Phytochemistry 72 (2011) 1538–1550

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Review

Plant lectins as defense proteins against phytophagous insects

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ARTICLE INFO

Article history:
Available online 21 March 2011

Keywords:
Agglutinins
Insecticidal proteins
Lectin
Plant defense
Pest control

ABSTRACT

One of the most important direct defense responses in plants against the attack by phytophagous insects is the production of insecticidal peptides or proteins. One particular class of entomotoxic proteins present in many plant species is the group of carbohydrate-binding proteins or lectins. During the last decade a lot of progress was made in the study of a few lectins that are expressed in response to herbivory by phytophagous insects and the insecticidal properties of plant lectins in general. This review gives an overview of lectins with high potential for the use in pest control strategies based on their activity towards pest insects. In addition, potential target sites for lectins inside the insect and the mode of action are discussed. In addition, the effect of plant lectins on non-target organisms such as beneficial insects as well as on human/animal consumers is discussed. It can be concluded that some insecticidal lectins are useful tools that can contribute to the development of integrated pest management strategies with minimal effect(s) on non-target organisms.

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doi:10.1016/j.phytochem.2011.02.024

1. Introduction

To cope with the continuous threat from different phytophagous insects, plants have evolved a whole arsenal of defense strategies. These defense mechanisms include morphological and structural features as well as the synthesis of chemical compounds. Chemical defense products may range from low molecular weight compounds called secondary metabolites to peptides and proteins that are active against pest insects. One particular group of such defense proteins is called plant lectins or plant agglutinins (Michiels et al., 2010; Van Damme, 2008; Vandenborre et al., 2009b). Plant lectins are a very heterogeneous group of proteins that all share one important biological property: they can recognize and bind reversibly to specific carbohydrate structures. Analyses at the level of the genome and the transcriptome have shown that lectin sequences are ubiquitous in the plant kingdom and hundreds of lectins have already been purified from various plant species (Van Damme et al., 2008). This review will focus on the role of plant lectins in the defense response and their activity against pest insects.

2. Definition and classification of plant lectins

The first discovery of a plant lectin dates back to the end of the nineteenth century, when a protein called ricin was found in castor bean (*Ricinus communis*) (Van Damme et al., 2008). Since ricin was shown to agglutinate red blood cells, the term hemagglutinin was introduced. Later, it was recognized that some hemagglutinins can selectively agglutinate human erythrocytes depending on their ABO blood group type. This finding gave birth to the word "lectin" which is derived from "legere", the Latin verb for "to select" (Van Damme et al., 2008). Afterwards, it was shown that this selectivity of plant lectins is based on the capacity to bind different carbohydrate structures present on the cell membrane of erythrocytes. Today, the term plant lectin is used to denote all plant proteins possessing at least one non-catalytic domain that binds reversibly to a specific mono- or oligosaccharide (Peumans and Van Damme, 1995).

Based on the overall domain architecture of plant lectins, four major groups can be distinguished: merolectins, hololectins, chimerolectins and superlectins (Van Damme et al., 1998a). Merolectins are proteins that contain only one carbohydrate-binding domain. Due to their monovalent nature, this group of lectins cannot agglutinate cells. Hololectins are lectins that are composed of two or more identical or very homologous carbohydrate binding domains which allow them to agglutinate cells and/or precipitate glycoconjugates. Most of the currently isolated and characterized plant lectins belong to the group of hololectins. In contrast to this lectin group, superlectins are composed of at least two carbohydrate-binding domains that recognize structurally unrelated carbohydrate structures. The group of chimerolectins comprises all plant lectins that are composed of one or more carbohydrate-binding domains fused to a domain that exerts a biological activity independent from the carbohydrate binding domain. Sequence analyses of complete plant genomes revealed that chimerolectins are very abundant in plants (Van Damme et al., 2008). Moreover, recent genome/transcriptome analyses of plants provided evidence for the occurrence of many proteins containing one or more lectin domain(s) embedded in a more or less complex multi-domain architecture (Van Damme et al., 2008). Because of this complexity and heterogeneity among all carbohydrate binding proteins, it is preferable to consider the class of plant lectins in terms of the carbohydrate-binding domains present in these proteins.

In the past, several attempts were made to organize this very heterogeneous group of plant proteins. Initially, lectins were classified into a number of specificity groups called "natural groups" based on their ability to recognize and bind specific sugars. However, such a classification based on carbohydrate-binding specificity was artificial and irrelevant with respect to the evolutionary relationships between plant lectins. In addition, different carbohydrate-binding motifs can recognize similar sugar structures. Therefore, a new classification system for plant lectins was elaborated taking into consideration all the sequence data that have become available in the last decades. Thorough genome/transcriptome analyses revealed that plant lectins can be classified into twelve distinct families of evolutionary and structurally related lectin domains (Van Damme et al., 2008) (Table 1). These different carbohydrate-binding domains were called in alphabetical order: (1) *Agaricus bisporus* agglutinin homologs, (2) amaranthins, (3) class V chitinase homologs, (4) cyanovirin family, (5) *Euonymus europaeus* agglutinin family, (6) *Galanthus nivalis* agglutinin family, (7) proteins with hevein domains, (8) jacalins, (9) proteins with a legume lectin domain, (10) LysM domains, (11) *Nicotiana tabacum*

Table 1
Overview of the plant carbohydrate-binding motifs according to Van Damme et al. (2008).

Lectin domain	Carbohydrate specificity	Examples	Insecticidal activity*
Agaricus bisporus agglutinin domain	T-antigen	ABA, MarpoABA	?
Amaranthins	T-antigen	Amaranthin, HFR2	+
Class V chitinase homologs	Blood group B, high-man N-glycans	RobpsCRP	?
Cyanovirin domain	High-man N-glycans	CV-N	?
Euonymus europaeus agglutinin domain	Blood group B, high-man N-glycans	EEA	?
Galanthus nivalis agglutinin domain	Man, oligomannosides, high-man N-glycans, complex, N-glycans	GNA, ASA II, ASAL, ACA, LOA	+++
Hevein domain	Chitin, high-man, Man, N-glycans	Hevein, UDA, WGA, HFR3	++
Jacalins	Gal, T-antigen, Man, N-glycans	Jacalin, Heltuba, HFR1	+
Legume lectin domain	Man/Glc, Gal/GalNAc, (GlcNAc)n, Fuc, Siaα2,3Gal/GalNAc, complex N-glycans	PHA, ConA, Gleheda, PSA, GS-II	++
LysM domain	Chitin-ologosaccharides	LysM, CEBiP	?
Nicotiana tabacum agglutinin domain	GlcNAc-oligomers, high-man *N*-glycans	NICTABA, PP2	+
Ricin-B domain	Gal/GalNAc, Siaα2–6Gal/GalNAc	Ricin, SNA-I	+

