

# Apoptosis induction by concanavalin A in gut cells of grain aphid

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**Abstract** In this study, the toxicity and mechanism of action of concanavalin A (ConA) in the grain aphid (*Sitobion avenae*) were studied. Feeding assays with *S. avenae* on an artificial diet containing different concentrations of ConA demonstrated an inhibitory effect on fecundity as well as high mortality caused by this lectin. ConA also increased the pre-reproductive period and the development time and reduced the intrinsic rate of natural increase. Moreover, an extract of the gut of treated *S. avenae* demonstrated an increase in caspase-3 activity together with DNA fragmentation, suggesting that ConA can induce the apoptotic pathway. These results suggest that ConA may be detrimental in insect gut tissues and the interaction of ConA with epithelial cells may be responsible for the observed insecticidal effects.

**Keywords** Apoptosis · Concanavalin A · Gut · Grain aphid

## Introduction

Plant lectins are defined as proteins possessing at least one non-catalytic domain, which binds reversibly to specific mono- or oligo-saccharides. These proteins can have severe effects on fecundity, growth, and development of an insect. Thus, lectins are consequently potentially useful as agents of

insect resistance when introduced into transgenic plants. (Van Damme et al. 2008). To date, little is known about the exact molecular mechanism for insecticidal activity of plant lectins (Michiels et al. 2010). Researchers have proposed that the insecticidal activity of plant lectins may be related to the sugar binding capacity of these proteins. Detailed analyses of the carbohydrate-binding properties have shown that many lectins recognize sugar structures that are not present in plants but can be found in other organisms. Furthermore, the recent technological advances in insect glycobiology and using glycan arrays showed that the binding specificity of different plant lectins toward sugars is not direct against simple sugars but rather against more complex structures like *O*- and *N*-glycans. Under normal circumstances, insects take up plant lectins through feeding. Therefore, the first candidate receptors (complex glycans) will thus be located in the digestive tract. Receptors for plant lectins can be defined as glycoconjugates/glycoligands that possess a carbohydrate moiety with a structure complementary to that of the binding site of the lectin. Moreover, in case the lectin is able to pass through the epithelial barrier, a whole new set of candidate receptors may come into focus (Vanderborre et al. 2009; Michiels et al. 2010).

Ultrastructural studies have shown that lectins can bind to gut epithelial cells in a number of pest species (Habibi et al. 2000; Fitches et al. 2001; Hopkins and Harper 2001; Sauvion et al. 2004; Majumder et al. 2004), which can cause damage to epithelial cells and disruption of nutrient assimilation (Michiels et al. 2010). Concanavalin A (ConA) has been found to bind to the entire digestive tract of the pea aphid *Acyrtosiphon pisum* and causes morphological changes to epithelial cells as well as increased secretion and detachment of the apical membrane (Sauvion et al. 2004). Moreover, clear morphological changes in midgut microvilli were observed after the uptake of wheat

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germ agglutinin (WGA) in the larval midgut of *Drosophila melanogaster* (Li et al. 2009).

A recent study that investigated the mechanism of action of lectins at the cellular level was able to demonstrate that binding of lectins (ribosome-inactivating proteins or legume lectin) to the midgut epithelium appeared to induce severe anatomical abnormalities with pathological consequences, such as apoptosis of epithelial cells, which may explain their cytotoxicity (Hamshou et al. 2010; Shahidi-Noghabi et al. 2010a; Sprawka et al. 2013). Apoptosis is a physiological mechanism that is required to maintain cell numbers and remove unnecessary cells. It is characterized by condensation of the cytoplasm and nucleus, DNA fragmentation, chromatin merging in the nuclear periphery, cell contraction, dynamic membrane blebbing, and cell phagocytosis (Kerr et al. 1972). The central component of the apoptotic machinery is a group cysteine proteases called caspases (Denault and Salvesen 2003). Information on apoptosis induction in insects by plant lectins is limited. However, the involvement of plant lectins in apoptotic processes, particularly in mammals, has been extensively studied because plant lectins/legume lectins elicit apoptosis in different cancer cell lines. For example, a previous study on the cytogenetic action of plant lectins with differing glycoligand specifications demonstrated that these proteins are capable of inducing apoptosis in a culture of mammalian cells (Kovalenko and Lukash 2007). Moreover, some legume lectins such as WGA, LCA (*Lens culinaris* agglutinin), PHA and ConA have been shown to induce apoptosis, which would explain their toxicity (Koyama et al. 2002).

