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Lectins and Protease Inhibitors as Plant Defenses against Insects

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Plants commonly accumulate lectins and proteinaceous protease inhibitors in their various tissues, sometimes in high concentrations. Much evidence suggests that one of the functions of these proteins is to serve as defenses against insects.

KEYWORDS: Lectins; protease inhibitors; insects; plant defense

PROTEASE INHIBITORS AS INSECT DEFENSES

Nature and Diversity of Insect Digest Proteases. Insects are an amazingly diverse group of animals with a million or more species. They feed on virtually anything organic, ranging from wood to leaves, flowers, roots, tubers, nectar, seeds, and fruits, to animal flesh, animal wastes, and blood, and of course, other insects, as well as fungi and bacteria. To grow, develop, and reproduce successfully, insects require the same 10 essential amino acids as do mammals. These essential amino acids must be obtained from dietary protein, and so insects make use of proteases in their major digestive organ, the midgut. Not surprisingly, given the enormous variety of their food, insects have a wide diversity of digestive proteases. This diversity was not fully appreciated until recent years. In the early 1980s, entomologists typically assumed that most insects use serine proteases to digest their dietary protein (*1*). A few exceptions to this rule were known, for example, cysteine proteases in blood-feeding insects (*2*, *3*) and aspartyl proteases in carrion-feeding beetles (*4*), but these exceptions were ascribable to the special nature of the food of these insects.

In the late 1980s, it became increasingly clear that a great many insect species utilize cysteine and aspartyl proteases for dietary protein digestion. The discovery that seed-feeding bruchid beetles utilize cysteine proteases (*5*, *6*) as well as aspartyl proteases called attention to plant-feeding insects that use these classes of enzymes for protein digestion. Subsequent studies led to the recognition that many (but not all) species of the order Coleoptera—the beetles, the largest group of insects by far—utilize cysteine proteases in their digestive tracts (*7*). Furthermore, a survey of pH optima curves from a range of insect orders revealed that some insects have optimal proteolytic activity in the acidic pH range of 2−4 (e.g., a leafhopper) and others in the strongly alkaline range of pH 11 (e.g., a cranefly) (*8*). The upshot of this is that no single inhibitor will ever be found that could be used to control all insect species. If protease inhibitors are to be used for control of insects, inhibitors effective against different groups of insects are likely to be different.

Inhibitors of Insect Digest Proteases. Proteinaceous protease inhibitors are widely dispersed in plant tissues, often occurring in quite high concentrations. These inhibitors have received an enormous amount of attention from food scientists and the medical community because they pose a potential threat to human and animal health if they are ingested without cooking or processing (*9*). The question that immediately comes to mind for the entomologist is: Do the inhibitors that occur abundantly in plants serve as plant defenses against insects? A corollary question is: Can the genes encoding these inhibitors be used to impart insect resistance to plants?

There is no question that proteinaceous inhibitors from plants can inhibit insect digestive proteases and that they may, in certain instances, suppress growth and development when they are fed to insects. One of the earliest papers bearing on this point was published in 1954 by Professor Irvin Liener with colleagues Herbert Lipke and Gottfried Fraenkel (*10*). They discovered that a preparation of proteinaceous inhibitor from soybean contained an inhibitor of the digestive proteolytic activity from the red flour beetle, activity that was not inhibited by the purified soybean inhibitor. This paper contained two firsts: (1) the first description of the inhibition of an insect enzyme by a plant protease inhibitor and (2) clues to the presence of a novel protease inhibitor in soybeans, which eventually led to the discovery and cloning of a novel gene with potential for insect control.

Transgenic Plants Expressing Digestive Protease Inhibitors Resist Insects. Given the substantial evidence that plant protease inhibitors can inhibit insect enzymes in vitro and inhibit insect growth, development, and survival in vivo when fed in their diets, it was natural to introduce genes encoding protease inhibitors into plants to ascertain if they would have the same effects. The first successful demonstration of genetic engineering using a protease inhibitor gene to confer insect resistance involved the transfer of the gene encoding cowpea trypsin inhibitor (CpTI) into tobacco (*11*). CpTI had originally caught the attention of entomologists and biochemists because it was thought to be the chemical basis of resistance in a cowpea weevil-resistant line (TVu 2027) of cowpeas (*12*). Subsequent research demonstrated that CpTI is not in fact the reason Tvu 2027 resists cowpea weevil (*13*, and others), but enthusiasm for this gene caused it to be used in early plant transformation efforts. Tobacco (*Nicotiana tabacum*) plants expressing high