*Insecticidal activity has been reported for many (+++), several (++), a few (+) or no (?) lectins from this family. T-antigen: Galβ(1,3)GalNAc; Man: mannose; Gal: galactose; Glc: glucose; GalNAc: N-acetylgalactosamine; GlcNAc: N-acetylglucosamine; Fuc: fucose; Sia: sialic acid; ABA: *Agaricus bisporus* agglutinin; MarpoABA: ABA homolog from *Marchantia polymorpha*; HFR: Hessian fly responsive; RobpsCRA: Chitinase-related agglutinin homolog from *Robinia pseudoacacia*; CV-N: cyanovirin-N; EEA: *Euonymus europaeus* agglutinin; GNA: *Galanthus nivalis* agglutinin; ASAL: *Allium sativum* leaf agglutinin; ASAII: *Allium sativum* bulb agglutinin II; ACA: *Allium cepa* agglutinin; LOA: *Listera ovata* agglutinin; WGA: wheat germ agglutinin; UDA: *Urtica dioica* agglutinin; Heltuba: *Helianthus tuberosus* agglutinin; PHA: *Phaseolus vulgaris* agglutinin; ConA: *Canavalia ensiformis* agglutinin; Gleheda: *Glechoma hederacea* agglutinin; PSA: *Pisum sativum* agglutinin; GS-II: *Griffonia simplicifolia* agglutinin II; LysM: Lysin domain; CEBiP: chitin elicitor binding protein; NICTABA: *Nicotiana tabacum* agglutinin; PP2: phloem protein 2; SNA-I: *Sambucus nigra* agglutinin I.

agglutinin family, and (12) ricin-B family. Each lectin domain has its own characteristic overall fold with one or more carbohydrate-binding sites. For a more detailed overview of this lectin classification system we refer to Van Damme et al. (2008). The occurrence of a particular carbohydrate-binding domain is in most cases not restricted to a certain plant family. Moreover, most of these domains are spread all over the plant kingdom. Recently, an analysis of the fully sequenced genomes of soybean, rice and *Arabidopsis* revealed the presence of at least hundreds of putative lectin genes belonging to all major lectin superfamilies (Jiang et al., 2010).

Although many of the characterized plant lectins certainly interact with monosaccharides, their affinity for simple sugars is usually low when compared to more complex carbohydrate structures such as *N*-glycans (Garcia-Pino et al., 2007; Mandal et al., 1994). Glycan array analyses with different plant lectins clearly demonstrated that one particular carbohydrate-binding domain can be characterized by a carbohydrate-binding profile rather than having one specific sugar structure that is recognized (Fouquaert et al., 2009; Lannoo et al., 2006; Taylor and Drickamer, 2009). Structural analyses revealed that a typical carbohydrate binding site consists of a shallow depression at the protein surface that is complementary to a specific sugar unit of a bulky *N*-glycan chain (Mandal et al., 1994; Schouppe et al., 2010). Additional amino acid residues in the vicinity of this primary binding site allow the formation of extra H-bonds with other sugar residues of the glycan structure creating a more extended binding site (Schwefel et al., 2010). This strongly increases the affinity for a particular complex carbohydrate structure (in the 10^{-6}–10^{-8} M range), while the affinity for simple sugars is usually much weaker (in the 10^{-3}–10^{-4} M range) (Bhattacharyya and Brewer, 1989; Garcia-Pino et al., 2007). The occurrence of these extended binding sites supports the idea that complex carbohydrate structures such as *N*-glycans are the natural target sites of most plant lectins (Van Damme et al., 2008).

3. Temporal and developmental control of lectin expression

Many plant lectins are abundantly present in seeds or various vegetative storage tissues such as tubers, bulbs, rhizomes or bark (Van Damme et al., 1998a). For example, different isoforms of the *Phaseolus vulgaris* agglutinin (PHA) are produced during seed development of the common bean where they can accumulate up to 10% of the total seed protein (Van Damme et al., 1998a). Other well known lectins are the *Galanthus nivalis* agglutinin (GNA) present in the bulbs of the snowdrop or the *Urtica dioica* agglutinin (UDA) which is found in the rhizomes of the stinging nettle. In general, these plant lectins accumulate in certain plant organs or tissues and their synthesis is independent of external environmental conditions. Most of these lectins are synthesized by the ER associated ribosomes and consequently accumulate in the vacuolar compartment of the plant cell or the extra/intercellular spaces.

In contrast to lectins with a developmentally regulated expression pattern, several plant lectins have been described in the last decades that are upregulated as a result of certain biotic or abiotic stress conditions such as insect herbivory, wounding, drought, cold or high salt concentrations. This group of lectins is referred to as 'inducible plant lectins' and was found to be expressed mainly in non-storage tissues such as leaves, roots or flowers. These inducible plant lectins are further characterized by their cellular localization and their relatively low expression levels. In contrast to the vacuolar/extracellular localization, inducible lectins are usually synthesized in the cytoplasm and reside in the cytoplasmic/nuclear compartment of the cell (Van Damme et al., 2008). The first inducible lectin reported was the mannose-specific jacalin-related lectin from salt-treated rice seedlings (*Oryza sativa*) called ORYSATA (Zhang et al., 2000). Under normal physiological conditions this lectin cannot be found in rice plants, but after exposure to salt or drought stress, ORYSATA is rapidly produced in roots and sheaths (Claes et al., 1990). Another lectin called NICTABA was shown to be expressed in the leaves of tobacco (*N. tabacum*) only after treatment with the plant hormone methyl jasmonate and after herbivory by different insect species (Chen et al., 2002; Vandenborre et al., 2009a,c).

4. Lectins as defense proteins in plants

Many plant lectins show a carbohydrate specificity for glycoconjugates present in organisms outside the plant kingdom while these glycoconjugates (e.g., galactose, sialic acid) are low abundant or absent from plants. Moreover, different carbohydrate structures present in organisms such as viruses, micro-organisms, fungi, nematodes or phytophagous insects were shown to interact with plant lectins (Hopkins and Harper, 2001; Ripoll et al., 2003; Wong et al., 2010). Often lectins are present in large quantities in storage organs and seeds that are particularly vulnerable to pathogens or pest insects (Peumans and Van Damme, 1995). In addition, most plant lectins are synthesized as inactive precursors that become active only after sequestration in specialized organelles. Because of these observations, it was proposed that plant lectins play a role in the general defense against a multitude of plant attackers (such as phytopathogenic micro-organisms, nematodes or pest insects) and are also used as storage proteins for the growth and development of the plant.

During the last two decades, important progress was made in the study of the activity of plant lectins against pathogens, nematodes and especially pest insects (Ma et al., 2010; Peumans and Van Damme, 1995; Vasconcelos and Oliveira, 2004). Some chitin-binding plant lectins were shown to affect growth and development of fungi. However, this antifungal activity is relatively weak in most cases compared to other antifungal/antimicrobial plant proteins (e.g., defensins, thionins) (Selitrennikoff, 2001; Wong et al., 2010). In contrast, many plant lectins are highly toxic for phytophagous insects. Numerous reports have already shown that ectopical expression of plant lectins in genetically engineered (crop) plants and/or incorporation of the purified lectins in artificial diets negatively affect the performance of many pest insects belonging to different orders such as Lepidoptera, Coleoptera, Diptera and Hemiptera (see Section 6). It can be envisaged that after ingestion of plant seeds or tissues by phytophagous insects, the plant lectins are released from the disrupted cellular structures and come into contact with carbohydrate structures present in the midgut of insects.

Next to their prominent role in defense against herbivores and pathogens, plant lectins are known to participate in various other (endogenous) biological processes. An abundantly present lectin in the latex of the rubber tree (*Hevea brasiliensis*) called 'hevein' is thought to play a role in the coagulation of latex by bringing together rubber particles (Girdol et al., 1994). Other lectins such as calreticulin and calnexin are important for proper folding of newly synthesized glycoproteins inside the ER or can be involved in transport of folded glycoproteins to the Golgi (ERGIC-53, VIP36, VIPL) (Banerjee et al., 2007; Carameldo and Parodi, 2008). As mentioned before, in the last decade several lectins were also shown to be expressed at very low concentrations in the cytosol and/or nucleus after biotic and abiotic stresses. This has led to the hypothesis that these lectins may also act as regulators for various intracellular signaling processes involved in plant stress physiology (Lannoo and Van Damme, 2010). For some extracellular root lectins it has been suggested that they play an important role in symbiotic interactions between host plants and symbiotic micro-organisms such as nitrogen-fixing rhizobia and mycorrhizal fungi (De Hoff et al., 2009).

