release of hormones which impair insulin synthesis/secretion. Alternatively, it is likely that the insulin-mimicking properties of lectins in vivo may interfere with the feedback regulation of pancreatic insulin production (Bardocz et al., 1996).

Liver enlargement was shown to be another systemic effect of dietary lectins (Oliveira et al., 1988; Pusztai, 1991). The increase in liver weight may be due to a response of this organ to the disturbance of the general metabolism that occurs when animals are fed with lectins. In fact some lectins induce body fat catabolism and glycogen loss, leading to depletion of the body reserves (Grant et al., 1987, 1995). Dietary PHA, for instance, induces an increase in body lipid utilization. A direct correlation between dietary PHA and increased amounts of urinary 3-hydroxybutyrate output of rats was observed, providing a strong evidence for the occurrence of a PHA-dependent increased lipolysis (Oliveira et al., 1988). The lipid depletion occurred primarily from the adipose tissues whereas little change was observed in liver lipid levels. In contrast, the glycogen content of the liver was halved whereas the glycogen concentration in skeletal muscle was not significantly affected during the same period (Oliveira et al., 1988; Pusztai, 1989). According to Pusztai et al. (1998) ML-1 reduced body fat reserves probably through depression of circulating insulin levels. Dietary PHA also alters the rate of muscle protein synthesis without significantly affecting the rate of protein degradation, resulting in loss of muscle weight (Palmer et al., 1987). It is possible that circulating lectin may have interacted directly with muscle cells leading to impairment of protein synthesis (Pusztai et al., 1989). Alternatively, this effect may have been indirect and hormonally mediated (Pusztai, 1991). Moreover, increased concentration of urinary N (mainly urea-N) was observed in PHA-fed rats suggesting disturbances in the protein metabolism (Oliveira et al., 1988). Increased activities of liver glutamic pyruvic transaminase (LGPT) and glutamic oxaloacetic transaminase (LGOT), indicative of increased catabolism of amino acids in the liver, were also observed when increasing amounts of dietary PLA (*Phaseolus lunatus*; lima bean) were offered to rats (Aletor and Fetuga, 1985). In summary, dietary lectins seem to interfere with the overall metabolism of body lipid, protein and carbohydrate (Pusztai, 1991; Grant, 1999). Such effects may have a bearing upon hepatomegaly. Although the significance of the morphological and metabolic changes in the liver of lectin-fed animals remains to be elucidated, such adverse effects upon this key organ certainly contribute to the overall toxicity of dietary lectin.

Lung hypertrophy was also detected in rats fed with diets containing ML-1 (Pusztai et al., 1998). Peanut lectin which displays affinity for glycoproteins found specifically on arterial smooth muscle cells stimulated the growth of smooth muscle and pulmonary arterial cells, suggesting that biologically active PNA present in peanut oil could possibly contribute significantly to its atherogenic properties (Kritchevsky et al., 1998).

Recently our group purified a toxic and hemagglutinating lectin (IAAL) from salsa (*Ipomoea asarifolia* R. et Schult., Convolvulaceae) leaves which agglutinates rabbit, horse and ovine red cells but not cow, sheep, goat, dog and human erythrocytes. This activity was not abolished by simple sugars but fetuin, avidin and *N*-acetyl-D-neuraminic acid were inhibitory (Santos, 2001). IAAL injected intraperitoneally into mice and rats induced dyspnoea, tonic–clonic convulsions and flaccid paralysis prior to death. IAAL was also toxic to orally fed mice. When IAAL was daily intubated into female mice, for 15 days, it induced stunted growth, diarrhoea, severe respiratory distress and muscle tremors. The liver of the female mice receiving IAAL showed megalocytose and hepatocytes with vacuolar degeneration and piknotic nuclei. In the kidneys, tubular liquefactive necrosis, atrophy and destruction of glomeruli and congestion of blood vessels occurred (data not shown). Surprisingly, the litter was also affected with some of these above symptoms (Fig. 3), although in a lesser extent.

Despite the adverse effects of dietary lectins, in recent years, considerable work has been done on the possible uses of them, at non-toxic oral doses, to obtain therapeutically beneficial effects, such as promotion of gut regrowth after total parenteral nutrition or exposure to elemental diets (Jordinson et al., 1999; Sasaki et al., 2002), use as drug delivery agents or oral vaccine adjuvants (Lavelle, 2001; Lavelle et al., 2001) or use in anti-cancer therapies (Pryme et al., 1998, 1999).

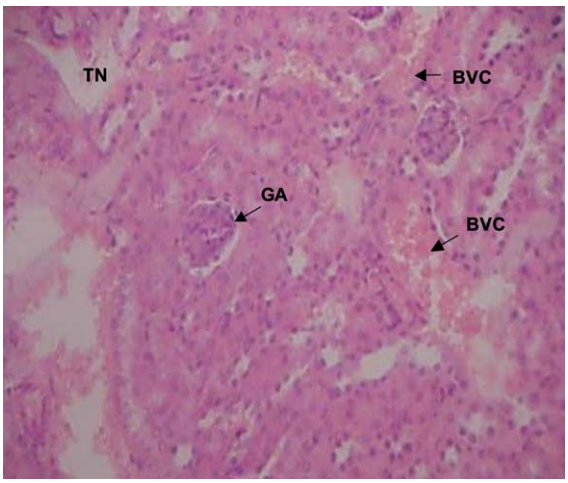

Fig. 3. Light micrograph (100 ×) of kidney section from a suckling mouse pup whose mother was intubated for 15 days with *Ipomea asarifolia* agglutinin (IAAL, 6–8 mg/day/mouse) showing blood vessel congestion (BVC), glomerulus atrophy (GA) and tubular necrosis (TN).

7. Insecticidal properties of plant lectins

The insecticidal activity (Table 1) of plant lectins against a large array of insect species belonging to the Coleoptera, Homoptera, Diptera and Lepidoptera order has been well documented (Gatehouse et al., 1995; Schuler et al., 1998; Carlini and Grossi-de-Sá, 2002). This feature represents a potential of using plant lectins as naturally occurring insecticide agents against the pests which restrain increased crop production. Generally the in vitro bioassay undertaken to judge this biological characteristic consists of inclusion of the studied lectin into artificial diets offered to the target insect during a given period of time. In general, the lectin levels incorporated into artificial diets to test oral toxicity have varied from 1 to 50 mg/g diet or from 5 to 1500 μg/ml diet to deliver these proteins to chewing and sucking insects, respectively. Then, the parameters which indicate the harmful effects of lectins on insects include larval weight, size, color, mortality, feeding inhibition, antimetabolic effects, honeydew excretion levels, pupation, delays in total developmental time, adult emergence and/or fecundity on the first and/or second generation of the insects which have been reared on lectin-containing artificial diets.

Although the precise mode of insecticidal action of plant lectins is not fully understood it appears that resistance to proteolytic degradation by the insect digestive enzymes and binding to insect gut structures are two basic prerequisites for lectins to exert their deleterious effects on insects. As in higher animal systems (Pusztai, 1991), the harmful effects of lectins on insects are attributed mainly to binding of them to the surface of the intestinal epithelial cells which lead to interference with the digestive, protective, or secretory functions of the intestine. Nevertheless, it is quite obvious that for binding to the intestine structures, cells and/or to any digestive enzyme or either to other insect component, the orally fed lectins have firstly to be refractory to the action of proteolytic enzymes and, in addition, to encounter satisfactory environmental conditions of pH and temperature to exert their effects. Indeed, structure/function analysis by site-directed mutagenesis indicated that the insecticidal and binding activities of the GlcNAc-specific lectin GSII (*Griffonia simplicifolia*) and its mutant forms were correlated with the resistance to proteolytic degradation by the midgut extracts and binding to the digestive tract of third or fourth instar larvae of *Callosobruchus maculatus* (Coleoptera: Bruchidae), the cowpea weevil. This insect when reared on a GSII-containing artificial diet had a within-seed prolonged development time and inhibited growth (Zhu-Salzman et al., 1998). These effects may have resulted from the binding of this lectin to the chitin of the peritrophic membrane, a structure existing in the gut of most phytophagous insects which protects the gut epithelial cells from abrasive food particles (Peumans and Van Damme, 1994). It is likely that other GlcNAc-specific lectins (Table 1) share similar mode of action on insects. The two chitin-binding lectins, TEL and KpLec, from *Talisia esculenta* and *Koelreuteria paniculata* seeds, respectively, negatively affected the larval development of *C. maculatus* and *Anagasta kuehniella* (Lepidoptera: Pyralidae; Mediterranean flour moth) through binding to glycan receptors on the surface of cells lining the insect gut (Macedo et al., 2002, 2003). Regression analysis showed that for every 0.1% increase in TEL dose given via artificial seeds, there were a 3.9% ($R^2 = 0.98$) and 4.1% ($R^2 = 0.99$) increase in mortality of *C. maculatus* and *Zabrotes subfasciatus* (Coleoptera: Bruchidae), respectively (Macedo et al., 2002) whereas for every 0.1% increase in KpLec dose, there were a 5.9% ($R^2 = 0.98$) and 5.3% ($R^2 = 0.96$) increase in mortality of *C. maculatus* and *Anagasta kuehniella*, respectively (Macedo et al., 2002). Immunological studies carried out to elucidate the mechanism of action of the mannose-specific lectin GNA on the rice brown planthoppers (*Nilaparvata lugens*; Homoptera: Delphacidae) showed that no significant proteolytic degradation occurred either in the gut or honeydew of insects fed on lectin-containing diet (Powell et al., 1998). The fully active GNA was able to bind to the luminal surface of the midgut epithelial cells within the planthopper, probably recognising cell surface carbohydrate moieties of glycoproteins and/or other glycoconjugates in the gut. For instance, the mannose-containing glycopolypeptide ferritin acts as the most abundant binding protein for GNA in the midgut of rice brown planthoppers (Du et al., 2000). Moreover the immunolabelling GNA assay revealed its presence in the fat bodies, the ovarioles, and throughout the haemolymph suggesting that GNA was able to cross the midgut epithelial barrier and pass into the insect's circulatory system resulting in a systemic toxic effect (Powell et al., 1998).