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levels of CpTI in their leaves (2.5−9.6 μg of CpTI/mg of soluble leaf protein) caused increased mortality (up to 50%) of tobacco budworm larvae (*Heliothis virescens*) feeding on the plants and stunted the growth of the surviving larvae. Clearly, a protease inhibitor can confer resistance to leaf-feeding insects. However, the resistance may be only relative, at best. Transgenic potato plants expressing CpTI at levels up to 2% of the leaf protein reduced the growth of the tomato moth larva (*Lacanobia oleracea*) by 45%, but there was no reduction in leaf damage (*14*). Evidently the insects compensated for inadequate protein digestion by consuming increased amounts of leaf tissue. CpTI-expressing tobacco plants can retard growth and development of the common cutworm, *Spodoptera litura*, as well (*15*). In field tests, rice (*Oryza sativa*) plants expressing CpTI exhibited marked resistance to two rice stem borers, *Chilo suppressalis* and *Sesamia inferens*. Under conditions of natural infestation, control (untransformed) plants were severely damaged and produced few or no panicles, whereas the transgenic plants had little or no infestation and more panicles produced seeds (*16*).

Another key early paper involved the transfer of potato protease inhibitors I and II into tobacco (*17*). Tobacco hornworm larvae (*Manduca sexta*) feeding on plants expressing potato inhibitor II (a trypsin/chymotrypsin inhibitor) at levels >100 μg/g of leaf tissue exhibited markedly retarded growth, whereas those feeding on leaves expressing similar levels of potato inhibitor I (a chymotrypsin inhibitor) were affected little, if at all. Potato protease inhibitor II, when transferred into rice, conferred substantial resistance to the pink stem borer, *Sesamia inferens* (*18*). Likewise, a trypsin inhibitor gene from winged bean expressed in transgenic rice plants retarded the growth of *Chilo suppressalis* larvae (*19*). A multidomain protease inhibitor from *Nicotiana alata* was introduced into tobacco and garden pea and accumulated at levels of 0.3 and 0.1%, respectively. When *Helicoverpa armigera* larvae fed on these plants, their growth and development was delayed, and mortality was increased (*20*). Similar results were obtained in a parallel study: *Helicoverpa punctigera* larvae exhibited increased mortality and decreased growth rates when fed on tobacco leaves expressing *N. alata* protease inhibitors at the level of 0.2% of the soluble leaf protein (*21*). Transgenic tobacco plants expressing Kunitz trypsin inhibitor from soybean were markedly resistant to *H. armigera* (*22*).

The discovery that many plant-feeding insects use cysteine proteases to digest their dietary protein (*7*) opened the path to exploring the use of genes encoding cysteine protease inhibitors (CPIs) for insect control. Among the best studied CPIs are the oryzacystatins isolated from mature rice seeds (*23*). Transgenic potatoes expressing oryzacystatin I (OCI) caused increased mortality of Colorado potato beetle larvae (*Leptinotarsa decemlineata*) feeding on the leaves (*24*). Poplar tree plantations used in paper production suffer severe damage due to *Chrysomela tremulae*, a beetle that causes severe losses in young plantations and in short-rotation intensive culture. Transgenic poplars expressing OCI were shown to resist attack by *C. tremulae* larvae (*25*). Transgenic oilseed rape expressing OCI in its seed was markedly more resistant to one strain of cabbage seed weevil (*Ceutorhynchus assimilis*) than to another strain of the insect from a different geographic orgin (*26*). This undoubtedly reflects the genetic diversity of populations of this species. Such variability in sensitivity to lectins and protease inhibitors is likely, in time, to be encountered with other species as well.