In summary, in the last few years it has become clear that different lectin domains have evolved to be functional in diverse physiological responses. Since this review focuses on the interaction between plant lectins and insects, lectin expression as part of the defense response and the activity of plant lectins towards phytophagous insects will be discussed in detail in the following sections. The effect(s) of lectins towards beneficial insects and mammals will also be reported.

5. Induction of lectin expression by insect feeding

Since plants have to deal with limited nutrient availability under normal environmental conditions, they cannot constantly invest their energy into the synthesis of defense compounds (Zavala and Baldwin, 2004). Consequently, inducible defense mechanisms have evolved that become activated upon insect attack (Chen, 2008). Because pest insects possess a diverse range of feeding behaviors and styles such as chewing, cell-content feeding or phloem feeding, different insects will be recognized differentially by a plant resulting in the synthesis of multiple signaling hormones. Especially jasmonic acid (JA) and ethylene are associated with the induced defense response against chewing and cell-content feeding insects, while salicylic acid (SA) was shown to be predominant for regulating defense responses towards phloem feeding insects (De Vos et al., 2005). The interplay between these plant hormones eventually results in the appropriate defense response that is active against the insect attacking at a given moment (Koornneef and Pieterse, 2008).

Different plant lectins have been described with a gene expression that is regulated by phytohormones important for the induced defense response. For instance, a lectin called NICTABA was discovered in tobacco (*N. tabacum*) leaves and was expressed only after hormonal treatment with JA or JA-related metabolites (Chen et al., 2002; Vandenborre et al., 2009a). Because insect feeding and more specifically chewing or biting insects such as caterpillars and beetles are known to increase JA levels in leaf tissues during feeding, NICTABA expression was analyzed after herbivory by larvae of the cotton leafworm (*Spodoptera littoralis*) and the tobacco hornworm (*Manduca sexta*). A strong increase in NICTABA expression was demonstrated in reaction to caterpillar feeding while mechanical wounding could not trigger lectin expression (Fig. 1). Furthermore, local insect herbivory also resulted in the systemic expression of NICTABA (Vandenborre et al., 2009a). Infestation of tobacco leaves with the two-spotted spider mites (*Tetranychus urticae*) could also trigger NICTABA expression, while phloem-feeding insects such as the greenhouse whitefly (*Trialeurodes vaporariorum*) and the tobacco aphid (*Myzus nicotianae*) were not able to affect the expression of NICTABA (Fig. 1) (Vandenborre et al., 2009c). This can be explained by the observation that phloem feeders try to avoid damage to plant tissues as much as possible, while mites lacerate plant cells causing more extensive tissue damage resulting in a defense response more similar to that of chewing herbivores (Walling, 2000, 2008). In addition, most phloem-feeding insects seem to induce defense signaling pathways largely resembling SA-mediated gene induction, whereas SA did not positively regulate NICTABA expression (De Vos et al., 2005; Moran and Thompson, 2001; Vandenborre et al., 2009c).

Previous studies in wheat (*Triticum aestivum*) plants have shown that resistant plants rapidly induce the expression of several lectin or lectin-like genes in sheaths of developing leaves in response to insect feeding (Giovanini et al., 2007; Puthoff et al., 2005; Subramanyam et al., 2006). Feeding by larvae of the Hessian fly (*Mayetiola destructor* Say) resulted in the expression of a mannose-binding jacalin-like lectin called Hessian fly responsive protein 1 (HFR1), and two chimerolectin-like proteins called HFR2 and HFR3 containing an *Amaranthus* domain and a chitin-binding hevein domain, respectively (Giovanini et al., 2007; Subramanyam et al., 2006). Subramanyam et al. (2008) have shown that the accumulation of the HFR1 protein in resistant wheat plants started within the first day after egg hatching of *M. destructor* larvae and corresponded to the critical period in which the first instars were attempting to establish feeding sites by injecting substances into the plant. Although no direct toxicity of HFR lectins has been shown due to the obligate nature of the Hessian fly to feed on wheat, the insecticidal activity of HFR1 was clearly demonstrated when fed to larvae of the fruit fly *Drosophila melanogaster* (Subramanyam et al., 2008). Wheat plants also responded with high expression levels of HFR2 and HFR3 after hatching of *M. destructor* larvae. When comparing the HFR expression after feeding by chewing or phloem-feeding insects, a different response was observed. Chewing larvae of the fall armyworm (*S. frugiperda*) induced HFR2, but not HFR3 expression (Giovanini et al., 2007; Puthoff et al., 2005). In contrast, the phloem-feeding bird cherry-oat aphids (*Rhopalosiphum padi*) induced HFR3 starting from 24 h after infestation, while HFR2 expression initiated only after 12 days (Giovanini et al., 2007; Puthoff et al., 2005). All together, these data suggest the involvement of a set of inducible lectins in the defense of resistant wheat against pest insects and in particular against Hessian fly infestation.

In addition, several other jasmonate-inducible lectins were found to be expressed in leaf tissues of important crop plants. Especially in the leaves of different monocots such as rice (*O. sativa*), barley (*Hordeum vulgare*), wheat (*Triticum vulgare*), rye (*Secale cereale*) and maize (*Zea mays*), mannose-binding jacalin-related lectins were shown to accumulate after treatment with methyl jasmonate (Jiang et al., 2006; Van Damme et al., 2004). For the latter jasmonate-inducible lectins, it remains to be shown if they are somehow involved in plant defense.

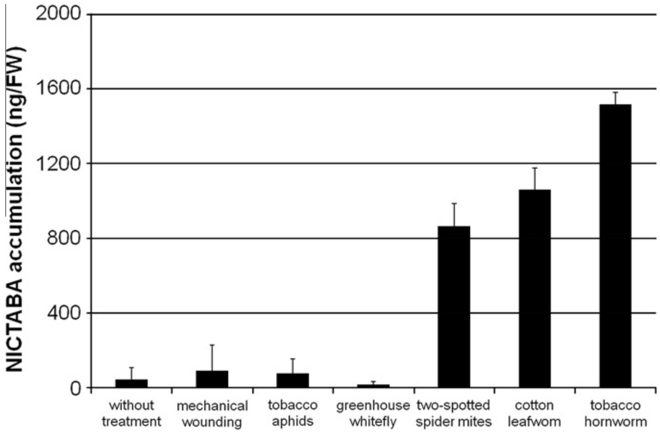

Fig. 1. Quantification of NICTABA accumulation in tobacco leaves after feeding by different herbivores. NICTABA levels were quantified 24 days post-infestation with tobacco aphids (*Myzus nicotianae*) and greenhouse whiteflies (*Trialeurodes vaporariorum*), whereas the lectin concentration in leaves subjected to herbivory by the two-spotted spider mites (*Tetranychus urticae*), the cotton leafworm (*Spodoptera littoralis*) and the tobacco hornworm (*Manduca sexta*) was quantified after 3 days. Mechanical wounding was performed by repeated puncturing of the leaves (redrafted from Vandenborre et al., 2009c).