Resistance to proteolytic degradation and binding of ASAL (mannose-binding leaf garlic lectin) to gut receptors in the luminal epithelium of two important homopteran insect pests, the mustard aphid (*Lypaphis erysimi*; Aphididae: Homoptera) and the red cotton bug (*Dysdercus cingulatus*; Hemiptera: Pyrrhocoridae) also correlated with its insecticidal activities (Bandyopadhyay et al., 2001). ASAL bound to the carbohydrate residue of the 55 and 45 kDa brush border membrane vesicle receptor proteins in the case of aphid and bug, respectively, possibly decreasing the insect membrane permeability. Zhu-Salzman and Salzman (2001) studying the digestion of recombinant GSII and its mutant protein variants with two purified cathepsin L-like gut proteases of *C. maculatus* larvae suggested that carbohydrate binding to the insect gut and proteolytic resistance are independent properties of rGSII. However they concluded that both properties facilitate GSII efficacy as a plant defensive molecule. GNA and ConA when included in artificial diets offered to the tomato moth (*Lacanobia oleracea*; Lepidoptera: Noctuidae) larvae accumulated in the gut, Malpighian tubules and haemolymph indicating that both lectins were internalised and distributed systemically after binding to glycoproteins present along the insect digestive tract (Fitches et al., 2001a).

Table 1
Plant lectins with oral toxicity to insect

Lectin (plant source)	Insect	Host	Reference
Mannose specific[a]			
ASA (*Allium sativum*)	*Laodelpha striatellus* (rice small brown planthopper); *Nilaparvata lugens* (rice brown planthopper);	Rice	Powell et al., 1995
	Myzus persicae (peach-potato aphid)	Peach, potato	Sauvion et al., 1996
	Dysdercus cingulatus (red cotton bug); *D. koenigii* (red cotton bug)	Cotton, okra, maize, pearl	Roy et al., 2002
ASA I, II	*D. cingulatus*; *D. koenigii*	Cotton, okra, maize, millet	Roy et al., 2002
ASAL (*Allium sativum*—leaf)	*D. cingulatus*; *Lipaphis erysimi* (mustard aphid)	Cotton, okra, maize, pearl	Bandyopadhyay et al., 2001
CEA (*Colocasia esculenta*)	*D. cingulatus*; *D. Koenigii*	Cotton, okra, maize, pearl	Roy et al., 2002
DEA (*Differenbachia sequina*)	*D. Cingulatus*; *D. Koenigii*	Cotton, okra, maize, pearl	Roy et al., 2002
GNA (*Galanthus nivalis*)	*Callosobruchus maculatus* (bruchid weevil)	Cowpea	Gatehouse et al., 1991
	Acyrthosiphon pisum (pea aphid)	Pea	Rahbé et al., 1995
	Antitrogus sanguineus (sugarcane whitegrub)	Sugarcane	Allsopp and McGhie, 1996
	Aulacorthum solani (glasshouse potato aphid)	Potato	Down et al., 1996
	M. persiacae	Peach, potato	Sauvion et al., 1996
	Lacanobia oleracea (tomato moth)	Tomato	Fitches and Gatehouse, 1998; Fitches et al., 2001a
	Maruca vitrata (legume pod-bore)	Cowpea	Machuka et al., 1999
	Tarophagous proserpina (taro planthopper)	Taro	Powell, 2001
	L. striatellus	Rice	Loc et al., 2002
	N. lugens	Rice	Powell et al., 1995, 1998; Loc et al., 2002
KPA (*Koelreuteria paniculata*)	*Anagasta kuehniella* (Mediterranean flour moth); *C. maculatus*	Beans, grains, fruits, nuts	Macedo et al., 2003
LOA (*Listera ovata*)	*M. vitrata*	Cowpea	Machuka et al., 1999
NPA (*Narcissus pseudonarcissus*)	*N. lugens*	Rice	Powell et al., 1995
	M. persiacae	Peach, potato	Sauvion et al., 1996
Mannose/glucose specific			
ConA (*Canavalia ensiformis*)	*A. pisum*	Pea	Rahbé and Febvay, 1993
	A. pisum	Pea	Rahbé et al., 1995
	Aphis gossypii (cotton and melon aphid)	Cotton, mellon	Rahbé et al., 1995
	Aulacorthum solani (glasshouse and potato aphid)	Potato	Rahbé et al., 1995
	Macrosiphum albifrons (lupin aphid)	Lupin	Rahbé et al., 1995
	Macrosiphum euphorbiae (potato aphid)	Apple, bean, broccoli, papaya	Rahbé et al., 1995
	M. persiacae	Peach, potato	Rahbé et al., 1995; Sauvion et al., 1996; Gatehouse et al., 1999
	L. oleracea	Tomato	Fitches and Gatehouse, 1998; Gatehouse et al., 1999; Fitches et al., 2001a

(*continued on next page*)

Table 1 (*continued*)

Lectin (plant source)	Insect	Host	Reference
	T. proserpina	Taro	Powell, 2001
LCA (*Lens culinaris*)	*A. pisum*	Pea	Rahbé et al., 1995
PSA (*Pisum sativum*)	*A. pisum*	Pea	Rahbé et al., 1995
	Hypera postica (clover leaf weevil)	Alfafa, lucerne	Elden, 2000
N-acetyl-D-glucosamine specific			
ACA (*Amaranthus caudatus*)	*A. pisum*	Pea	Rahbé et al., 1995
BSA (*Bandeiraea simplicifolia*)	*Diabrotica undecimpunctata* (Southern corn rootworm); *Ostrinia nubilaris* (European corn borer)	Corn	Czapla and Lang, 1990
BSAII	*A. pisum*	Pea	Rahbé et al., 1995
GSII (*Griffonia simplicifolia*)	*C. maculatus*	Cowpea	Zhu et al., 1996; Zhu-Salzman et al., 1998; Zhu-Salzman and Salzman, 2001
PAA (*Phytolacca americana*)	*D. undecimpunctata*; *O. nubilaris*	Corn	Czapla and Lang, 1990
TEL (*Talisia esculenta*)	*C. maculatus; Zabrotes subfasciatus* (Mexican dry bean weevil)	Beans	Macedo et al., 2002
WGA (*Triticum aestivum*)	*D. undecimpunctata*; *O. nubilaris*	Corn	Czapla and Lang, 1990
	Antitrogus sanguineus (sugarcane whitegrub)	Sugarcane	Allsopp and McGhie, 1996
	H. postica	Alfafa	Elden, 2000
	L. erysimi	Mustard	Kanrar et al., 2002
Galactose specific			
AHA (*Artocarpus hirsuta*)	*Tribolium castaneum* (red flour beetle)	Large number of grains	Gurjar et al., 2000
AIA (*Artocarpus integrifolia*)	*D. undecimpunctata*; *O. nubilaris*	Corn	Czapla and Lang, 1990
GHA (*Glechoma hederacea* - leaf)	*Leptinotorsa decemlineata* (colorado potato beetle)	Potato	Wang et al., 2003
RCA^{120} (*Ricinus communis*)	*D. undecimpunctata*; *O. nubilaris*	Corn	Czapla and Lang, 1990
YBA (*Sphenostylis stenocarpa*)	*Clavigralla tomentosicollis* (coreid bug)	*Vigna* spp.	Okeola and Machuka, 2001
	C. maculatus; *M. vitrata*	Cowpea	Machuka et al., 2000
N-acetyl-D-galactosamine specific			
ACA (*Amaranthus caudatus*)	*A. pisum*	Pea	Rahbé et al., 1995
BFA (*Brassica fructiculosa*)	*Brevicoryne brassicae* (cabbage aphid)	Broccoli, Brussels sprouts, cauliflower, head cabbage	Cole, 1994
BPA (*Bauhinia purpurea*)	*D. undecimpunctata*; *O. nubilaris*	Corn	Czapla and Lang, 1990
CFA (*Codium fragile*)	*D. undecimpunctata*; *O. nubilaris*	Corn	Czapla and Lang, 1990
EHA (*Eranthis hyemalis*)	*D. undecimpunctata*	Corn	Kumar et al., 1993
MPA (*Maclura pomifera*)	*D. undecimpunctata*; *O. nubilaris*	Corn	Czapla and Lang, 1990
PTA (*Psophocarpus tetragonolobus*)	*C. maculatus*	Cowpea	Gatehouse et al., 1991
	N. lugens	Rice	Powell, 2001
SNA-II (*Sambucus nigra*)	*A. pisum*	Pea	Rahbé et al., 1995
VVA	*D. undecimpunctata*; *O. nubilaris*	Corn	Czapla and Lang, 1990
Complex[b]			
PHA (*Phaseolus vulgaris*)	*L. hesperus* (Western tarnished plant bug)	Cotton, alfafa, legumes	Habibi et al., 2000

[a] Sugar specificity is represented by the best monosaccharide inhibitor.
[b] Complex carbohydrate structure bearing terminal galactose residues (Goldstein and Poretz, 1986).