Expression of protease inhibitor genes in plants does not always reduce damage or confer protection against insects. When the Kunitz trypsin inhibitor (SBTI) gene from soybean was expressed in transgenic tobacco plants, larvae of *H. armigera* grew normally, despite the accumulation of the inhibitor in the plant leaves and despite its ability to inhibit *H. armigera* gut proteolytic activity (*27*). This curious result requires further explanation. However, similar observations have been made with transgenic potatoes expressing OCI (*28*). Colorado potato beetle (CBP) larvae feeding on these plants consumed leaf material more quickly, gained weight more quickly, and were 20% heavier at the end of the third larval instar than controls feeding on untransformed plants. CBP larvae have the remarkable ability to switch the digestive enzyme complement in their midgets in the presence of a protease inhibitor (*29*), and there is evidence that this may explain the ability of CBP larvae to perform well in the presence OCI. The insects simply switch off the production of cysteine proteases sensitive to OCI and elaborate a different complement of proteases that permit normal protein digestion. Two lepidopterous species, *Lymantria dispar* and *Closter anastomosis*, grew normally on transgenic poplar leaves expressing a Kunitz inhibitor gene from soybean. The proteolytic enzymes in the midgets of both of the insects were susceptible to the inhibitor, yet growth was unaffected (*30*). *H. armigera* larvae feeding on tobacco expressing a giant taro (*Alocasia macrorrhiza*) protease inhibitor at 0.3% of the soluble leaf protein grew slightly more slowly than the controls, but no mortality above that of the control was observed (*31*). Total gut proteolytic activity was reduced by 13%, mostly because of the marked inhibition of trypsin (58%), but chymotrypsin and elastase were concomitantly increased by 26 and 16%, respectively, largely compensating for the trypsin inhibition by the giant taro inhibitor. This is another example of the ability of insects to adapt to the presence of protease inhibitors in their diets. A chrysomelid beetle, *Psyllioides chrysophala*, actually grew more rapidly on transgenic oilseed rape plants than they did on the untransformed controls. Larvae feeding on the transgenic plants had elevated levels of both cysteine and serine proteases in their guts (*32*). Expression of SKTI in transgenic potato plants had little effect on tomato moth larval feeding, growth, and survival (*33*).

LECTINS AS PLANT DEFENSES

Characteristics of Lectins. Plant lectins are proteins with at least one noncatalytic domain that binds reversibly to specific mono- or oligosaccharides (*34*). A recent review by Peumans and Van Damme (*34*) places the lectins in four major and three minor families.

Best characterized is the legume lectin family (*35*), the lectins of which have been studied extensively thanks to their presence in common legume foods, where they may act as antinutritional factors. Legume lectins are unique in that they contain Mn^{2+} and Ca^{2+} ions associated with a series of highly conserved amino acids which participate in carbohydrate binding. These amino acids are scattered through the primary structure of the protein but are brought together through folding to form a binding pocket. Legume lectins are commonly glycosylated and are composed of two or four protomers held together by noncovalent bonds, so the functional lectin molecule has multiple carbohydrate binding sites. Various legume lectins may bind galactose, *N*-acetylgalactosamine, mannose, glucose, *N*-acetylglucosamine (GleNAc), fucose, *N*-acetylneuraminic acid, oligomers of *N*-acetylglucosamine, or still more complex carbohydrates.

Chitin-binding lectins contain one or more hevein domains, the term "hevein" referring to a 43 amino acid chitin-binding polypeptide present in latex of the rubber tree (*Hevea brasil-*

iensis). They are ubiquitous in plants. They bind *N*-acetylglucosamine and oligomers and polymers of GlcNAc.

Type 2 ribosome-inactivating proteins (RIP) are lectins that catalytically inactivate ribosomes of eucaryotes and thereby irreversibly shut down protein synthesis. They are composed of two chains, one of them binding carbohydrate and the other being an enzyme that cleaves a key ADP-ribose moiety in elongation factor 2, which is essential for protein synthesis. Ribosome inactivation causes cell death and eventual death of the organism. Type 2 RIPs are among the most toxic substances known. Carbohydrate specificity is restricted to galactose, *N*-acetylgalactosamine, and *N*-acetylneuraminic acid.

Monocot mannose-binding lectins bind only mannose and oligosaccharides of mannose and are found only in a subgroup of monocot plants, the Alliaceae, Amaryllidaceae, Araceae, Bromeliaceae, Liliaceae, and Orchidaceae. A variety of molecular forms are known, ranging from monomers through homo- and heterodimers and homo- and heterotetramers.

Three minor families are (a) the jacalin-related lectins, (b) the amaranthin family, and (c) the cucurbit phloem lectins. Jacalin is a lectin that occurs in the seeds of the jackfruit, where it probably serves as a storage protein. Jacalin-like lectins have thus far only been found in the Moraceae and Convulvulaceae families. Amaranthin lectins are a group of *N*-acetylgalactosamine-specific lectins found only in various *Amaranthus* species. Cucurbitaceae phloem lectins are a small group of chitin-binding lectins known only from the phloem of cucurbits.