6. The use of plant lectins for pest control in plant biotechnology

Genetically engineered crop plants with enhanced insect resistance have revolutionized agriculture and have lead to a reduction in pesticide usage and lower production costs worldwide (Christou et al., 2006). Nowadays, the commercial transgenic plants expressing insecticidal toxins mostly depend on the ectopical expression of toxins derived from the bacterium *Bacillus thuringiensis* (Bt). However, there are as yet no Bt toxins which could be used to control sucking pests such as mirids, thrips, bugs, hoppers or aphids (Malone et al., 2008). Therefore, the search for alternatives to Bt toxins that can be used for engineering insect resistance has received a lot of attention. One of the most promising groups of candidate proteins exerting insecticidal properties are plant lectins. In the last two decades it has been shown for many plant lectins that they have entomotoxic properties (Table 2).

6.1. Insecticidal properties of plant lectins

In the last two decades, many plant lectins were shown to be toxic to various pest insects belonging to economically important insect orders such as Lepidoptera, Coleoptera, Diptera or Hemiptera. Experiments were set up for testing the insecticidal properties of plant lectins by feeding insects on artificial diets containing the purified lectin. For example, 25 lectins from 15 different plant families were screened for their effects on the development of the legume pod borer (*Maruca vitrata*) (Machuka et al., 1999). Although a total of 16 lectins had negative effects on larval survival, weight, feeding ability, pupation, adult emergence and/or fecundity, only GNA and the *Listera ovata* agglutinin (LOA) were effective against the pod borer larvae for all six parameters examined. To analyze the entomotoxic properties of lectins under more natural conditions, many transgenic (crop) plants that ectopically express a plant lectin have been generated. Depending on the insect species, various detrimental effects have been demonstrated on their performance ranging from a severe delay in development to high mortality rates.

6.1.1. GNA-related lectins

The strong entomotoxic properties of many lectins purified from bulbs of Amaryllidaceae species such as snowdrop (*Galanthus*) or daffodils (*Narcissus*) suggest a major role for these lectins in the protection of bulbs against phytophagous insects. GNA purified from snowdrop bulbs is probably the best studied plant lectin for its insecticidal properties. The snowdrop lectin is a plant lectin that specifically binds to terminal mannose residues from high-mannose *N*-glycans which were shown to occur very frequently on insect glycoproteins (Schachter, 2009). Although its toxicity has been shown for pest insects belonging to a wide range of insect orders, especially Hemiptera were shown to be very sensitive to GNA. Further support for the defensive role of GNA-related lectins against sucking insects is the observation that some lectins also accumulate in the phloem sap in natural situations. For instance, in phloem exudates of flowering stalks from leek plants (*Allium porrum*) the lectin called APA is the most abundant protein (Peumans et al., 1997).

GNA has been successfully engineered into a variety of crops including sugarcane, rice, wheat, potatoes or tobacco to give them a higher resistance against many pest insects. Transgenic wheat plants expressing GNA, either constitutively or under control of a phloem-specific promotor, were shown to exert severe entomotoxic effects on the development and survival of the grain aphid (*Sitobion avenae*) (Stoger et al., 1999). Field trials using transgenic maize plants with a phloem-specific GNA expression demonstrated a clearly enhanced resistance against corn leaf aphids (*Rhopalosiphum maidis*) (Wang et al., 2005). Also transgenic rice ectopically expressing the garlic leaf lectin ASAL (*Allium sativum* agglutinin) caused a significant increase in nymphal mortality of Hemipteran pest insects such as the brown leafhopper (*Nilaparvata lugens*), the green leafhopper (*Nephotettix virescens*) and the whitebacked planthopper (*Sogatella furcifera*) (Yarasi et al., 2008). In another

Table 2
Lectin families with proven insecticidal activity.

Lectin family	Examples	Pest insect	References
GNA-related lectins	GNA, APA, LOA, ASAL, ACA	*Maruca vitrata*	Machuka et al. (1999)
		Sitobion avenae	Stoger et al., 1999)
		Rhopalosiphum maidis	Wang et al. (2005)
		Nilaparvata lugens	Yarasi et al. (2008)
		Nephotettix virescens	Saha et al. (2006)
		Sogatella furcifera	Yarasi et al. (2008)
		Lipaphis erysimi	Hossain et al. (2006)
		Eoreuma loftini	Sétamou et al. (2002)
		Lacanobia oleracea	Gatehouse et al. (1997)
		Spodoptera littoralis	Sadeghi et al. (2008a, 2009)
Legume lectins	PSA, Gleheda, ConA	*Meligethes aeneus*	Melander et al. (2003)
		Leptinotarsa decemlineata	Wang et al. (2003)
		Callosobruchus maculatus	Zhu et al. (1996)
		Acyrthosiphon pisum	Sauvion et al. (2004a)
		Tarophagous proserpina	Sauvion et al. (2004a)
Hevein-related lectins	WGA, OSA, UDA	*Callosobruchus maculatus*	Murdock et al. (1990)
		Diabrotica undecimpunctata	Czapla and Lang (1990)
		Ostrinia nubilalia	Hopkins and Harper (2001)
Nictaba-related lectins	NICTABA, PP2	*Spodoptera littoralis*	Vandenborre et al. (2010a)
		Manduca sexta	Vandenborre et al. (2010a)
		Acyrthosiphon pisum	Beneteau et al. (2010)
		Myzus persicae	Beneteau et al. (2010)
Ricin-related lectins	SNA-I, SNA-I', cinnamomin, Maize RIP	*Spodoptera exigua*	Shahidi-Noghabi et al. (2009)
		Helicoverpa armigera	Zhou et al. (1999)
		Helicoverpa zea	Dowd et al. (2003)
		Lasioderma serricorne	Dowd et al. (2003)
		Myzus nicotianae	Shahidi-Noghabi et al. (2009)
		Culex pipiens pallens	Zhou et al. (1999)
Amaranthins	ACA	*Aphis gossypii*	Wu et al. (2006)
Jacalins	Heltuba, HFR-1	*Myzus persicae*	Chang et al. (2003)
		Drosophila melanogaster	Subramanyam et al. (2008)

study, the insecticidal activity of GNA, ASAL and the onion lectin ACA (*Allium cepa* agglutinin) were compared for their activity on mustard aphid nymphs (*Lipaphis erysimi*), and ACA was found to be the most toxic (Hossain et al., 2006). Interestingly, transgenic rice expressing ASAL exhibited less or no incidence of tungro disease after infestation with the green leafhopper (*N. virescens*) (Saha et al., 2006). This may be of importance because sap-sucking insects not only deprive the plant of nutrients, but they also cause a lot of damage by transmitting many plant viruses.

Next to the activity of GNA-related lectins against Hemiptera, these lectins were also reported to have entomotoxic activity on the larval growth and development of several Lepidoptera such as the Mexican rice borer (*Eoreuma loftini*), the tomato moth (*Lacanobia oleracea*) or the cotton leafworm (*S. littoralis*) when genetically engineered into crops (Gatehouse et al., 1997; Sadeghi et al., 2008a, 2009; Sétamou et al., 2002).

6.1.2. Legume lectins

Legume lectins have been purified especially from seeds and several legume lectins were shown to bind to carbohydrate structures that are not present in plants such as the Thomsen-nouveau antigen (Tn) antigen or complex *N*-glycan structures with terminal galactose and sialic acid residues.