Therefore, in this study, we hypothesized that legume lectins may be detrimental in the gut tissues that form the first barrier after feeding by an insect, and as such, the interaction of plant lectins with epithelial cells may be responsible for the observed insecticidal effects. Thus, grain aphid (*Sitobion avenae* F.) females were exposed to ConA, which was the first identified plant legume lectins with a mannose/glucose-binding specificity. Subsequently, the occurrence of apoptosis in treated insects was investigated. The guts were dissected from treated *S. avenae* and analyzed first for DNA fragmentation and second for the induction of caspase-3 activity. In addition, the toxicity of ConA in the grain aphid was studied. In insect bioassays, ConA was added to the diet and fed to neonates and adults of *S. avenae*.

## Materials and methods

### Insect culturing

Grain aphid *S. avenae* F. parthenogenetic females were maintained on winter wheat (*Triticum aestivum* L. cv. Liwilla) seedlings in an environmental chamber at  $21 \pm 1$  °C,

L16:D8 photoperiod and 70 % RH. Aphids were transferred to liquid artificial diet as required for bioassays.

### Chemicals

Lectin ConA was purchased from MP Biomedicals (CN.150710). Genomic DNA was extracted with the application of *Genomic Mini AX Tissue* kit (A&A Biotechnology, Gdynia, Poland, www.aabiotech.com). All dietary components and other chemical reagents were obtained from Sigma (Sigma Chemical Co., Poznań, Poland) and were of analytical or best available grade.

### Aphid artificial diet bioassays

The liquid diet used for aphid feeding bioassays was prepared as described by Kieckhefer and Derr (1976). Adult aphids were removed from plants 48 h before starting the feeding assay and placed in plastic feeding chambers ( $h = 1.5$  cm<sup>3</sup>,  $\phi = 3.5$  cm<sup>3</sup>) covered by two sheets of Parafilm, with 500 mm<sup>3</sup> control diet (without lectin) sandwiched between two layers. Feeding chambers with aphids were maintained under the same environmental conditions as cultures on plants. Nymphs produced after 24 h were removed and fed for another 24 h on control diet, prior to exposure to diet containing added ConA at concentrations 50, 500, 1,000, 1,500 µg cm<sup>-3</sup>. Ten nymphs per treatment were then transferred to each feeding chamber (five chambers per replicate) containing test diets (with lectin or without lectin–control). The diet was refreshed as required. Aphids were monitored for the duration of pre-reproductive period, the fecundity, and mortality daily for 15 days. The collected data were used to calculate the average time of generation development ( $T$ ) and the intrinsic rate of natural increase ( $r_m$ ) according to the equations of Wyatt and White (1977):

$$T = d/0.74$$

$$r_m = [0.74(\ln Md)]/d$$

where  $d$  is the length of pre-reproductive period,  $Md$  is the number of larvae born during the reproduction period which equals the  $d$  period, 0.74 is the correction factor.

### Induction of apoptosis by ConA in the grain aphid

The aphids were exposed to the Con A for 48 h to investigate whether this protein is able to induce apoptosis. Thus, adult *S. avenae* were placed on an artificial control diet (without PHA) or a diet containing 1,500 µg cm<sup>-3</sup> of ConA as described above. For feeding chamber 30 apterae morphs were placed on feeding sachets and the experiment

was repeated three times. After diet probing, aphids were collected. Next, the entire guts of adult aphids were dissected under the binocular microscope and analyzed for both DNA fragmentation and caspase-3-like activity.