Lectins Are Common and Often Abundant in Plants. Hundreds of different lectins have been identified in plants. They are sometimes found in high abundance. A low molecular weight (13 kDa) mannose-binding lectin makes up 75% of the protein in the nectar of leek (*Allium porrum)* flowers (*36*). The most abundant protein in the bark of yellowwood (*Cladrastis lutea)* is a mannose/glucose-binding lectin (*37*). The major protein in the tubers of *Arum maculatum* is a lectin, and the same is true for tubers of numerous other species (*38*). The most abundant protein in the fruits of elderberry is a lectin (*39*). Some 1−10% of the total soluble protein of legume seeds is composed of lectins (*34*). Approximately 3−5% of the proteins in the seeds of amaranth are lectins (*40*).

Biological Roles Lectins Play. Over the past several years it has gradually become clear that lectins play two major roles in plants. First, they are stores of proteins that can be mobilized for plant growth and development. Second, they are plant defenses against herbivores and pathogens. They play additional roles in plants as well.

When lectins occur at high concentrations in plant tissues, they undoubtedly represent a storage form of protein. Peumans and Van Damme (*34*) recount the arguments for this role. First, lectins often occur most abundantly in seeds and vegetative storage tissues. Second, they are found in subcellular organelles widely believed to represent storage sites. Third, they accumulate during the growth and development or reproductive phase of the plant life cycle and are mobilized and utilized later.

That lectins serve a storage role is perfectly compatible with the idea that the same lectins can serve as plant defenses, too. Indeed, nature has found, in lectins, a way to get two for the price of one, as it were. Tubers and seeds especially are repositories of the energy and amino acid reserves the plant needs for subsequent growth. Seeds represent, in addition, the vehicle that many plants use to transfer their DNA to the next generation. These vital parts of the plant require protection if the plant is to survive. Lectins—as well as protease inhibitors and numerous other defensive molecules—aid in plant defense, but when the time is right, these same proteins supply material and energy for needed growth, development, and reproduction.

LECTINS AS PLANT DEFENSES AGAINST INSECTS

Several tentative generalizations have emerged regarding the effects of lectins on insects.

(1) There is no obvious correlation between the sugar specifies of lectins and their toxicity when fed to insects. It is true that certain trends have been observed. Lectins that bind *N*-acetylglucosamine and its oligomers often retard growth and development when fed to certain beetles, yet some lectins that bind GlcNAc are not very toxic (*41*). Similarly, mannospecific lectins are quite toxic to certain aphids and sucking bugs, yet other lectins from *Vicia villosa* and *V. faba* are not (*42*). Similar results have been found with *Chilo partellus*, a lepidopterous stem borer that seriously damages maize and grain sorghum in Asia and Africa. The mannose-binding lectin from the vegetative tissues of peanut (*Arachis hypogaea*) causes substantial *C. partellus* larval mortality and larval growth depression at dietary levels of 5000 and 10000 mg/kg of diet, yet the mannose-binding lectin from garden pea, *Pisum sativum*, had no significant effect on mortality or growth (*43*).

(2) There is no correlation between the ability of lectins to bind to insect midgut tissues (possibly the initial site of action) and their toxicity. Numerous lectins bind to midgut tissues and yet are not toxic. Binding to carbohydrates may be necessary for toxicity (see below), but it is not sufficient. Presumably there is a wide spectrum of carbohydrate moieties in the insect midgut to which lectins can bind, but for most of these, lectin binding has no functional impact.

(3) All lectins that are toxic to insects exert their toxicity via binding to specific carbohydrate moieties.

(4) A prerequisite for toxicity is that the lectin is able to survive the hostile proteolytic environment of the insect midgut.

(5) There is no such thing as a lectin that is toxic to "insects". A given lectin may be quite toxic to one insect species and innocuous to another. A good example is phytohemagglutinin (PHA) from common bean, *Phaseolus vulgaris*. Purified PHA is not toxic to cowpea weevil larvae when fed in the diet at levels as high as 1% (w/w) (*44*). Earlier results indicated that PHA is toxic to this insect (*45*, *46*) but were later shown to have resulted from the presence of α-amylase inhibitor as an impurity of the PHA preparation used by the earlier authors (*44*). By contrast, purified PHA caused rapid, dose-dependent mortality when fed in the liquid diet of the potato leafhopper (*Empoasca fabae*) (*47*).