Melander et al. (2003) evaluated the effect of the pea lectin PSA (*Pisum sativum* agglutinin) on the growth and survival of the pollen beetle (*Meligethes aeneus*). An *in planta* feeding experiment was performed using transgenic oilseed rape (*Brassica napus*) with a tissue-specific expression of PSA in the anthers and pollen. Feeding of the pollen beetle on the modified anthers resulted in a lower mass gain for the larvae (Melander et al., 2003), but had no effect on the adult beetles (Lehrman et al., 2007). Insect feeding trials using the legume lectin called Gleheda purified from ground ivy (*Glechoma hederacea*) demonstrated that Gleheda is a potent insecticidal protein for larvae of the Colorado potato beetle (*Leptinotarsa decemlineata*) (Wang et al., 2003). Moreover, none of the larvae fed with potato leaves dipped in a Gleheda solution reached the pupal instar, indicating that the lectin caused complete mortality. In addition, the cowpea weevil (*Callosobruchus maculates*) was found to be very sensitive to the seed lectin GS-II from *Griffonia simplicifolia* (Zhu et al., 1996).

Next to a clear toxicity of several legume lectins for Coleoptera, the mannose-binding legume lectin from jackbean concanavalin A (ConA) was shown to be toxic to the Hemipteran pea aphid (*Acyrthosiphon pisum*) (Sauvion et al., 2004a,b). When the effect of ConA and PSA was studied towards the tara planthopper (*Tarophagous proserpina*), a high mortality rate was observed after ingestion of ConA, while PSA did not exert any significant effect towards the survival of the aphids (Powell, 2001). This clearly illustrated the selectivity of a plant lectin in insecticidal activity against a given pest insect, even when both lectins have a similar sugar binding specificity.

6.1.3. Hevein-related lectins

Hevein-related plant lectins have also been studied intensively for their entomotoxic properties. Most of these lectins exhibit specificity for chitin, a polymer known to be synthesized specifically by arthropods, nematodes and fungi (Merzendorfer, 2006). As such, hevein-related lectins are assumed to have no target structures in mammal organisms and are considered as safe tools for the use in genetically modified crop plants.

Feeding trials with artificial diets clearly showed that wheat germ agglutinin (WGA) and UDA had a negative effect on the development of the cowpea weevil (*Callosobruchus maculatus*) larvae when added to artificial seeds (Huesing et al., 1991; Murdock et al., 1990). Also the larval growth of the southern corn rootworm (*Diabrotica undecimpunctata*) was clearly inhibited after ingesting WGA (Czapla and Lang, 1990). Next to pest insects belonging to the order of the Coleoptera, WGA was shown to be very active against Lepidopteran insect larvae such as the neonate larvae from the European corn borer (*Ostrinia nubilalis*) (Czapla and Lang, 1990; Hopkins and Harper, 2001). In contrast, only few entomotoxic effects were reported for hevein-related proteins towards Hemipteran insects. This low toxicity may be attributed to a differential ultrastructural organization of the midgut between the different insect orders. Hemipteran insects lack a functional peritrophic matrix (PM) in their midgut in contrast to insect species belonging to the order of Lepidoptera or Coleoptera (see Section 7).

6.1.4. Other interesting lectins

Next to the lectin families discussed above that have been studied intensively in the last two decades, several plant lectins belonging to other lectin families were also shown to have entomotoxic properties. One example is the tobacco leaf lectin NICTABA that was found to be expressed after folivory by pest insects. Feeding trials using transgenic tobacco plants with ectopical NICTABA expression clearly demonstrated that NICTABA is detrimental for larvae of two Lepidopteran larvae, the cotton leafworm (*S. littoralis*) and the tobacco hornworm (*M. sexta*) (Vandenborre et al., 2010a). As illustrated in Fig. 2, *S. littoralis* larvae clearly showed a reduction in growth and development after feeding on tobacco leaves with a strong NICTABA expression. Recently, another protein belonging to the Nictaba family called phloem protein 2 (PP2) was found to exert entomotoxic activity (Beneteau et al., 2010; Dinant et al., 2003). Negative effects were reported on the nymphal weight gain of pea aphids (*A. pisum*) and green peach aphids (*Myzus persicae*) when the recombinant PP2 lectin from *Arabidopsis* was tested in an *in vitro* diet assay (Beneteau et al., 2010).

Within the group of lectins with a ricin domain (previously referred to as type-2 ribosome inactivating proteins or RIPs) a lectin called cinnamomin present in seeds of the camphor tree (*Cinnamomum camphora*) was found to be toxic for the bollworm (*Helicoverpa armigera*) and the common mosquito (*Culex pipiens pallens*) (Zhou et al., 1999). Insecticidal activity was also shown for a RIP found in maize (*Z. mays*) towards the larvae of the cigarette beetle (*Lasioderma serricorne*) and the corn earworm (*H. zea*) when ectopically expressed in tobacco plants (Dowd et al., 2003). Recently, Shahidi-Noghabi et al. (2009) revealed clear entomotoxic effects of the type-2 RIP SNA-I' from elderberry bark (*Sambucus nigra*) towards larvae of the beet armyworm (*Spodoptera exigua*) and the tobacco aphids (*M. nicotianae*).

The amaranthins and the jacalin-related lectins are two lectin families that were shown to have potential as insect control agents, especially against sap-sucking pest insects. When the *Amaranthus caudatus* agglutinin (ACA) was ectopically expressed in cotton under the control of a phloem-specific promoter, a strongly enhanced resistance was observed against nymphs of the cotton aphid (*Aphis gossypii*) (Wu et al., 2006). Transgenic tobacco plants expressing Heltuba, a lectin from the Jerusalem artichoke (*Helianthus tuberosus*) belonging to the family of jacalin-related lectins, showed a decrease in development and fecundity of the peach-potato aphid (*M. persicae*) (Chang et al., 2003). Another promising jacalin-related lectin for use in transgenic crop plants is HFR1 which was shown to be induced in resistant wheat (*T. aestivum*) during infestation by the Hessian fly larvae. Although no toxicity has been demonstrated to the Hessian fly, a strong insecticidal activity was reported to larvae of the fruit fly (*D. melanogaster*) (Subramanyam et al., 2008).

6.2. Effects of plant lectins on beneficial insects

As discussed above, plant lectins have the potential to play an important role in the development of integrated pest management

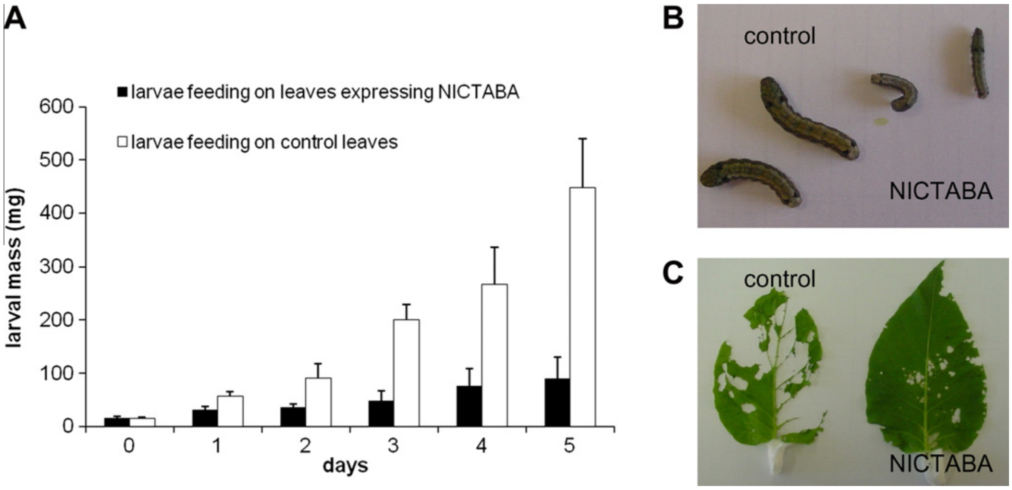

Fig. 2. Performance of the cotton leafworm (*Spodoptera littoralis*) larvae after feeding on NICTABA expressing leaves. (A) Increase in mean larval mass gain during a 5-day feeding period on tobacco leaves treated with methyl jasmonate compared to control leaves. (B) Picture of the corresponding larvae from (A) on day 4. (C) Comparison of leaf damage between tobacco leaves expressing NICTABA and control leaves.

strategies. However, by introducing genetically modified crops expressing insecticidal proteins like lectins into agriculture, unwanted side effects can occur for beneficial insects. Beneficial insects may be exposed to lectins produced in transgenic plants either directly by consuming parts of the plant (e.g., honeybees), or indirectly by consuming the target pest insect itself as a prey (e.g., predatory beetles or lacewings), or by parasitizing the insects that have been feeding from these plants by using them as a host (e.g., parasitic wasps). To address this concern several studies have been conducted to analyze the performance of these beneficial insects after being exposed to lectin expressing transgenic crops.