#### Isolation and analysis of DNA fragmentation

The dissected aphid guts (60 guts) were collected in sterile deionized water. Genomic DNA was extracted from aphid guts using a *Genomic Mini AX Tissue* kit (A&A Biotechnology, Gdynia, Poland, [www.aabiot.com](http://www.aabiot.com)), according to manufacturer's instructions. Quantification of DNA was conducted using an Epoch Microplate spectrophotometer (BioTek Instruments, Inc.). Additionally, A260/280 and A260/230 ratios were calculated to evaluate sample integrity and contamination of proteins or other organic substances. DNA samples of high integrity and purity were subjected to electrophoretic analysis. Separation of DNA samples (8 µg) was performed using horizontal gel electrophoresis (2 % agarose) under standard conditions. Electrophorograms were stained with ethidium bromide and screened in transilluminator under UV light and photographed.

#### Caspase-3 activity assay

The caspase-3 activity was measured using a *Caspase-3 Colorimetric Assay Kit* (Sigma-Aldrich, Poznań, Poland, PC CASP-3-C). This assay is based on the amount *p*-nitroaniline released from hydrolysis of the peptide substrate acetyl-Asp-Glu-Val-Asp-*p*-nitroanilide (Ac-DEVD-*p*NA) by caspase-3. Dissected gut tissues of *S. avenae* adults were incubated in ice-cold lysis buffer (50 mM HEPES pH 7.4, 5 mM CHAPS, 5 mM DTT) for 15 min, homogenized with a small homogenizer for 10 min, and centrifuged at 14,000g for 10 min at 4 °C. Mixture for determining the activity of caspase-3 which contained supernatants of gut homogenates (10 mm<sup>3</sup>), assay buffer (20 mM HEPES pH 7.4, 2 mM EDTA, 0.1 % CHAPS, 5 mM DTT) (980 mm<sup>3</sup>), and caspase-3 substrate (10 mm<sup>3</sup>) was incubated at 37 °C. Optical density was measured at 405 nm after 2 h. To verify the signal detected was contributed by caspase-3 activity 10 mm<sup>3</sup> supernatant, 970 mm<sup>3</sup> assay buffer, 10 mm<sup>3</sup> 2 mM Ac-DEVD-CHO (acetyl- Asp-Glu-Val-Asp-al, the inhibitor of caspase-3) and 10 mm<sup>3</sup> of caspase-3 substrate were added into quartz cuvettes in order. The caspase-3 activity could not be detected when Ac-DEVD-CHO was included in the quartz cuvettes.

Activity of caspase-3 was expressed as nmol of released *p*-nitroaniline per min per cm<sup>3</sup>. Three insect samplings were made for each assay.

#### Statistical analysis

The effect of dose of lectin ConA on grain aphid population parameters (the pre-reproductive period, mean daily fecundity, the intrinsic rate of natural increase, the average time of generation development, and mortality) was assessed by Kruskal–Wallis test followed by the multiple comparisons of mean ranks for all groups. Effect of ConA on caspase-3 activity was determined with two-tailed unpaired Student's *t* tests. All statistical analyses used Statistica for Windows v.9.0 (Statsoft 2011).

## Results

#### Insect bioassays: toxicity of ConA to the grain aphid

Values of population parameters indicated that the addition of the lectin ConA to the diets clearly affected the grain aphid population development. The lectin ConA statistically affected the pre-reproductive period (Kruskal–Wallis test;  $H_{4,25} = 22,51$ ;  $p = 0.0001$ ), fecundity (Kruskal–Wallis test;  $H_{4,25} = 23,18$ ;  $p = 0.0001$ ),  $r_m$  (Kruskal–Wallis test;  $H_{4,25} = 23,09$ ;  $p < 0.0001$ ), T (Kruskal–Wallis test;  $H_{4,25} = 21,51$ ;  $p = 0.0001$ ), and mortality (Kruskal–Wallis test;  $H_{4,25} = 22,99$ ;  $p = 0.0001$ ). The analysis showed an effect of increasing the concentration of the lectin ConA. Higher concentrations of tested compound increased the pre-reproductive period, decreased fecundity, and increased mortality of adult *apterae* (Table 1, 2). Higher concentrations of tested compound in the diet also increased T and reduced  $r_m$  (Table 1).