Snowdrop Lectin. The most extensively studied anti-insect lectin is the mannose-binding lectin from snowdrop (*Galanthus nivalis*) bulbs. It is a homotetramer having a molecular weight of ∼50000, with each non-covalently bonded protomer containing three highly homologous mannose binding sites. It is commonly referred to as GNA on the basis of the name *Galanthus nivalis* agglutinin. At 1000 mg/L (= ∼20 uM) of liquid diet, GNA caused 79% mortality to first-instar numphs of the rice brown plant hopper (*Nilaparvata lugens*) and 89% mortality to the rice green leafhopper (*Nephottetix cinciteps*), whereas most other lectins had little or no effect on these important rice pests (*48*). In a later study, GNA strongly suppressed feeding of the rice brown planthopper when the dietary concentration was 1000 mg/L (*49*). Half of a cohort of pea aphids (*Acyrthosiphon pisum*) died when they fed on an artificial diet containing GNA at 144 mg/L, and higher concentration in the diet retarded growth and caused increased mortality of the sugarcane whitegrub (*Antitrogus sanguineus*)

(*50*). Snowdrop lectin fed in the liquid diet of nymphs of the glasshouse potato aphid (*Aulocothum solani*) at 1000 mg/L slowed nymphal development and caused, after a delay of several days, a high percentage of mortality. Adult insects fed GNA were little affected with regard to mortality, but their fecundity was sharply reduced (*51*). GNA also has been shown to have antinutritive effects when fed in the diet of a lepidopteran, the tomato moth (*Lacanobia oleracea*), but the required dose was high. With dietary levels of 20000 μg/g growth was markedly retarded, but survivorship was not reduced (*52*).

One factor that may affect the anti-insect properties of mannose-specific lectins is the number of subunits per molecule. GNA, a homotetramer, was toxic (LC50 = 4 μM) when fed to *N. lugens* than was the trimeric *Narcissus pseudonarcissus* lectin (LC50 = 11 μM), which was in turn more toxic than the heterodimeric lectin from *Allium sativum* (LC50 > 40 uM) (*53*).

Researchers studying the interactions of lectins and insects have sought to understand the roles lectins naturally play in the interactions of insects and plants, but their research also has been conducted with an eye to the possibility of conferring resistance on plants by genetically engineering lectin genes into plants. The anti-insect activity of GNA, together with evidence that it may be less toxic than other lectins to mammals, has made it the leading candidate lectin gene for transfer into crop plants. Several plant species have been transformed with the GNA gene: potato (*Solanum tuberosum*) (*54*); rice (*Oryza sativa*) (*55*); wheat (*Triticum aestivum*) (*56*); and tobacco (*57*). In every case, plants that were high expressers of the transgene exhibited a degree of resistance to insects feeding on them. Thus, transgenic potato plants expressing GNA at 0.3−0.4% of the total soluble protein were much less favorable as a food source for the potato aphid (*Aulocothum solani*) than were untransformed control plants. In greenhouse studies, the rate of population buildup over multiple generations was only one-fourth that of the control plants (*51*). The aphid *Myzus persicae* likewise performed less well on the GNA-expressing plants versus the controls (*54*), and the same was found to be true for the tomato moth, *L. oleraceae* (*14*, *52*). The presence of GNA in transgenic rice plants expressing the protein at levels as high as 2.0% of the soluble total protein retarded development of the rice brown planthopper (*N. lugens*) and deterred its feeding (*55*). Grain aphids (*Sitobion avenae*) suffered reduced fecundity when they fed on wheat plants expressing GNA at ≥0.04% of the total protein, but their survivorship was not affected (*56*). In tobacco plants, the presence of GNA causes reduced fecundity of the aphid *M. persicae*.

Transgenic potato plants expressing concanavalin A from jackbean (*Canavalia ensiformis*) retarded development of the tomato moth and decreased larval weight but had no effect on survival (*58*). Fecundity of the potato aphid was markedly reduced.

Site and Mode of Action of Anti-insect Lectins. There are three likely sites where dietary lectins disrupt feeding, digestion, and thereby growth and development. Food recognition by insects depends on sensory receptors commonly located on the tips of the feet, the tarsi, and on the antennae and mouthparts. Binding of lectins to carbohydrate moieties associated with the membranes of the chemosensory sensillae could block access of food chemical signals to their actual receptor proteins. Alternatively, lectins could disrupt the integrity of the sensory membranes as well, thus interfering with the ability of the insect to detect food. Systematic studies of this potential area of interaction are needed.