A toxic dose of GNA was also able to induce morphological changes in the midgut region of planthoppers with disruption of the microvilli brush border region (Powell et al., 1998). Habibi et al. (2000) showed that the gut epithelial cells of *Lygus hesperus* (Hemiptera: Miridae), the Western tarnished plant bug, were severely disrupted by PHA. Strong binding of PHA to the brush border microvilli of the midgut at the first and third, but not the second ventriculus, and lectin internalisation were detected. Moreover the first ventriculus showed severe disruption, disorganisation and swelling toward the proximal and distal gut lumen regions which were completely closed. Complete closure of the gut lumen also occurred in the hindgut region as a consequence of severe disruption and swelling of hindgut epithelial cells. However, only slight disruption with enlargement of nuclei occurred in the foregut epithelial cells clearly indicating that *Lygus hesperus* contains PHA receptors in three specific regions of its digestive tract.

Another additional antinutritional complication for the insects fed on lectin-containing artificial diets of plants, which also occurs in lectin-fed higher animals, is the possibility that lectins may destabilise insect metabolism by interfering with gut enzymatic function either indirectly or by binding to glycosylated digestive enzymes in the gut (Van Damme et al., 1998). Orally-fed GNA and ConA, for example, affected the activities of soluble and brush border membrane enzymes in the midgut of *Lacanobia oleracea* larvae. Furthermore significantly elevated total aminopeptidase activity, both in terms of total activity per larval gut and activity per mg gut protein, was observed. Similarly, both GNA and ConA treatments resulted in elevated levels of trypsin activity per gut. GNA, but not ConA, induced a significant increase in α-glucosidase activity per gut. Thus lectins upon binding to the gut may indirectly affect the enzyme regulatory mechanisms as a consequence of perturbation of the peritrophic matrix and/or brush border membrane environment (Fitches and Gatehouse, 1998).

The major insecticide resistance mechanism in the brown planthopper *Nilaparvata lugens* involves overproduction of esterase isoenzymes. All the esterases purified from an insecticide resistant strain of this insect were shown to be glycosylated. As GNA, MAA (*Maackia amurensis* agglutinin) and DAS (*Diffenbachia sequina* agglutinin) bind to this family of esterases at the terminally linked mannose (Small and Hemingway, 2000). It is possible that in transgenic plants expressing mannose-binding lectins, if they were not fully effective in protecting the plant against the brown planthopper (or other insecticide resistant pest), they could interact with and inhibit the enzymes thereby

Table 2
Plant species transformed with lectin genes to confer resistance against insects

Transformed plant	Lectin[a]	Target pest	Reference
Maize	WGA	*Ostrinia nubilaris*; *Diabrotica undecimpunctata*	Maddock et al., 1991
Mustard (*B. juncea*)	WGA	*Lipaphis erysimi*	Kanrar et al., 2002
Arabidopsis thaliana	PHA-E, L[b]	*Lacanobia oleracea*	Fitches et al., 2001b
Potato	GNA	*Aulacorthum solani*	Down et al., 1996
Potato	GNA	*Myzus persicae*	Gatehouse et al., 1996; Couty et al., 2001b
Potato	GNA	*L. oleracea*	Fitches et al., 1997; Gatehouse et al., 1997
Potato	GNA	*L. oleracea*	Bell et al., 1999, 2001; Down et al., 2001
Potato	GNA	*Aphidius ervi* (parasitoid of *M. persicae*)	Couty et al., 2001b
Potato	ConA	*L. oleracea*; *M. persicae*	Gatehouse et al., 1999
Rice	GNA	*Nilaparvata lugens*	Rao et al., 1998; Foissac et al., 2000; Tinjuangjun et al., 2000; Maqbool et al., 2001; Tang et al., 2001; Loc et al., 2002
Rice	GNA	*Nephotettix virescens* (green leafhopper)	Foissac et al., 2000
Rice	GNA	*Cnaphalocrocis medinalis* (rice leaffolder); *Scirpophaga incertulas* (yellow stemborer)	Maqbool et al., 2001
Rice	GNA	*Laodelphax striatellus* (rice small brown planthopper)	Sun et al., 2002; Wu et al., 2002
Sugarcane	GNA	*Eoreuma loftini* (Mexican rice borer); *Diatraea saccharalis* (sugarcane borer)	Setamou et al., 2002
Sugarcane	GNA	*Parallorhogas pyralophagus* (parasitoid of *E. loftini*)	Tomov and Bernal, 2003
Tobacco	PSA	*Heliothis virescens* (tobacco budworm)	Boulter et al., 1990[c]
Tobacco	GNA	*M. persicae*	Hilder et al., 1995
Tobacco	GNA	*Helicoverpa zea* (cotton bollworm)	Wang and Guo, 1999
Wheat	GNA	*Sitobion avenae* (grain aphid)	Stoger et al., 1999

[a] For lectin abbreviations see Table 1.
[b] Transgenic *Arabidopsis* plants expressing the *Phaseolus vulgaris* erythro- and leucoagglutinating isolectins (PHA-E and PHA-L) either at 0.4–0.6% of total soluble proteins were not toxic to *L. oleracea*.
[c] First demonstration of insect enhanced resistance of transgenic plants expressing a foreign lectin.

rendering the insect more susceptible to the action of insecticide. This could reduce the amounts of pesticide used.

Despite the large number and varieties of insecticidal plant lectins described (Table 1) the real allure of this field of research is the possibility of using plant lectins as potent control agents to engineer crop plants for insect resistance (Jouanin et al., 1998; Vaughan et al., 1999). Currently, the two major groups of plant-derived genes used to confer insect resistance on crops are those of inhibitors of digestive enzymes (proteases and amylase inhibitors) and lectins (Schuler et al., 1998). Several bioassay studies of lectin-expressing plants, in particular GNA-containing, have been reported recently (Hilder et al., 1995; Down et al., 1996; Gatehouse et al., 1996; Czapla, 1997; Rao et al., 1998; Foissac et al., 2000; Couty et al., 2001a). Overall, transgenic plants expressing high levels of lectins exhibited some degree of resistance to the target insect. It is worth mentioning that the great interest on transgenic crop plants expressing the gene for GNA or other mannose-binding lectins such as ASA resides in the fact that although these lectins show toxicity against species of various insect orders they are non toxic to mammals and birds (Powell et al., 1995; Down et al., 1996; Bandyopadhyay et al., 2001). Therefore GNA has been transferred and expressed in several crop plants (Table 2).

In parallel studies on the possible toxic or antinutritive effects of lectins, being considered for introduction into transgenic crops, on parasitoids of insect pests have been carried out to evaluate the ecological problems that such transgenic plants could bring to dependent secondary species or symbiotes in the field (Bell et al., 1999, 2001; Down et al., 2000; Couty et al., 2001bc; Tomov and Bernal, 2003). Fortunately, the studies conducted so far with this purpose have demonstrated that the effects of lectins on parasitoids are not acute. For example, no acute toxicity of GNA-containing aphid towards its parasitoid two-spot ladybird (*Adalia bipunctata* L.; Coleoptera: Coccinellidae) larvae fed on *Myzus persicae* (Homoptera: Aphididae) was observed, although there were small effects on development which led the authors to hypothesise that dietary GNA had reduced the nutritive quality of the aphids thereby limiting subsequent nutrient intake by the ladybirds and slowing their development (Down et al., 2000). The results obtained by Couty et al. (2001b) indicated that GNA delivered via artificial diet to *M. persicae* was transferred to its parasitoid *Aphidius ervi* (Hymenoptera: Aphidiidae) and that the parasitoid larvae excreted most of the ingested GNA in the meconium. Tomov and Bernal (2003) concluded that although GNA transgenic sugarcane, ingested via *Eoreuma loftini* tissues, was not acutely toxic to *Parallorhogas pyralophagus* (Hymenoptera: Braconidae), sublethal effects on life history parameters occurred both in the first and second generation of the parasitoid. These effects might be considered in a broader context to determine their possible ecological significance.

8. Conclusions

Lectins are highly antinutritional and/or toxic substances being detrimental to various plant-eating organisms. This property is associated, in part, to their high resistance to proteolysis in gastrointestinal tract of monogastric, ruminants and insects, to their capacity of binding to epithelial cells lining the small intestine and to their ability to modulate intestinal and systemic metabolism. It has been extensively demonstrated that plant lectins are effective biological agents against insect attack. In fact, insect-resistant plants produced by expression of lectin genes in transgenic plants are already a reality. This approach could increase crop productivity and reduce the usage of pesticide. However, few lectins tested for this purpose have been specifically selected on the basis that they convey high resistance to disease or predators in plants, whilst simultaneously having a low toxicity to higher mammals, including man. Hence, future research priority must be given to finding other lectins with these above characteristics hoping to draw definitive conclusions to two basic questions: (a) What effects do lectins have in a diet? (b) What effects do insect-resistant transgenic plants have when pest and their parasitoids or symbiotes encounter them in the field?

Acknowledgements

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Programa de Apoio a Núcleos de Excelência (PRONEX), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Programa Nacional de Cooperação Acadêmica (PROCAD) and Fundação de Amparo à Pesquisa do Estado do Ceará (FUNCAP).

References

Aletor, V.A., Fetuga, B.L., 1985. Responses of the rats hepatic and serum transaminases to lectin extracted from lima bean (*Phaseolus lunatus*). Z. Tierphysiol. Tierer. 54, 249–252.