In order to analyze the effect of an insecticidal lectin in transgenic pollen on the development of honeybee larvae (*Apis mellifera*), pollen derived from two transgenic lines of oilseed rape (*B. napus*) were mixed into a larval diet and compared to a control pollen diet (Lehrman, 2007). The transgenic oilseed rape selectively produced PSA in its anthers and pollen. After feeding the larvae of *A. mellifera*, no negative effect could be detected on larval survival, mass gain or developmental time. In another study, it was tested whether bumblebee (*Bombus terrestris*) workers are able to detect GNA dissolved in a sucrose solution and whether consumption of this lectin affects survival and offspring production (Babendreier et al., 2007). No difference was found in the number of visits and the duration of the visits, indicating that bumblebees do not discriminate between GNA containing and control solutions in a choice experiment. However, when GNA was fed to bumblebees that were kept in microcolonies, these insects appear to be strongly affected by ingestion of GNA. Mortality of both workerbees and drones increased and offspring production was significantly reduced (Babendreier et al., 2007). The potential effect of GNA was also analyzed on the larvae of the solitary bee *Osmia bicornis* (Konrad et al., 2008). Low doses of GNA in the larval diet (0.01% of total protein) failed to affect overall development, while a high dose of GNA (0.1% of total protein) resulted in a prolonged development time and reduced efficiency in conversion of pollen food into larval body weight. However, it is unlikely that bees will be exposed to GNA doses that are ⩾0.1% of the total protein content under field conditions.

Predators or parasitoids are beneficial insects that play an important role in controlling certain pest populations on several crop plants. The direct effect of GNA on larvae of different aphid predators such as lady beetles (*Adalia bipunctata* and *Coccinella septempunctata*) and green lacewings (*Chrysoperla carnea*) was evaluated by Hogervorst et al. (2006). In this study, no difference in mass gain was detected after feeding on an artificial diet containing 1% GNA, but the longevity was negatively affected for all three predators (Hogervorst et al., 2006). In addition, no significant effect could be observed on the development of *A. bipunctata* when fed on aphids containing GNA delivered though an artificial diet (Down et al., 2003). In contrast, a study by Birch et al. (1999) reported reduced fecundity, egg viability and a reduction in female longevity of *A. bipunctata* preying on *M. persicae* which was reared on GNA-producing potatoes. These sublethal effects were probably caused by a combination of both direct (caused by GNA toxicity) and indirect (caused by GNA effect on the aphids) effects. Recently Li and Romeis (2009) also reported the impact of GNA on adults of green lacewing. They showed that although adults that had emerged from GNA-fed larvae did not differ from the controls, the fertility of these adults was significantly reduced, in that the proportion of hatching eggs was much lower.

Parasitoids comprise endophagous insects that lay their eggs into the body of the insect host, which is then used as a food source for the developing larvae. The performance of *Eulophus pennicornis*, a parasitic wasp for Lepidoptera, was studied under simulated field conditions with larvae of the tomato moth (*L. oleracea*) after feeding on GNA expressing tomato/potato plants (Bell et al., 1999, 2001). The ability of the wasp to parasitize and subsequently develop on the pest larvae of *L. oleracea* was not altered by the presence of GNA in the diet of the host (Bell et al., 2001). *E. pennicornis* progeny that developed in *L. oleracea* reared on GNA expressing plants showed no significant alteration in fecundity when compared to wasps that had developed on hosts fed on control plants. In contrast, sublethal effects were observed on larval development of the wasp *Aphelinus abdominalis* after parasitizing on aphids that were fed on transgenic plants expressing GNA or GNA-containing artificial diets (Couty et al., 2001). Most probably this observation was an indirect host-size-mediated effect on the sex ratio and the size of parasitoids developing in the smaller host aphids that were fed on a GNA-containing diet (Couty et al., 2001). Moreover, it was found that GNA is excreted in high amounts by the parasitoid after its uptake. The adult stage of *A. abdominalis* is also a predator of aphids, but it was shown that the adult females were not affected by feeding on GNA-dosed aphids (Couty and Poppy, 2001).

Many beneficial insects use honeydew secreted by phloem-feeding insects as their main carbohydrate source. Honeydew produced by insects feeding on transgenic crop plants expressing plant lectins may be an alternative route for beneficial insects to come into contact with lectins. To study this concern, the toxicity of GNA was examined on different parasitic wasps (*Aphidius colemani*, *Trichogramma brassicae* and *Cotesia glomerata*) known to feed on honeydew (Romeis et al., 2003). In all three species, feeding on a sucrose solution supplemented with GNA resulted in significantly reduced survival rates. However, it remains questionable whether the GNA concentrations (0.1% and 1%) used in this study are representative for the GNA present in honeydew of aphids fed on transgenic plants in the field. Moreover, GNA could not be detected in the honeydew of the whitebacked planthopper (*Sogatella furcifera*) after feeding on transgenic rice expressing GNA at levels of 0.3% of total soluble protein, although the plant lectin exerted severe insecticidal effects on the planthoppers (Nagadhara et al., 2004).

6.3. Variability in insecticidal properties of plant lectins

As shown by the examples given above, many plant lectins have insecticidal properties or can reduce the insect performance. Although some lectins possess insecticidal activity for a particular pest insect, several lectins have a broad insecticidal activity targeting insects belonging to phylogenetically distant insect orders, possibly also including beneficial insects. This observed variability in lectin effect(s) on different insect species may be due to different reasons.

To fulfill an insecticidal activity after ingestion, plant lectins need some degree of resistance to the hostile environment of the insect digestive tract. Dependent on the insect species, midgut conditions can be very acidic or strongly alkaline (Chen et al., 2007; Hakim et al., 2010). Biochemical analysis indicated that most lectins discussed above are remarkably stable in a wide pH range (pH 2 to pH 12) (Van Damme et al., 1998b), while others such as NICTABA are only stable under alkaline conditions (Chen et al., 2002). This implies that NICTABA will most probably be active against insects with alkaline conditions in their midgut such as Lepidopterans (Chen et al., 2007; Vandenborre et al., 2010a). Another factor that may create the observed variability in toxicity of plant lectins is their resistance against proteolysis (Zhu-Salzman et al., 1998). Each insect species has its own set of enzymes specialized in proteolytic degradation, such as serine, cysteine or metalloproteases (Felton, 2005). Dependent on the resistance of a given plant lectin to the proteases present in the insect midgut, it can exert an insecticidal activity or it will be cleaved and lose its activity.