#### DNA fragmentation is characteristic for apoptosis

The DNA laddering method was adopted, the presence of a nucleosomal ladder being an accepted characteristic for identifying the initiation of apoptosis (Tilly 1993; Sumithra et al. 2010). To examine DNA fragmentation, genomic DNA was extracted, electrophoresed on 2 % agarose gel, and examined under UV light. A clear DNA laddering pattern of low molecular weight fragments was observed in the gut tissues. In contrast, control tissues (no treatment) showed no DNA fragmentation (Fig. 1).

#### Caspase-3 activity is involved in the induced apoptosis

To further investigate the mechanisms of ConA-mediated apoptosis, we tested the ability of ConA to trigger caspase activation.

As illustrated in Fig. 2, the exposition of aphids to ConA caused significant increase in caspase-3 activity in gut

**Table 1** Aphicidal activity of ConA on grain aphid

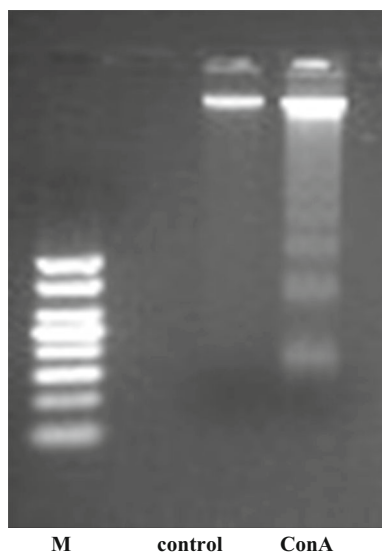
| Studied parameters (means $\pm$ SD)            | Control           | Lectin ConA                  |                               |                                 |                                 |
|--|-------------------|------------------------------|-------------------------------|---------------------------------|---------------------------------|
|  |                   | 50 ( $\mu\text{g cm}^{-3}$ ) | 500 ( $\mu\text{g cm}^{-3}$ ) | 1,000 ( $\mu\text{g cm}^{-3}$ ) | 1,500 ( $\mu\text{g cm}^{-3}$ ) |
| Mean daily fecundity/female                    | 1.59 $\pm$ 0.03a  | 1.41 $\pm$ 0.01ab            | 1.27 $\pm$ 0.01abc            | 1.20 $\pm$ 0.01bc               | 1.12 $\pm$ 0.008c               |
| Pre-reproductive period (days)                 | 6.20 $\pm$ 0.44c  | 7.20 $\pm$ 0.44bc            | 8.40 $\pm$ 0.54abc            | 9.40 $\pm$ 0.54ab               | 11.60 $\pm$ 0.54a               |
| Intrinsic rate of natural increase ( $r_m$ )   | 0.27. $\pm$ 0.01a | 0.24 $\pm$ 0.008ab           | 0.22 $\pm$ 0.005abc           | 0.19 $\pm$ 0.006bc              | 0.16 $\pm$ 0.005c               |
| Average time of generation development ( $T$ ) | 8.40 $\pm$ 0.60c  | 9.75 $\pm$ 0.06bc            | 11.38 $\pm$ 0.73abc           | 12.73 $\pm$ 0.74ab              | 13.32 $\pm$ 1.45a               |

Mean in row followed by different letters is different at  $p < 0.05$  (Kruskal–Wallis test)

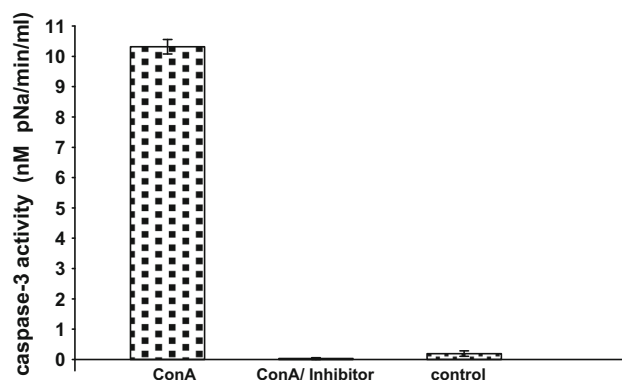
**Table 2** Mortality of *S. avenae* fed on artificial diet without (control) and with different concentrations of ConA (after 15 days of treatment)

| Concentration of lectin ( $\mu\text{g cm}^{-3}$ ) | Mortality (mean $\pm$ SD) (%) |
|---|-------------------------------|
| Control   | 1.00 $\pm$ 2.23c              |
| 50  | 22.00 $\pm$ 4.47bc            |
| 500   | 29.00 $\pm$ 2.23abc           |
| 1,000   | 48.00 $\pm$ 2.73ab            |
| 1,500   | 79.40 $\pm$ 3.78a             |