Allsopp, P.G., McGhie, T.K., 1996. Snowdrop and wheatgerm lectins and avidin as antimetabolities for the control of sugarcane whitegrubs. Entomol. Exp. Appl. 80, 409–414.

Baintner, K., Jakab, G., Gyori, Z., Kiss, P., 2000. Binding of FITC-labelled lectins to the gastrointestinal epithelium of the rat. Pathol. Oncol. Res. 2000, 179–183.

Bandyopadhyay, S., Roy, A., Das, S., 2001. Binding of garlic (*Allium sativum*) leaf lectin to the gut receptors of homopteran pests is correlated to its insecticidal activity. Plant Sci. 161, 1025–1033.

Barbieri, L., Battelli, M.G., Stirpe, F., 1993. Ribosome-inactivating proteins from plants. Biochim. Biophys. Acta 1154, 237–282.

Bardocz, S., Ewen, S.W.B., Grant, G., Pusztai, A., 1995. Lectins as growth factors of the small intestine and the gut. In: Pusztai, A.,

Bardocz, S. (Eds.), Lectins: Biomedical Perspectives, Taylor and Francis, London, pp. 103–116.

Bardocz, S., Grant, G., Pusztai, A., Franklin, M.F., Carvalho, A.F.F.U., 1996. The effect of phytohaemagglutinin on the growth, body composition, and plasma insulin of the rat at different dietary concentrations. Brit. J. Nutr. 76, 613–626.

Begbie, R., King, T.P., 1985. The interaction of dietary lectin with porcine small intestine and production of lectin-specific antibodies, In: Bog-Hansen, T.C., Breborovicz, J. (Eds.), Lectins: Biology, Biochemistry, Clinical Biochemistry, Walter de Gruyter, Berlin, pp. 15–27.

Bell, H.A., Fitches, E.C., Down, R.E., Marris, G.C., Edwards, J.P., Gatehouse, J.A., Gatehouse, A.M.R., 1999. The effect of snowdrop lectin (GNA) delivered via artificial diet and transgenic plants on *Eulophus pennicornis* (Hymenoptera: Eulophidae), a parasitoid of the tomato moth *Lacanobia oleracea* (Lepidoptera: Noctuidae). J. Insect Physiol. 45, 983–991.

Bell, H.A., Fitches, E.C., Marris, G.C., Bell, J., Edwards, J.P., Gatehouse, J.A., Gatehouse, A.M.R., 2001. Transgenic, G.N.A. expressing potato plants augment the beneficial biocontrol of *Lacanobia oleracea* (Lepidoptera; Noctuidae) by the parasitoid *Eulophus pennicornis* (Hymenoptera; Eulophidae). Transgenic Res. 10, 35–42.

Bender, A.E., Reaidi, G.B., 1982. Toxicity of kidney beans (*Phaseolus vulgaris*) with particular reference to lectins. J. Plant Foods 4, 15–22.

Boulter, D., Edwards, G.A., Gatehouse, A.M.R., Gatehouse, J.A., Hilder, V.A., 1990. Additive protective effects of incorporating two different higher plant derived insect resistance genes in transgenic tobacco plants. Crop Prot. 9, 351–354.

Boyd, W.C., Reguera, R.M., 1949. Studies on haemagglutinins present in seeds of some representatives of the family Leguminoseae. J. Immunol. 62, 333–339.

Boyd, W.C., Shapleigh, E., 1954. Separation of individuals of any blood group into secretors and non-secretors by use of a plant agglutinin (lectin). Blood 9, 1195–1198.

Brady, P.G., Vannier, A.M., Banwell, J.G., 1978. Identification of the dietary lectin, wheat germ agglutinin, in human intestinal contents. Gastroenterology 75, 236–239.

Broekaert, W.F., Allen, A.K., Peumans, W.J., 1987. Separation and partial characterization of isolectins with different subunit compositions from *Datura stramonium* seeds. FEBS Lett. 220, 116–120.

Bryk, S.G., Gheri, G., 2002. Lectin histochemistry of enterocytes sugar residues in the gut of the chick embryo and of the newborn. Ital. J. Anat. Embryol. 107, 37–49.

Carbonaro, M., Cappelloni, M., Nicoli, S., Lucarini, M., Carnovale, E., 1997. Solubility-digestibility relationship of legume proteins. J. Agric. Food Chem. 45, 3387–3394.

Carbonaro, M., Grant, G., Cappeloni, M., Pusztai, A., 2000. Perspectives into factors limiting in vivo digestion of legume proteins: antinutritional compounds or storage proteins? J. Agric. Food Chem. 48, 742–749.

Carlini, C.R., Grossi-de-Sá, M.F., 2002. Plant toxic proteins with insecticidal properties. A review on their potentialities as bioinsecticides. Toxicon 20, 1515–1539.

Caygill, J.C., 1999. Antinutritional compounds: background and introduction. In: Caygill, J.C., Mueller-Harvey, I. (Eds.), Secondary Plant Products: Antinutritional and Beneficial Actions in Animal Feeding, Nottingham University Press, Nottingham, UK, pp. 1–6.

Chrispeels, M.J., Raikhel, N.V., 1991. Lectins, lectin genes, and their role in plant defense. Plant Cell 3, 1–91.

Cole, R.A., 1994. Isolation of a chitin-binding lectin with insecticidal activity in chemically-defined synthetic diets from two wild Brassica species with resistance to cabbage aphid *Brevicoryne brassicae*. Entomol. Exp. Appl. 72, 181–187.

Cordain, L., Toohey, L., Smith, M.J., Hickey, M.S., 2000. Modulation of immune function by dietary lectins in rheumatoid arthritis. Brit. J. Nutr. 83, 207–217.

Couty, A., de-la-Vina, G., Clark, S.J., Kaiser, L., Pham, D.M.H., Poppy, G.M., 2001a. Direct and indirect sublethal effects of *Galanthus nivalis* agglutinin (GNA) on the development of a potato-aphid parasitoid, *Aphelinus abdominalis* (Hymenoptera: Aphelinidae). J. Insect Physiol. 47, 553–561.

Couty, A., Down, R.E., Gatehouse, A.M.R., Kaiser, L., Pham-Delegue, M.H., Poppy, G.M., 2001b. Effects of artificial diet containing GNA and GNA-expressing potatoes on the development of the aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae). J. Insect Physiol. 47, 1357–1366.

Couty, A., Clark, S.J., Poppy, G.M., 2001c. Are fecundity and longevity of female *Aphelinus abdominalis* affected by development in GNA-dosed *Macrosiphum euphorbiae*? Physiol. Entomol. 26, 287–293.

Cuadrado, C., Grant, G., Rubio, L., Muzquiz, M., Bardocz, S., Pusztai, A., 2002. Nutritional utilization by the rat of diets based on lentil (*Lens culinaris*) seed meal or its fractions. J. Agric. Food Chem. 50, 4371–4376.

Czapla, T.H., 1997. Plant lectins as insect control proteins in transgenic plants. In: Carozzi, N., Koziel, M. (Eds.), Advances in Insect Control: The Role of Transgenic Plants, Taylor and Francis, Basingstoke, UK, pp. 123–138.

Czapla, T.H., Lang, B.A., 1990. Effect of plant lectins on the larval development of European corn borer (Lepidoptera: Pyralidae) and southern corn rootworm (Coleoptera: Crysomelidae). J. Econ. Entomol. 83, 2480–2485.

Does, M.P., Houterman, P.M., Dekker, H.L., Cornelissen, B.J.C., 1999. Processing, targeting, and antifungal activity of stinging nettle agglutinin in transgenic tobacco. Plant Physiol. 120, 421–431.

Douglas, M.W., Parsons, C.M., Hymowitz, T., 1999. Nutritional evaluation of lectin-free soybeans for poultry. Poultry Sci. 78, 91–95.

Down, R.E., Gatehouse, A.M.R., Hamilton, W.D.O., Gatehouse, J.A., 1996. Snowdrop lectin inhibits development and decreases fecundity of the glasshouse potato aphid (*Aulacorthum solani*) when administered in vivo and via transgenic plants both in laboratory and glasshouse trials. J. Insect Physiol. 42, 1035–1045.

Down, R.E., Ford, L., Woodhouse, S.D., Raemaekers, R.J.M., Leitch, B., Gatehouse, J.A., Gatehouse, A.M.R., 2000. Snowdrop lectin (GNA) has no acute toxic effects on a beneficial insect predator, the 2-spot ladybird (*Adalia bipunctata* L.). J. Insect Physiol. 46, 379–391.

Down, R.E., Ford, L., Bedford, S.J., Gatehouse, L.N., Newell, C., Gatehouse, J.A., Gatehouse, A.M.R., 2001. Influence of plant development and environment on transgene expression in potato and consequences for insect resistance. Transgenic Res. 10, 223–236.

Du, J., Foissac, X., Carss, A., Gatehouse, A.M.R., Gatehouse, J.A., 2000. Ferritin acts as the most abundant binding protein for

snowdrop lectin in the midgut of rice brown planthoppers (*Nilaparvata lugens*). Insect Biochem. Mol. Biol. 30, 297–305.

Duranti, M., Gius, C., 1997. Legume seeds: protein content and nutritional value. Field Crop Res. 53, 31–45.

El-Adawy, T.A., 2002. Nutritional composition and antinutritional factors of chickpeas (*Cicer arietinum* L.) undergoing different cooking methods and germination. Plant Food Hum. Nutr. 57, 83–97.