Taking into account that each lectin has its own characteristic carbohydrate binding specificity, different lectins will interact with different target structures in the insect body. Mannose-specific lectins such as the GNA-related lectins will target especially high-mannose or oligo-mannose *N*-glycans present on many insect glycoproteins, while chitin-specific lectins such as WGA will bind to carbohydrate structures such as the chitin-microfibrils in the peritrophic membrane (PM) (see Section 7). Depending on the presence of these carbohydrate structures in a specific insect species, a particular lectin can be toxic or not. This was clearly illustrated by the observation that WGA was not toxic to the pea aphid which lacks a functional PM, while both GNA and ConA showed clear insecticidal properties (Rahbé et al., 1995).

Another source of variability in insect toxicity between different plant lectins is the fact that glycosylation profiles in insect tissues strongly depend on the developmental stage (Aoki et al., 2007). As a consequence plant lectins can be toxic for the larval stage, while they have a less profound effect on the adult insect. This may explain the observed toxicity of PSA for the larvae of the pollen beetle, while adult beetles were not influenced after ingesting PSA (Lehrman et al., 2007; Melander et al., 2003).

7. Toxicity of plant lectins towards mammals

Many plant lectins are present in a wide range of vegetables/crops (e.g. tomato, potato, pea, bean, garlic, leek, lentil, soybean, peanut, rice, corn, wheat) and fruits (e.g. banana, mullberry, breadfruit), and are consumed by humans and animals on a regular base. Since many of these plants are eaten raw, these plant lectins are considered to be non-toxic for humans and mammals in general. However, some legume lectins e.g. ConA and PHA are known to be toxic for mammals (Vasconcelos and Oliveira, 2004). For example, PHA was shown to be toxic for humans especially when kidney beans were not sufficiently cooked before consumption. The acute symptoms of PHA poisoning are nausea, vomiting or diarrhea and are most likely due to the ability of PHA to bind to the epithelial cells from the digestive tract which can cause changes in cellular morphology and metabolism. It should be noted that several lectins will survive digestion by gastrointestinal enzymes. Consequently, the interaction of these plant lectins with glycoproteins in the digestive tract was reported to result in both local and systemic reactions (Pusztai et al., 1993; Vasconcelos and Oliveira, 2004). Although toxicity was clearly shown for the broad bean (*P. vulgaris*) lectin considerable variation in lectin activity was observed for different beans (De Mejía et al., 2005). Interestingly, the bioactivity of some plant lectins against mammalian tissues and cells could also be exploited for other applications, e.g. the use of plant lectins as potential anticancer drugs (De Mejía and Prisecaru, 2005).

Other well known examples of plant lectins with a severe toxicity towards mammals are ricin and abrin present in castor beans (*R. communis*) and the seeds of *Abrus precatorius* (jequirity bean), respectively (Dickers et al., 2003). However, it should be mentioned that not all ricin-B lectins are equally toxic as ricin and abrin. It has been clearly shown that ricin-B lectins from elderberry (*Sambucus* sp.) can be considered as virtually non-toxic compared to ricin (Battelli et al., 1997; Girbes et al., 2003).

Lectins related to the snowdrop lectin GNA have been studied in detail for their activity on insects. One of the major reasons for this large interest in GNA-related lectins is that several of these lectins are found in edible plants (e.g. leek, garlic), which will reduce the problems related to consumer acceptability whenever these lectins would be used in crop plants. A report by Fenton et al. (1999) reported the binding of the snowdrop lectin to human white cells. However, these data are contradicted by other studies reporting very low if any mitogenic and immunogenic activity of GNA (Kilpatrick et al., 1990; Huskens et al., 2008; François and Balzarini, 2011). Since the proliferative response of the GNA-related lectin from daffodil was shown to be age-related with weak mitogenicity observed for adult human lymphocytes but more than sevenfold increased effects on lymphocytes from umbilical cord blood, it is important to check different age groups when testing the response of lectins on cells (Summers et al., 2002).

Obviously, health safety assessment for each lectin is necessary before plant lectins could be introduced into crop plants for commercial purposes. In a 90-day feeding study with rats designed to assess the safety of genetically modified rice expressing the kidney bean lectin PHA-E, clear abnormalities were observed in rats after PHA-E ingestion (Poulsen et al., 2007b). In contrast, a similar 90-day feeding study using transgenic rice expressing GNA revealed no adverse effects on rats after continuous dietary GNA uptake (Poulsen et al., 2007a). This clearly illustrates the case specificity of plant lectins concerning safety assessment for genetically modified crops. It will be very important to perform these long-term oral toxicity studies to predict the potentially harmful effects of a plant lectin after dietary uptake by animals or humans when introduced in genetically modified crop plants.

8. Mode of action of plant lectins

8.1. Target structures or tissues for plant lectins in the insect body

The luminal side of the midgut epithelium of many insects is lined up with a physical barrier called the PM that is secreted by certain epithelium cells (Hegedus et al., 2009). This PM consists of a chitineous grid-like network held together by chitin-binding glycoproteins such as peritrophins. The PM-associated glycoproteins contain many glycan structures that fill the interstitial spaces creating a molecular sieve (Hegedus et al., 2009). Since both the chitin fibrils and many glycoproteins are present in the PM, this midgut structure is an obvious target for lectins. Indeed, studies that analyzed the effect of plant lectins on the ultrastructural organization of the insect midgut have shown clear abnormalities in the formation of a functional PM and disruption of microvilli structures. This lectin effect is clearly observed in a transmission electron graph of a dissected PM from the midgut of cotton leafworm larvae fed on a Gleheda-containing diet (Fig. 3). Similarly, analysis of the midgut structures in the European corn borer (*O. nubilalis*) after feeding on a WGA-containing diet showed hypersecretion of many disorganized PM layers into the midgut lumen and the presence of many disintegrated microvilli (Harper et al., 1998; Hopkins and Harper, 2001). Clear morphological changes of midgut microvilli were also observed after uptake of WGA in the larval midgut of *Drosophila* (Li et al., 2009).

Next to preventing the synthesis of a functional PM or disruption of microvilli structures, it was illustrated for some plant lectins that they can enter the insect body after ingestion by transcytosis across the midgut epithelium. When larvae of the tomato moth (*L. oleracea*) were fed on GNA- or ConA-containing diets both lectins were found in the digestive tract, but the uptake of the lectins inside the insect body was also demonstrated. GNA and ConA were shown to accumulate in the hemolymph and the Malpighian tubules of *L. oleracea*, while ConA was also observed in the fat tissue (Fitches et al., 2001). Similarly, GNA was found in the hemolymph, fat tissue and ovarioles of the rice brown planthopper (*N. lugens*) after ingestion of the lectin (Powell et al., 1998). These analyses illustrate that additional target sites for plant lectins inside the insect body are likely (e.g., fat tissue, hemocytes, Malpighian tubules, ovaries).

8.2. Insect glycoproteins as targets for lectins

Binding studies using glycan arrays have shown that many plant lectins have a strong affinity for glycans that are frequently present on insect proteins. Because most of the digestive enzymes or transport proteins secreted in the midgut of insects or proteins embedded in the epithelial cell membrane contain such glycan structures, these glycoproteins are all potential targets for plant lectins. Indeed, one of the major target proteins for GNA was shown to be a subunit of ferritin, an important protein for iron transport (Du et al., 2000; Sadeghi et al., 2008b). Another enzyme that was targeted in the midgut of the pea aphid (*A. pisum*) by both GNA and ConA was identified as a membrane-bound aminopeptidase (Cristofoletti et al., 2006). Several plant lectins were also shown to bind to α-amylases present in the digestive tract (Macedo et al., 2007; Sadeghi et al., 2008b). Moreover, a recent report that analyzed the presence of all potential target proteins for GNA in *Drosophila* clearly illustrated that plant lectins probably act on insects through the interaction with multiple target glycoproteins (Vandenborre et al., 2010b). This was also shown for pest insects such as beetles, aphids and caterpillars, and for non-target insects such as honeybees (Vandenborre et al., 2011).