Mean in column followed by different letters is different at  $p < 0.05$  (Kruskal–Wallis test)

**Fig. 1** Concanavalin A induced DNA fragmentation in gut cells of grain aphid. DNA was isolated from gut cells of *S. avenae*, which fed on a artificial diet with 1,500  $\mu\text{g cm}^{-3}$  of ConA (ConA) or diet without ConA (control) for 48 h; M-DNA molecular weight marker (50, 100, 150, 200, 250, 300, 400, 500 bp). Approximately 8  $\mu\text{g}$  of DNA was analyzed on the 2 % agarose gel

cells. In contrast, no enzymatic activity was detected in samples of guts of adults treated with ConA in the presence of 2 mM of the specific caspase-3 inhibitor Ac-DEVD-CHO.

**Fig. 2** Caspase-3 activity in *S. avenae* gut extracted from adult after 48 h of feeding on artificial diet containing 1,500  $\mu\text{g cm}^{-3}$  of ConA (ConA) or diet without ConA (control). The addition of 2 mM Ac-DEVD-CHO completely blocked caspase-3 activity (ConA/inhibitor). Values are presented as mean ( $\pm$ SD) based on three individual repetitions, and 90 *apterae* morphs were exposed to ConA

## Discussion

Lectins have been suggested as promising agents against insect pests and have been successfully engineered into a variety of crops. This approach could be used as a part of integrated pest management strategies and to prevent pest attack (Lam and Ng 2011). The strong effects observed for lectins against aphids are of particular interest in view of the fact that aphids are important pests of crops and ornamental plants, and are not sufficiently sensitive to known Bt Cry toxins, and so cannot be controlled by existing plant engineering technologies using Bt toxin genes (Fitches et al. 2008). However, more in-depth studies are necessary to investigate the toxicity of lectins from different sources toward aphids and to understand their mode of action. The mechanisms by which lectins exercise their toxic effects in insects remain poorly defined. In the past two decades, many efforts have been undertaken to unravel the mechanism behind the toxic properties of plant lectins. Researchers have postulated that binding of the ingested lectin to exposed carbohydrates in the epithelial membrane of the insect or the peritrophic membrane is the predetermining factor for insecticidal activity

(Bandyopadhyay et al. 2001, Singh et al. 2008; Vandenborre et al. 2011; Sprawka et al. 2012). Studies that analyzed the effect of plant lectins on the ultrastructural organization of the insect gut have shown the disruption of epithelial cells, including elongation of the striated border microvilli and swelling of epithelial cells into the lumen of the gut, leading to complete closure of the lumen and impaired nutrient assimilation by cells. This allows for the absorption of potentially harmful substances from the intestine into the circulatory system, fat bodies, ovarioles, and throughout the hemolymph (Jaber et al. 2010). In particular, Fitches and Gatehouse (1998) fed ConA in a semi-artificial diet to *L. oleracea* caterpillars. ConA was found to bind primarily to gut tissues. Fitches et al. (2001, 2004) showed that binding of ConA to microvilli is followed by transport of the proteins into the cells of the gut and Malpighian tubules. Immunolocalization studies on possible mechanisms of lectin toxicity in insects at the cellular level showed that ConA interacts with glycosylated receptors present at the cell surface or within the midgut epithelial cells. Moreover, immunohistochemical and electron microscopy studies revealed that ConA induced severe cellular swelling of epithelial cells, accompanied by hypersecretion and progressive detachment of the apical membrane in the pea aphid (Sauvion et al. 2004). However, Miyake et al. (2007) reported that ConA binds to the surface of gut cells and inhibits membrane repair by the inhibition of exocytosis.