Elden, T.C., 2000. Influence of a cysteine proteinase inhibitor on alfafa weevil (Coleptera: Curculionidae) growth and development over successive generations. J. Entomol. Sci. 35, 70–76.

Endo, Y., Tsurugi, K., 1987. RNA N-glycosidase activity of ricin A-chain: mechanism of action of the toxic lectin ricin on eukaryotic ribosomes. J. Biol. Chem. 263, 8735–8739.

Erickson, R.H., Kim, J., Sleisenger, M.H., Kim, Y.S., 1985. Effects of lectins on the activity of brush border membrane-bound enzymes of rat small intestine. J. Pediatr. Gastroenter. Nutr. 4, 984–991.

Fitches, E., Gatehouse, J.A., 1998. A comparison of the short and long term effects of insecticidal lectins on the activities of soluble and brush border enzymes of tomato moth larvae (*Laconobia oleracea*). J. Insect Physiol. 44, 1213–1224.

Fitches, E., Gatehouse, A.M.R., Gatehouse, J.A., 1997. Effects of snowdrop lectin (GNA) delivered via artificial diet and transgenic plant on the development of tomato moth (*Lacanobia oleracea*) larvae in laboratory and glasshouse trials. J. Insect Physiol. 8, 727–739.

Fitches, E., Woodhouse, S.D., Edwards, J.P., Gatehouse, J.A., 2001a. In vitro and in vivo binding of snowdrop (*Galanthus nivalis* agglutinin; GNA) and jackbean (*Canavalia ensiformis*; Con A) lectins within tomato moth (*Lacanobia oleracea*) larvae; mechanisms of insecticidal action. J. Insect Physiol. 47, 777–787.

Fitches, E., Ilett, C., Gatehouse, A.M.R., Gatehouse, L.N., Greene, R., Edwards, J.P., Gatehouse, J.A., 2001b. The effects of *Phaseolus vulgaris* erythro- and leucoagglutinating isolectins (PHA-E and PHA-L) delivered via artificial diet and transgenic plants on the growth and development of tomato moth (*Lacanobia oleracea*) larvae; lectin binding to gut glycoproteins in vitro and in vivo. J. Insect Physiol. 47, 1389–1398.

Fitzgerald, A.J., Jordinson, M., Rhodes, J.M., Singh, R., Calam, J., Goodlad, R.A., 2001. Comparison of the effects of concanavalin-A and epidermal growth factor on epithelial cell proliferation in the rat intestine. Aliment. Pharm. Therap. 15, 1077–1084.

Foissac, X., Loc, N.T., Christou, P., Gatehouse, A.M.R., Gatehouse, J.A., 2000. Resistance to green leafhopper (*Nephotettix virescens*) and brown planthopper (*Nilaparvata lugens*) in transgenic rice expressing snowdrop lectin (*Galanthus nivalis* agglutinin; GNA). J. Insect Physiol. 46, 573–583.

Friedman, M., 1996. Nutritional value of proteins from different food sources: a review. J. Agric. Food Chem. 44, 6–20.

Gatehouse, A.M.R., Howe, D.S., Flemming, J.E., Hilder, V.A., Gatehouse, J.A., 1991. Biochemical basis of insect resistance in winged bean (*Psophocarpus tetragonolobus*) seeds. J. Sci. Food Agric. 55, 63–74.

Gatehouse, A.M.R., Powell, K.S., Van Damme, E.J.M., Peumans, W.J., Gatehouse, J.A., 1995. Insecticidal properties of plant lectins: their potential in plant protection. In: Pustzai, A., Bardocz, S. (Eds.), Lectins: Biomedical Perspectives, Taylor and Francis, London, pp. 35–58.

Gatehouse, A.M.R., Down, R.E., Powell, K.S., Sauvion, N., Rahbé, Y., Newell, C.A., Merryweather, A., Hamilton, W.D.O., Gatehouse, J.A., 1996. Transgenic potato plants with enhanced resistance to the peach-potato aphid *Mirzus persicae*. Entomol. Exp. Appl. 79, 295–307.

Gatehouse, A.M., Davison, G.M., Newell, C.A., Hamilton, W.D.O., Burgess, E.P.J., Gilbert, R.J.C., Gatehouse, J.A., 1997. Transgenic potato plants with enhanced resistance to the tomato moth, *Lacanobia oleracea*: growth room trials. Mol. Breed. 3, 49–63.

Gatehouse, A.M., Davison, G.M., Stewart, J.N., Gatehouse, L.N., Kumar, A., Geoghegan, I.E., Birch, A.N.E., Gatehouse, J.A., 1999. Concanavalin A inhibits development of tomato moth (*Lacanobia oleracea*) and peach-potato aphid (*Myzus persicae*) when expressed in transgenic potato plants. Mol. Breed. 5, 153–165.

Gilbert, R.J., 1988. Healthy eating day. Commun. Dis. Rep. 33, 3–4.

Goldstein, I.J., Poretz, R.D., 1986. Isolation, physicochemical characterization, and carbohydrate-binding specificity of lectins. In: Liener, I.E., Sharon, N., Goldstein, I.J. (Eds.), The Lectins, Academic Press, Orlando, pp. 33–247.

Goldstein, I.J., Winter, H.C., Poretz, R.D., 1997. Plant lectins: tools for the studying of complex carbohydrates. In: Montreuil, J., Vliegenthart, J.F.G., Schacter, H. (Eds.), Glycoproteins II, Elsevier, Amsterdam, pp. 403–474.

Grangeiro, T.B., Schriefer, A., Calvete, J.J., Raida, M., Urbanke, C., Barral-Netto, M., Cavada, B.S., 1997. Molecular cloning and characterization of ConBr, the lectin of *Canavalia brasiliensis* seeds. Eur. J. Biochem. 248, 43–48.

Grant, G., 1991. Lectins. In: D'Mello, J.P.F., Duffus, C.M. (Eds.), Toxic Substances in Crop Plants, pp. 49–67, The Royal Society of Chemistry.

Grant, G., 1999. Plant lectins. In: Caygill, J.C., Mueller-Harvey, I. (Eds.), Secondary Plant Products: Antinutritional and Beneficial Actions in Animal Feeding, Nottingham University Press, Nottingham, UK, pp. 87–110.

Grant, G., More, L.J., McKenzie, N.H., Stewart, J.C., Pusztai, A., 1983. A survey of the nutritional and haemagglutination properties of legume seeds generally available in the UK. Brit. J. Nutr. 50, 207–214.

Grant, G., Greer, F., McKenzie, N.H., Pusztai, A., 1985. The nutritional response of mature rats to kidney bean (*Phaseolus vulgaris*) lectins. J. Sci. Food Agric. 36, 409–414.

Grant, G., Oliveira, J.T.A., de Dorward, P.M., Annand, M.G., Waldron, M., Pusztai, A., 1987. Metabolic and hormonal changes in rats resulting from consumption of kidney bean (*Phaseolus vulgaris*) or soyabean (*Glycine max*). Nutr. Rep. Int. 36, 763–772.

Grant, G., More, L.J., McKenzie, N.H., Dorward, P.M., Buchan, W.C., Telek, L., Pusztai, A., 1995. Nutritional and haemagglutination properties of several tropical seeds. J. Agric. Sci. 124, 437–445.

Grant, G., Edwards, J.E., Ewan, E.C., Murray, S., Atkinson, T., Farningham, D.A.H., Pusztai, A., 1999. Secretion of pancreatic digestive enzymes induced in rats by first-time oral exposure to kidney bean E_2L_2 lectin is mediated only in part by cholecystokinin (CCK). Pancreas 19, 382–389.

Grant, G., Alonso, R., Edwards, J.E., Murray, S., 2000. Dietary soya beans and kidney beans stimulate secretion of cholecystokinin and pancreatic digestive enzymes in 400-day-old Hooded-Lister

rats but only soya beans induce growth of the pancreas. Pancreas 20, 305–312.

Gupta, A., Sandhu, R.S., 1998. Effect of garlic agglutinin and garlic extracts on the rat jejunum. Nutr. Res. 18, 841–850.

Gurjar, M.M., Gaikwad, S.M., Salokhe, S.G., Mukherjee, S., Khan, M.I., 2000. Growth inhibition and total loss of reproductive potential in *Tribolium castaneum* by *Artocarpus hirsut* lectin. Invertebr. Reprod. Dev. 38, 95–98.

Haas, H., Falcone, F.H., Schramm, G., Haisch, K., Gibbs, B.F., Klaucke, J., Poppelmann, M., Becker, W.M., Gabius, H.J., Schlaak, M., 1999. Dietary lectins can induce in vitro release of IL-4 and IL-13 from human basophils. Eur. J. Immunol. 29, 918–927.

Habibi, J., Backus, E.A., Huesing, J.E., 2000. Effects of phytohemagglutinin (PHA) on the structure of midgut epithelial cells and localization of its binding sites in western tarnished plant bug, *Lygus hesperus* Knight. J. Insect Physiol. 46, 611–619.

Hajos, G., Gelencser, E., Pusztai, A., Grant, G., Sakhri, M., Bardocz, S., 1995. Biological effects and survival of trypsin-inhibitors and the agglutinin from soybean in the small intestine of the rat. J. Agric. Food Chem. 43, 165–170.