Secreted glycoproteins that are targeted by plant lectins in the midgut will tend to cluster, resulting in large complexes with a molecular size that is too big to pass through the PM. These macromolecular insect protein-lectin complexes may prevent larval enzymes to diffuse back across the PM for being recycled in the digestive system and will result in leakage of digestive enzymes. Accordingly, lectin interaction with digestive enzymes and/or transport proteins in the midgut lumen may also be part of an antinutritive mechanism.

8.3. Effect of plant lectins on gene expression

To study the observed structural changes of the insect midgut microvilli after uptake of dietary plant lectins in more detail, the differential expression of genes was analyzed in epithelium cells of *D. melanogaster* larvae after feeding on a WGA-containing diet (Li et al., 2009). Microarray analysis revealed that 61 transcripts were differentially expressed in midgut cells after feeding of *Drosophila* larvae on a WGA-rich diet (Li et al., 2009). The genes

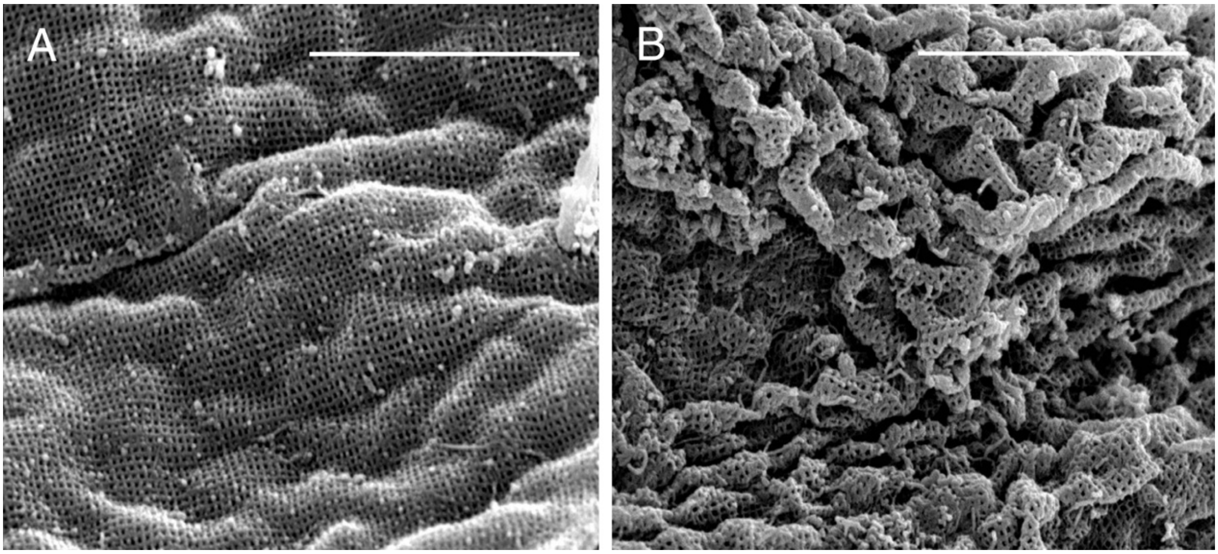

Fig. 3. Transmission electron micrograph of the peritrophic matrix (PM) of the last larval instar of the cotton leafworm *Spodoptera littoralis* (Lepidoptera). (A) Normal PM of *S. littoralis* larvae after feeding on a control diet. (B) PM of *S. littoralis* larvae with clear aberrations after feeding on a Gleheda containing diet. Scale bars represent 6 μm. (unpublished data from E. Viñuela, F. Budia, P. Medina, E.J.M. Van Damme, G. Smagghe).

with altered expression levels in response to WGA uptake were associated with cytoskeleton organization, chitin metabolism, digestive enzymes, detoxification reactions and energy metabolism. Differential expression of genes involved in cytoskeleton organization is in agreement with the morphological changes in microvilli after WGA ingestion. The WGA-induced genes associated with chitin metabolism may be a response of the midgut cells to produce more chitin for the repair or replacement of the damaged PM. Also α-amylase and an endopeptidase were over-expressed as a response to ingestion of WGA. The expression of glutathione *S*-transferases which are typical stress proteins was also upregulated. More analyses of protein expression data in insect bodies as a response to the uptake of toxic plant lectins are needed and will provide clear insights into the molecular mechanisms involved during the complex interaction between plant lectins and phytophagous insects.

9. Perspectives

Until the mid 1990s, most known plant lectins were abundant proteins present in seeds or vegetative storage tissues belonging to only a few lectin families (e.g., GNA family, legume family, hevein family). For practical reasons, this strongly biased the search for plant lectins with insecticidal properties. In the last decade several novel lectin families were discovered. These lectin families are largely unexplored for their biochemical characteristics and may become a source of new insecticidal lectins. Moreover, some of these plant lectins such as NICTABA are synthesized in response to insect herbivory which indicates a major role for these carbohydrate binding proteins in the plant defense response. It is thought therefore that further purification and exploration of these uncharacterized lectins will result in new tools for genetic engineering of crop plants.

Although plant lectins are known for more than hundred years, even today many aspects concerning the physiological role *in planta* remain to be elucidated. However, it has been established now that many lectins can be considered as part of the naturally occurring defense responses against phytophagous insects. Many lectins were shown to have clear entomotoxic properties, but at this moment it remains very difficult to predict whether a specific plant lectin will be active against a given pest insect and/or non-target insect. This is mainly due to the fact that the actual targets for the lectins in insects are mostly unknown. Consequently, the presence and biochemical properties of the target structures inside the insects cannot be studied. Although our knowledge on the glycosylation patterns in insects is increasing, these glycans are difficult targets to study. Glycan structures are very diverse because of the branched structure, differences in glycosidic linkages, heterogeneity in glycan profile for a specific glycoprotein and multiple modifications that may occur on specific sugar monomers. Moreover, the glycan profile of a given protein may depend on the developmental stage and is tissue specific. Furthermore, the presence and structure of glycans are not written in the genomic code. To unravel this glycocode several new techniques have been developed recently. Analytical tools such as anti-carbohydrate antibodies, lectin microarrays, glycan arrays and advanced mass spectrometry techniques are currently being explored but are still in their validation phase (Gemeiner et al., 2009).

Once the targets for plant lectins will be identified, it will be very interesting to link these target structures to the observed physiological effects in pest insects following lectin ingestion. This requires integration of microscopical techniques with molecular analysis for the differential regulation at the transcriptional level in insect tissue. Such analyses will reveal new clues for understanding the observed insecticidal effects of lectins and are needed to provide a scientific basis for the development of new pest management strategies based on lectin research. As already indicated above, before introducing plant lectins in genetically modified crop plants it will be necessary to check the possible toxicity of each lectin in long term studies to determine the potentially harmful effects of a plant lectin after dietary uptake by animals or humans.

Acknowledgments

This research was supported by the Research Council of Ghent University (Project BOF10/GOA/003) and the Fund for Scientific Research-Flanders (Project 3G016306).

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