A second potential site of lectin action is the peritrophic matrix (PM), a protective envelope secreted by the epidermal cells of the midgut and composed of proteins, glycoproteins, chitin, and glycosaminoglycans. Ingestion of wheat germ agglutinin (WGA) by European corn borer larvae (*Ostrinia nubilalis*) caused abnormalities to appear in the PM structure (*59*). The matrix, which is normally a single layer in the anterior midgut, was observed to form a mass of convoluted PMs in WGA-fed insects. There was evidence that the chitin meshwork, which is an integral part of the matrix, was disrupted, allowing large holes to appear in the envelope. In WGA-fed larvae food particles were observed contacting the delicate surface of the digestive epithelium, something that does not normally happen, and bacterial penetration was observed through the matrix. One of the functions of the PM is to protect against these threats to the integrity of the delicate midgut epithelium. The microvillar structure of the midgut is likewise disrupted by WGA, with disintegrated microvillae being common. Harper and colleagues (*59*) suggested two explanations for the mode of action of WGA. First, WGA, because of its high affinity for oligomers of GlcNAc, may bind the nascent chitin oligosaccharide chains, which are essential for the production of the chitin polymer, a major component of the PM. Second, by binding to glycoproteins making up the PM, the assembly of glycoprotein−chitin linkages needed for normal PM structure may be disrupted. In sum, WGA appears to interfere with the secretion and assembly of the chitin network that is the backbone of the PM. This disruption results in hypersecretion of PM by the microvilli, with subsequent disintegration of this tissue, which acts both as a template and as the engine of PM production.

A third possible site of action of lectins is the surface of the digestive epithelial cells in the insect midgut. These cells secrete digestive enzymes and absorb the chemical products of digestion. Numerous studies have demonstrated that dietary lectins can bind to the surfaces of epithelial cells (e.g., refs *49*, *60*, and *61*). For example, Harper et al. (*61*) observed that GNA binds very strongly to brush border membrane vesicles made from the midgut of the European corn borer, yet was nontoxic when fed to the insect. The fact that some lectins that bind to the midgut epithelium are nontoxic proves that binding alone is not sufficient to cause disruption of physiological function. Presumably there is a special subpopulation of cell surface carbohydrates that cause toxicity when they bind a lectin. This subpopulation may be present or absent in a given insect species or in a particular developmental stage of a species. In general, however, the results of Harper et al. (*61*) indicate that lectins causing significant mortality to *O. nubilalis* also bound strongly to brush border membrane vesicles. A better indicator of the essentiality of binding for toxicity may be the correlation between the two when using molecular variants of a single lectin. This has been done with GSII, an *N*-acetylglucosamine-specific lectin from the African legume *Griffonia simplicifolia.* Zhu-Salzman et al. (*60*) prepared a series of site-specific mutants of the recombinant GSII protein. Those mutant forms that retained relatively high binding capacity to GlcNAc also maintained their ability to bind to the isolated midguts and slowed growth and development of the cowpea weevil *Callosobruchus maculatus*. Those mutant forms that lost binding capacity to GlcNAc and the midgut wall failed to depress growth and development of the insect.

In numerous instances, exposure of insects to dietary lectins has been shown to cause ultrastructural changes in the gut epithelium. Disruption of the microvillae and abnormalities in epithelial cells have been observed in the rice brown planthopper

(*N. lugens*) fed a diet containing GNA (*49*). Similar lesions were seen in the midgut epithelia of the pea aphid (*A. pisum*) fed a diet containing concanavalin A (Sauvion et al., 1995, quoted in ref *49*) and in cowpea weevil larvae fed low levels of WGA (*62*).

There is some evidence that chronic ingestion of lectins can cause hypertrophy of the insect gut. This has been seen with tomato moth larvae fed for 16 days on a diet containing concanavalin A; those insects receiving the con A diet had gut weight/whole-body weight ratios indicative of disproportionate growth of the midgut relative to the rest of the body. (*52*). Short-term exposure of insects of dietary lectins (GNA and Con A) triggered increases in aminopeptidase and trypsin activities associated with the gut, indicating some kind of compensatory response of the gut epithelial cells that produce these enzymes. These increases in enzyme levels were not sustained during chronic exposure to the dietary lectins.