Hara, T., Mukunoki, Y., Tsukamoto, I., Miyoshi, M., Hasegawa, K., 1984. Susceptibility of kintoki bean lectin to digestive enzymes in vitro and its behaviour in the digestive organs of mouse in vivo. J. Nutr. Sci. Vitaminol. 30, 381–394.

Harper, S.M., Crenshaw, R.W., Mullins, M.A., Privalle, L.S., 1995. Lectin binding to insect brush border membranes. J. Econ. Ecol. 88, 1197–1202.

Herzig, K.H., Bardocz, S., Grant, G., Nustede, R., Folsch, U.R., Pusztai, A., 1997. Kidney bean lectin is a potent cholecystokinin releasing stimulus in the rat inducing pancreatic growth. Gut 41, 333–338.

Higuchi, M., Suga, M., Iwai, K., 1983. Participation of lectin in biological effects of raw winged bean seeds on rats. Agric. Biol. Chem. 47, 1979–1986.

Higuchi, M., Tsuchiya, I., Iwai, K., 1984. Growth inhibition and small intestinal lesions in rats alter after feeding with isolated winged bean lectin. Agric. Biol. Chem. 48, 695–701.

Hilder, V.A., Boulter, D., 1999. Genetic engineering of crop plants for insect resistance—a critical review. Crop Prot. 18, 177–191.

Hilder, V.A., Powell, K.S., Gatehouse, A.M.R., Gatehouse, J.A., Gatehouse, L.N., Shi, Y., Hamilton, W.D.O., Merryweather, A., Newell, C.A., Timans, J.C., 1995. Expression of snowdrop lectin in transgenic tobacco plants resulted in added protection against aphids. Transgenic Res. 4, 18–25.

Huisman, J., van der Poel, A.F.B., Van Leeuwen, P., Verstegen, M.W.A., 1990a. Comparison of growth, nitrogen-metabolism and organ weights in piglets and rats fed on diets containing *Phaseolus vulgaris* beans. Brit. J. Nutr. 64, 743–753.

Huisman, J., van der Poel, A.F.B., Mouwen, J.M.V.M., Vanweerden, E.J., 1990b. Effect of variable protein contents in diets containing *Phaseolus vulgaris* beans on performance, organ weights and blood variables in piglets, rats and chickens. Brit. J. Nutr. 64, 755–764.

Jaffé, W.G., 1960. Studies on phytotoxins in beans. Arzneimittel Forschung 10, 1012–1016.

Jordinson, M., Playford, R.J., Calam, J., 1997. Effects of a panel of lectins on cholecystokinin release in rats. Am. J. Physiol. 273, 946–950.

Jordinson, M., Goodlad, R.A., Brynes, A., Bliss, P., Ghatei, M.A., Bloom, S.R., Fitzgerald, A., Grant, G., Bardocz, S., Pusztai, A., Pignatelli, M., Calam, J., 1999. Gastrointestinal responses to a panel of lectins in rats maintained on total parenteral nutrition. Am. J. Physiol. 276, 1235–1242.

Jouanin, L., Bonade, B.M., Girard, C., Morrot, G., Giband, M., 1998. Transgenic plants for insect resistance. Plant Sci. 131, 1–11.

Kanrar, S., Venkateswari, J., Kirti, P.B., Chopra, V.L., 2002. Transgenic Indian mustard (*Brassica juncea*) with resistance to the mustard aphid (*Lipaphis erysimi* Kalt.). Plant Cell Rep. 20, 976–981.

Kelsall, A., Fitzgerald, A.J., Howard, C.V., Evans, R.C., Singh, R., Rhodes, J.M., Goodlad, R.A., 2002. Dietary lectins can stimulate pancreatic growth in the rat. Int. J. Exp. Pathol. 83, 203–208.

Kilpatrick, D.C., Pusztai, A., Grant, G., Graham, C., Ewen, S.W.B., 1985. Tomato lectin resists digestion in the mammalian alimentary canal and binds to intestinal villi without deleterious effects. FEBS Lett. 185, 299–305.

Kordás, K., Burghardt, B., Kisfalvi, K., Bardocz, S., Pusztai, A., Varga, G., 2000. Diverse effects of phytohaemagglutinin on gastrointestinal secretions in rats. J. Physiol. 94, 31–36.

Kordás, K., Szalmay, G., Bardocz, S., Pusztai, A., Varga, G., 2001. Phytohaemagglutinin inhibits gastric acid but not pepsin secretion in conscius rats. J. Physiol. 95, 309–314.

Koshte, V.L., Vandijk, W., Vanderstelt, M.E., Aalberse, R.C., 1990. Isolation and characterization of BanLec-I, a mannoside-binding lectin from *Musa paradisiac* (banana). Biochem. J. 272, 721–726.

Koshte, V.L., Aalbers, M., Calkhoven, P.G., Aalberse, R.C., 1992. The Potent IgG4-inducing antigen in banana is a mannose-binding lectin, BanLec-I. Int. Arch. Allergy Immunol. 97, 17–24.

Kritchevsky, D., Tepper, S.A., Klurfeld, D.M., 1998. Lectin may contribute to the atherogenicity of peanut oil. Lipids 33, 821–823.

Kumar, M.A., Timm, D.E., Neet, K.E., Owen, W.G., Peumans, W.J., Rao, A.G., 1993. Characterization of the lectin from the bulbs of *Eranthis hyemalis* (winter aconite) as an inhibitor of protein synthesis. J. Biol. Chem. 268, 25176–25183.

Lajolo, F.M., Genovese, M.I., 2002. Nutritional significance of lectins and enzyme inhibitors from legumes. J. Agric. Food Chem. 50, 6592–6598.

Lavelle, E.C., 2001. Targeted delivery of drugs to the gastrointestinal tract. Crit. Rev. Ther. Drug 18, 341–386.

Lavelle, E.C., Grant, G., Pusztai, A., Pfuller, U., O'Hagan, D.T., 2000. Mucosal immunogenicity of plant lectins in mice. Immunology 2000, 30–37.

Lavelle, E.C., Grant, G., Pusztai, A., Pfuller, U., O'Hagan, D.T., 2001. The identification of plant lectins with mucosal adjuvant activity. Immunology 102, 77–86.

Leontowicz, H., Leontowicz, M., Kostyra, H., Kulasek, G., Gralak, M.A., Krzeminski, R., Podgurniak, M., 2001. Effects of raw or extruded legume seeds on some functional and morphological gut parameters in rats. J. Anim. Feed Sci. 10, 169–183.

Liener, I.E., 1953. Soyin, a toxic protein from the soybean. I. Inhibition of rat growth. J. Nutr. 49, 529–539.

Liener, I.E., 1986. Nutritional significance of lectins in the diet. In: Liener, I.E., Sharon, N., Goldstein, I.J. (Eds.), The Lectins, Academic Press, London, pp. 527–552.

Liener, I.E., 1994. Implication of antinutritional components in soybean foods. Crit. Rev. Food Sci. Nutr. 34, 31–67.

Liener, I., Young, M., Lovrien, R., 1986. Lectins inhibit digestive enzymes and so survive alimentary passage. FASEB J. 45, 1573–1573.

Lis, H., Sharon, N., 1998. Lectins: carbohydrate-specific proteins that mediate cellular recognition. Chem. Rev. 98, 637–674.

Loc, N.T., Tinjuangjun, P., Gatehouse, A.M.R., Christou, P., Gatehouse, J.A., 2002. Linear transgene constructs lacking vector backbone sequences generate transgenic rice plants which accumulate higher levels of proteins conferring insect resistance. Mol. Breed. 9, 231–244.

Macedo, M.L.R., Mello, G.C., Freire, M.G.M., Novello, J.C., Marangoni, S., Matos, D.G.G., 2002. Effect of a trypsin inhibitor from *Dimorphandra mollis* seeds on the development of *Callosobruchus maculatus* larval. Plant Physiol. Bioch. 40, 891–898.

Macedo, M.L.R., Freire, M.G.M., Cabrini, E.C., Toyama, M.H., Novello, J.C., Marangoni, S., 2003. A trypsin inhibitor from *Peltophorum dubium* seeds active against pest proteases and its effects on the survival of *Anagasta kuehniellla* (Lepidoptera: Pyralidae). Biochim. Biophys. Acta 1621, 170–182.

Machuka, J., Van Damme, E.J.M., Peumans, W.J., Jackai, L.E.N., 1999. Effect of plant lectins on survival development of the pod borer *Maruca vitrata*. Entomol. Exp. Appl. 93, 179–187.

Machuka, J.S., Okeola, O.G., Chrispeels, M.J., Jackai, L.E.N., 2000. The African yam bean seed lectin affects the development of the cowpea weevil but does not affect the development of larvae of the legume pod borer. Phytochemistry 53, 667–674.

Maddock, S.E., Hufman, G., Isenhour, D.J., Roth, B.A., Raikhel, N.V., Howard, J.A., Czapla, T.H., 1991. Expression in maize plants of wheatgerm agglutinin, a novel source of insect resistance, Third International Congress in Plant Molecular Biology, Tucson, Arizona.

Maqbool, S.B., Riazuddin, S., Loc, N.T., Gatehouse, A.M.R., Gatehouse, J.A., Christou, P., 2001. Expression of multiple insecticidal genes confers broad resistance against a range of different rice pests. Mol. Breed 7, 85–93.

Murdock, L.L., Shade, R.E., 2002. Lectins and protease inhibitors as plant defense against insects. J. Agric. Food Chem. 50, 6605–6611.

Nachbar, M.S., Oppenheim, J.D., 1980. Lectins in the United States diet: a survey of lectins in commonly consumed foods and a review of the literature. Am. J. Clin. Nutr. 33, 2338–2345.

Nakata, S., Kimura, T., 1985. Effect of ingested toxic bean lectins on the gastrointestinal tract in the rat. J. Nutr. 115, 1621–1629.

Noah, N.D., Bender, A.E., Reaidi, G.B., Gilbert, R.J., 1980. Food poisoning from red kidney beans. Brit. Med. J. 281, 236–237.

Nowell, P.C., 1960. Phytohemagglutinin: an initiator of mitosis in cultures of normal human leukocytes. Cancer Res. 20, 462–466.

Oka, Y., Chet, I., Spiegel, Y., 1997. Accumulation of lectins in cereal roots invaded by the cereal cyst nematode *Heterodera avenae*. Physiol. Mol. Plant P. 51, 333–345.

Okeola, O.G., Machuka, J., 2001. Biological effects of African yam bean lectins on *Clavigralla tomentosicollis* (Hemiptera: Coreidae). J. Econ. Entomol. 94, 724–729.

Oliveira, J.T.A., Pusztai, A., Grant, G., 1988. Changes in organs and tissues induced by feeding of purified kidney Bean (*Phaseolus vulgaris*) lectins. Nutr. Res. 8, 943–947.

Oliveira, J.T.A., Vasconcelos, I.M., Gondim, M.J.L., Cavada, B.S., Moreira, R.A., Santos, C.F., Moreira, L.I.M., 1994. *Canavalia brasiliensis* seeds. Protein quality and nutritional implications of dietary lectin. J. Sci. Food Agric. 64, 417–424.

Oliveira, J.T.A., Vasconcelos, I.M., Bezerra, L.C.N.M., Silveira, S.B., Monteiro, A.C.O., Moreira, R.A., 2000. Composition and nutritional properties of seeds from *Pachira aquatica* Aubl, *Sterculia striata* St Hil et Naud and *Terminalia catappa* Linn. Food Chem. 70, 185–191.

Olsnes, S., Wesche, J., Falnes, P., 1999. Binding, uptake, routing and translocation of toxins with intracellular sites of action. In: Alouf, J.E., Freer, J.H. (Eds.), The Comprehensive Sourcebook of Bacterial Protein Toxins, Academic Press, London, pp. 73–93.

Otte, J.M., Chen, C., Brunke, G., Kiehne, K., Schimitz, F., Foelsch, U.R., Herzig, K.H., 2001. Mechanisms of lectin (phytohemagglutinin)-induced growth in small intestinal epithelial cells. Digestion 64, 169–178.

Palmer, R.M., Pusztai, A., Bain, P., Grant, G., 1987. Changes in rates of tissue protein synthesis in rats induced in vivo by consumption of kidney bean lectins. Comp. Biochem. Phys. 88, 179–183.

Peumans, W.J., Van Damme, E.J.M., 1994. The role of lectins in the plant defense against insects. In: Van Driessche, E., Fisher, J., Beeckmans, S., Bog-Hansen, T.C. (Eds.), Lectins: Biology, Biochemistry, Clinical Biochemistry, Textop, Hellerup, Denmark, pp. 128–141.

Peumans, W.J., Van Damme, J.M., 1995. Lectins as plant defense proteins. Plant Physiol. 109, 347–352.

Peumans, W.J., Winter, H.C., Bemer, V., Van Leuven, F., Goldstein, I.J., Truffa-Cachi, P., Van Damme, E.J.M., 1997. Isolation of a novel plant lectin with an unusual specificity from *Calystegia sepium*. Glycoconj. J. 14, 259–265.

Powell, K.S., 2001. Antimetabolic effects of plant lectins towards nymphal stages of the planthoppers *Tarophagous proserpina* and *Nilaparvata lugens*. Entomol. Exp. Appl. 99, 71–77.

Powell, K.S., Gatehouse, A.M.R., Hilder, V.A., Van Damme, E.J.M., Peumans, W.J., Boonjawat, J., Horsham, K., Gatehouse, J.A., 1995. Different antimetabolic effects of related lectins towards nymphal stages of *Nilaparvata lugens*. Entomol. Exp. Appl. 75, 61–65.

Powell, K.S., Spence, J., Bharathi, M., Gatehouse, J.A., Gatehouse, A.M.R., 1998. Immunohistochemical and developmental studies to elucidate the mechanism of action of the snowdrop lectin on the rice brown planthopper, *Nilaparvata lugens* (Stal). J. Insect Physiol. 44, 529–539.

Preet, K., Punia, D., 2000. Proximate composition, phytic acid, polyphenols and digestibility (in vitro) of four brown cowpea varieties. Int. J. Food Sci. Nutr. 51, 189–193.

Pryme, I.F., Pusztai, A., Bardocz, S., Ewen, S.W.B., 1998. The induction of gut hyperplasia by phytohaemagglutinin in the diet and limitation of tumour growth. Histol. Histopathol. 13, 575–583.

Pryme, I.F., Pusztai, A., Bardocz, S., Ewen, S.W.B., 1999. A combination of dietary protein depletion and PHA-induced gut growth reduce the mass of a murine non-Hodgkin lymphoma. Cancer Lett. 139, 145–152.

Pusztai, A., 1989. Effects on gut structure, function and metabolism of dietary lectins. The nutritional toxicity of the kidney bean lectin. In: Franz, H., (Ed.), Advances in Lectin Research, VEB Verlag Volk und Gesundheit, Berlin, pp. 74–86.

Pusztai, A., 1991. General effects on animal cells. In: Pusztai, A., (Ed.), Plant Lectins, Cambridge University Press, Cambridge, pp. 105–205.

Pusztai, A., Bardocz, S., 1996. Biological effects of plant lectins on the gastrointestinal tract: metabolic consequences and applications. Trends Glycosci. Glyc. 8, 149–165.

Pusztai, A., Clarke, E.M.W., King, T.P., 1979. The nutritional toxicity of *Phaseolus vulgaris* lectins. Proc. Nutr. Soc. 38, 115–120.

Pusztai, A., Oliveira de, J.T.A., Bardocz, S., Grant, G., Wallace, H.M., 1988. Dietary kidney bean lectin-induced hyperplasia and increased polyamine content of the small intestine. In: Bog-Hansen, T.C., Freed, D.L.J. (Eds.), Lectins, Biology, Biochemistry and Clinical Biochemistry, Sigma Library, St Louis, MO, pp. 117–120.

Pusztai, A., Greer, F., Grant, G., 1989. Specific uptake of dietary lectins into the systemic circulation of rats. Biochem. Soc. T. 17, 481–482.

Pusztai, A., Grant, G., Spencer, R.J., Duguid, T.J., Brown, D.S., Ewen, S.W.B., Peumans, W.J., Van Damme, E.J.M., Bardocz, S., 1993a. Kidney bean lectin-induced *Escherichia coli* overgrowth in the small-intestine is blocked by GNA, a mannose-specific lectin. J. Appl. Bacteriol. 75, 360–368.

Pusztai, A., Ewen, S.W.B., Grant, G., Brown, D.S., Stewart, J.C., Peumans, W.J., Van Damme, E.J.M., Bardocz, S., 1993b. Antinutritive effects of wheat-germ-agglutinin and other *N*-acetylglucosamine-specific lectins. Brit. J. Nutr. 70, 313–321.

Pusztai, A., Ewen, S.W.B., Carvalho, A.F.U., Grant, G., Brantner, K., Bardocz, S., 1995. Dietary lectins affect hormone balance of body and modulate its general metabolism. In: Singer, M.V., Ziegler, R., Rohr, G. (Eds.), Gastrointestinal Tract and Endocrine System, Kluwer Academic Publishers, Lancaster, pp. 457–463.

Pusztai, A., Koninkx, J., Hendriks, H., Kok, W., Hulscher, S., Van Damme, E.J.M., Peumans, W.J., Grant, G., Bardocz, S., 1996. Effect of the insecticidal *Galanthus nivalis* agglutinin on metabolism and the activities of brush border enzymes in the rat small intestine. J. Nutr. Biochem. 7, 677–682.

Pusztai, A., George, G., Gelencsar, E., Ewen, S.W.B., Pfueller, U., Eifler, R., Bardocz, S., 1998. Effects of an orally administered mistletoe (type-2 RIP) lectin on growth, body composition, small intestinal structure, and insulin levels in young rats. J. Nutr. Biochem. 9, 31–36.

Rahbé, Y., Febvay, G., 1993. Protein toxicity to aphids—an in vitro test on *Acyrthosiphon pisum*. Entomol. Exp. Appl. 67, 149–160.

Rahbé, Y., Sauvion, N., Febvay, G., Peumans, W.J., Gatehouse, A.M.R., 1995. Toxicity of lectins and processing of ingested proteins in the pea aphid *Acyrthosiphon pisum*. Entomol. Exp. Appl. 76, 143–155.

Radberg, K., Biernat, M., Linderoth, A., Zabielski, R., Pierzynowski, S.G., Westrom, B.R., 2001. Enteral exposure to crude red kidney bean lectin induces maturation of the gut in suckling pigs. J. Anim. Sci. 79, 2669–2678.

Rao, K.V., Rathore, K.S., Hodges, T.K., Fu, X., Stoger, E., Sudhakar, D., Williams, S., Christou, P., Bharathi, M., Bown, D.P., Powell, K.S., Spence, J., Gatehouse, A.M.R., Gatehouse, J.A., 1998. Expression of snowdrop lectin (GNA) in transgenic rice plants confers resistance to rice brown planthopper. Plant J. 15, 469–477.