Another possible site of lectin action is within the insect body. If the dietary lectin can survive its passage through the insect alimentary tract and be absorbed unchanged into the circulation, then it might pass to any distant site within the body via the circulating hemolymph. There is evidence that this may happen. In the rice brown planthopper fed a diet containing GNA, immunohistochemical studies revealed the presence of GNA associated with fat bodies and hemolymph. This GNA must have come from the diet and, therefore, passed into the circulating hemolymph, which carried it to the fat body (*49*). In rats, there is evidence that dietary WGA may be able to reach systemic circulation (*63*). However, in cowpea weevils fed recombinant GSII, there was no evidence of any of the lectin reaching the circulation (*60*).

Practical Applications of Genes Encoding Lectins and Digestive Protease Inhibitors for Plant Protection? Is There a Future? Lectins and protease inhibitors undoubtedly do serve as natural plant protectants. The fact that insect damage to plants can induce protease inhibitors supports the idea of a defensive role (*64*). Genes encoding these proteins have been successfully transferred into plants and expressed, and the transformed plants show varying degrees of insect resistance. Thus far, no crop plant cultivars carrying lectin- or protease-based resistance have been deployed commercially. There are several reasons for this.

First, in most cases, the degree of insect resistance imparted by lectins and protease inhibitors is only moderate. Although some insect mortality may be observed, the more common effect is stunting of growth and slowing of development. These relatively modest effects may actually contribute a substantial degree of plant protection, but the degree of protection is not always very striking. The insects continue to live on the transformed plants, and leaf damage is still obvious. In some cases, indeed, insects eat more plant tissue rather than less. This stands in sharp contrast to what we see with plants transformed with genes encoding *Bacillus thuringiensis* delta endotoxins (Bts). Such plants are practically immune to the target insects and commonly show virtually no damage. The biotechnology industry currently is gripped by the Bt model and is not much interested in genes that confer only a modest degree of protection.

Second, the concentrations of lectins and protease inhibitors needed to confer even this modest degree of protection are high, commonly $\geq 1\%$ of the soluble protein in the plant tissues. This high level of expression and accumulation of the protein product imposes a substantial physiological load on the plant, which has to divert significant material and energy resources to these proteins. Yield reductions may be a consequence. It is likewise a challenge to molecular biologists to obtain and sustain these high degrees of accumulation.

Third, insects will probably be able to adapt rather quickly, both physiologically and genetically, to the presence of lectins or protease inhibitors in their diets. Some insects utilize multiple proteases for protein digestion, one or more of which is unaffected by a specific inhibitor. Compensatory production of uninhibited proteases can circumvent the effects of any single inhibitor (*64*). Insect populations are diverse, and they have a vast genetic history of interacting with plants containing protease inhibitors and lectins. This means that pest insect populations are likely to include individuals that carry genetic variation that enables them to survive an encounter with a new lectin or protease inhibitor in their food—which is exactly what would happen if a new crop cultivar expressing a novel lectin or protease inhibitor were to be deployed at some point in the geographic range of the insect. The adapted genetic variants might be rare initially, but deployment of a cultivar expressing an effective lectin or protease inhibitor could soon weed out susceptible individuals, leaving the resistant ones to mate with one another, so that the pest population would soon be dominated by resistant individuals. At that point the new lectin—or protease inhibitor-based resistance—would be practically useless.

Fourth, different insect species vary widely in their susceptibility to given lectins and protease inhibitors. Most crop plant species are attacked by multiple pest species, not just one. A good example is grain sorghum in Africa, which is attacked by at least five different species of stalk borer. Finding single lectins or protease inhibitors that are effective against the multiple insect pests of a crop will be difficult, if not impossible.

Fifth, and perhaps most importantly of all, lectins and protease inhibitors are potentially toxic to humans, livestock, and other organisms in the environment. Although this may not be an absolute barrier to the use of lectins and protease inhibitors as plant defenses—after all, we eat food every day made from plant materials containing lectins and protease inhibitors—it will demand extremely thorough testing to ensure a high degree of food safety. If there is indeed a potential hazard to consumers of a candidate lectin or protease inhibitor gene, then elaborate and thorough precautions in the form of processing will have to be taken to eliminate any significant risk. The burden of using these genes is thus very heavy, and unless they confer an enormous advantage, it will likely collapse on those who try to carry it. This need for extensive safety testing and processing precautions should be contrasted with the 40 plus years of experience with Bt-based insecticide preparations—now widely used by producers of organically grown crops—which have a safety record as close to perfect as can be imagined.

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