Ratanapo, S., Ngamjunyaporn, W., Chulavatnatol, M., 1998. Sialic acid binding lectins from leaf of mulberry (*Morus alba*). Plant Sci. 139, 141–148.

Renkonnen, K.O., 1948. Studies on hemagglutinins present in seeds of some representatives of the family Leguminoseae. Ann. Med. Exp. Fenn. 26, 66.

Reynoso-Camacho, R., De-Mejia, E.G., Loarca-Pina, G., 2003. Purification and acute toxicity of a lectin extracted from tepary bean (*Phaseolus acutifolius*). Food Chem. Toxicol. 41, 21–27.

Rios, F.J.B., Cavada, B.S., Medeiros, D.A., Moreira, R.A., Vasconcelos, I.M., Oliveira, J.T.A., 1996. Digestibility of plant lectins from *Canavalia, Cratylia, Dioclea* and *Artocarpus* Genera. In: Van Driessche, E., Rougé, P., Beeckmans, S., Bog-Hansen, T.C. (Eds.), Lectins: Biology, Biochemistry, Clinical Biochemistry, Textop, Denmark, pp. 277–284.

Ripoll, C., Favery, B., Lecomte, P., Van Damme, E., Peumans, W., Abad, P., Jouanin, L., 2003. Evaluation of the ability of lectin from snowdrop (*Galanthus nivalis*) to protect plants against root-knot nematodes. Plant Sci. 164, 517–523.

Rouanet, J.M., Besancon, P., Lafont, J., 1983. Effect of lectins from leguminous seeds on rat duodenal enterokinase activity. Experientia 39, 1356–1358.

Roy, A., Banerjee, S., Majumder, P., Das, S., 2002. Efficiency of mannose-binding plant lectins in controlling a homopteran insect, the red cotton bug. J. Agric. Food Chem. 50, 6775–6779.

Rüdiger, H., 1998. Plant lectins—more than just tools for glycoscientists: occurrence, structure and possible functions of plant lectins. Acta Anat. 161, 130–152.

Santos, L.F.L., 2001. Toxina da salsa (*Ipomoea asarifolia* R. et Schult.): aspectos bioquímicos, estruturais, funcionais e potencial biotecnológico. Doctoral thesis. Federal University of Ceará, Fortaleza, Brazil, pp. 142.

Sasaki, M., Fitzgerald, A.J., Grant, G., Ghatei, M.A., Wright, N.A., Goodlad, R.A., 2002. Lectins can reverse the distal intestinal atrophy associated with elemental diets in mice. Aliment. Pharm. Therap. 16, 633–642.

Sauvion, N., Rahbé, Y., Peumans, W.J., Van Damme, E.J.M., Gatehouse, J.A., Gatehouse, A.M.R., 1996. Effects of GNA and other mannose binding lectins on development and fecundity of the peach-potato aphid *Myzus persicae*. Entomol. Exp. Appl. 79, 285–293.

Sayegh, A.I., Ritter, R.C., 2003. Cholecystokinin activates specific enteric neurons in the rat small intestine. Peptides 24, 237–244.

Schuler, T.H., Poppy, G.M., Kerry, B.R., Denholm, I., 1998. Insect-resistant transgenic plants. Trends Biotechnol. 16, 168–174.

Setamou, M., Bernal, J.S., Legaspi, J.C., Mirkov, T.E., Legaspi, B.C. Jr., 2002. Evaluation of lectin-expressing transgenic sugarcane against stalkborers (Lepidoptera: Pyralidae): effects on life history parameters. J. Econ. Entomol. 95, 469–477.

Sgarbieri, V.C., 1996. Propriedades nutricionais das proteínas. In: Sgarbieri, V.C., (Ed.), Proteinas em Alimentos Protéicos: Propriedades, Degradações e Modificações, Livraria Varela Ltda, São Paulo, pp. 337–386.

Small, G.J., Hemingway, J., 2000. Molecular characterization of the amplified carboxylesterase gene associated with organophosphorus insecticide resistance in the brown planthopper, *Nilaparvata lugens*. Insect Mol. Biol. 9, 647–653.

Stillmark, H., 1888. Ueber Ricin, ein giftiges Ferment aus dem Samen von *Ricinus communis* L. und einigen anderen Euphorbiaceen. Arb. Pharmak. Inst. Dorpat 3, 59–151.

Stoger, E., Williams, S., Christou, P., Down, R.E., Gatehouse, J.A., 1999. Expression of the insecticidal lectin from snowdrop (*Galanthus nivalis* agglutinin; GNA) in transgenic wheat plants:

effects on predation by the grain aphid *Sitobion avenae*. Mol. Breed. 5, 65–73.

Sun, X., Wu, A., Tang, K., 2002. Transgenic rice lines with enhanced resistance to the small brown planthopper. Crop Prot. 21, 511–514.

Tang, K., Zhao, E., Sun, X., Wan, B., Qi, H., Lu, X., 2001. Production of transgenic rice homozygous lines with enhanced resistance to the rice brown planthopper. Acta Biotechnol. 21, 117–128.

Tchernychev, B., Wilchek, M., 1996. Natural human antibodies to dietary lectins. FEBS Lett. 397, 139–142.

Thompson, L.U., Tenebaum, A.V., Hui, H., 1986. Effect of lectins and the mixing of proteins on rate of protein digestibility. J. Food Sci. 51, 150–152.

Tinjuangjun, P., Loc, N.T., Gatehouse, A.M.R., Gatehouse, J.A., Christou, P., 2000. Enhanced insect resistance in Thai rice varieties generated by particle bombardment. Mol. Breed. 6, 391–399.

Tomov, B.W., Bernal, J.S., 2003. Effects of GNA transgenic sugarcane on life history parameters of *Parallorhogas pyralophagus* (Marsh) (Hymenoptera: Braconidae), a parasitoid of Mexican rice borer. J. Econ. Entomol. 96, 570–576.

Triadou, N., Audran, E., 1983. Interaction of the brush-border hydrolases of the human small intestine with lectins. Digestion 27, 1–7.

Van Damme, E.J.M., Peumans, W.J., Barre, A., Rougé, P., 1998. Plant lectins: a composite of several distinct families of structurally and evolutionary related proteins with diverse biological role. Crit. Rev. Plant Sci. 17, 575–692.

Van Damme, E.J.M., Hao, Q., Charels, D., Barre, A., Rougé, P., Van Leuven, F., Peumans, W.J., 2000. Characterization and molecular cloning of two different type 2 ribosome-inactivating proteins from the monocotyledonous plant *Polygonatum multiflorum*. Eur. J. Biochem. 267, 2746–2759.

Vasconcelos, I.M., Maia, A.A.B., Siebra, E.A., Oliveira, J.T.A., Carvalho, A.F.F.U., Melo, V.M.M., Carlini, C.R., Castelar, L.I.M., 2001. Nutritional study of two Brazilian soybean (*Glycine max*) cultivars differing in the contents of antinutritional and toxic proteins. J. Nutr. Biochem. 12, 1–8.

Vaughan, A., Hilder, V.A., Boulter, D., 1999. Genetic engineering of crop plants for insect resistance—a critical review. Crop Prot. 18, 177–191.

Wang, Z.B., Guo, S.D., 1999. Expression of two insect resistant genes cryIA (bandc) GNA in transgenic tobacco plants results in added protection against both cotton bollworm and aphids. Chinese Sci. Bull. 44, 2051–2058.

Wang, W., Hause, B., Peumans, W.J., Smagghe, G., Mackie, A., Fraser, R., Van Damme, E.J.M., 2003. The Tn antigen-specific lectin from ground ivi is an insecticidal protein with an unusual physiology. Plant Physiol. 132, 1322–1334.

Williams, P.E.V., Pusztai, A., MacDearmid, A., Innes, G.M., 1984. The use of kidney beans (*Phaseolus vulgaris*) as protein supplements in diets for young rapidly growing beef steers. Anim. Feed Sci. Tech. 12, 1–10.

Wilson, A.B., King, T.P., Clarke, E.M.W., Pusztai, A., 1980. Kidney bean (*Phaseolus vulgaris*) lectin-induced lesions in the small intestine. II. Microbiological studies. J. Comp. Pathol. 90, 597–602.

Wu, A., Sun, X., Pang, Y., Tang, K., 2002. Homozygous transgenic rice lines expressing GNA with enhanced resistance to the rice sap-sucking pest *Laodelphax striatellus*. Plant Breed. 121, 93–95.

Zhu, K., Huesing, J.E., Shade, R.E., Bressan, R.A., Hasegawa, P.M., Murdock, L.L., 1996. An insecticidal *N*-acetylglucosamine-specific lectin gene from *Griffonia simplicifolia* (Leguminosae). Plant Physiol. 110, 195–202.

Zhu-Salzman, K., Salzman, R., 2001. A functional mechanic of the plant defensive *Griffonia simplicifolia* lectin II: resistance to proteolysis is independent of glycoconjugate binding in the insect gut. J. Econ. Entomol. 94, 1280–1284.

Zhu-Salzman, K., Shade, R.E., Koiwa, H., Salzman, R.A., Narasimhan, M., Bressan, R.A., Hasegawa, P.M., Murdock, L.L., 1998. Carbohydrate binding and resistance to proteolysis control insecticidal activity of *Griffonia simplicifolia* lectin II. Proc. Natl Acad. Sci. USA 95, 15123–15128.