A recent reports that analyzed the interaction between lectins and midgut epithelial cells led to new insights into the interactions between plant lectins, especially legume lectins and insects. In our previous work, apoptosis induction occurred in the gut cells of *S. avenae* apterous females upon feeding phytohemagglutinin (PHA, legume lectins) (Sprawka et al. 2013). These observations agree with the current results for ConA. Samples of the gut of *S. avenae* showed the two main characteristics of apoptosis. We noted clear DNA fragmentation in grain aphid guts. DNA fragmentation analysis is a classical and characteristic feature of apoptotic cells (Wyllie et al. 1980). In addition, we also showed that caspase-3 activity was induced in *S. avenae* tissue. Caspase-3 plays a central role in mediating apoptosis, including chromatin condensation, DNA fragmentation, and cell blebbing (Porter and Janicke 1999). This increase in caspase activity concurred with the detection of DNA fragmentation, suggesting that the activation of caspase-3 activity may induce DNA fragmentation. Thus, we can conclude that apoptosis was induced in *S. avenae* upon feeding a diet containing the typical legume lectin ConA.

Apoptosis is a protective reaction of biological systems to numerous forms of damage affecting an individual cell or an entire population of cells; it is designed to ensure the

integrity and viability of the entire organism (Kovalenko and Lukash 2007). We speculated that ConA is capable of inducing such damage and this, in turn, is responsible for the entomotoxicity of ConA in insects. Moreover, the process of apoptosis can be induced by environmental stress, such as binding of the nuclear receptors by glucocorticoids, heat, radiation, certain chemotherapeutic agents, nutrient deprivation, viral infection, hypoxia, and chemical agents (Zhuang et al. 2011). This subcategory of apoptosis is called toxic apoptosis (Levin 1995).

There is not much information on apoptosis induction in insect by plant lectins, especially for legume lectin. Only recently, Shahidi-Noghabi et al. (2010b) and Hamshou et al. (2010) reported that lectins (*Sambucus nigra* agglutinin SNA, *Sclerotinia sclerotiorum* agglutinin SSA) belong to the ribosome-inactivating proteins ((RIPs, another class of lectins) are capable of inducing cell death by apoptosis. The induction of apoptosis under the influence of plant lectin/legume lectins in mammalian cells has been studied intensively because these proteins elicit apoptosis in various cancer cell lines (Fu et al. 2011). It has been reported that some legume lectins, such as LCA, WGA, ConA, and PHA, are highly cytotoxic and induce apoptosis (Kim et al. 1993; Bussing et al. 1996; Gastman et al. 2004). Furthermore, other reports have demonstrated that another typical legume lectin *Phaseolus coccineus* lectin possesses marked cytotoxicity and induces apoptosis in murine fibrosarcoma L 929 cells (Chen et al. 2009). A legume lectin named *Sophora flavescens* lectin induces cell death through a caspase-dependent apoptotic pathway (Liu et al. 2008). The mechanisms by which legume lectins induce apoptosis in insects are unknown. In mammals, caspase-dependent apoptosis is mediated by two main pathways, the death receptor pathway and mitochondrial pathway (Denton et al. 2013). Researchers examining the induction of apoptosis by legume lectins in cancer cell lines have proposed that these lectins induce apoptosis by binding to the carbohydrate portion of cell surface glycoproteins or glycolipid, since preventing the binding of these compounds to cell surface by haptenic sugar abrogates their apoptotic effects (Kim et al. 1993). Thus, binding to glycoligands is important to trigger apoptosis. It is possible that lectins trigger apoptosis by binding to the glycosylated portion of cell death receptors (tumor necrosis factor, TNF). This is thought to induce crosslinking and which triggers the apoptotic cascade. Recent studies have demonstrated that ConA bears apoptosis-inducing activities and initiates apoptotic cell death mediated by mitochondria (Liu et al. 2009; Li et al. 2009). Additionally, it has been suggested that, after ConA administration, human melanoma A375 cells and hepatoma HepG2 cells commit to death through a mitochondria-mediated apoptotic pathway involving: mitochondrial membrane potential (MMP) collapse,



cytochrome c release, and caspase-9/3 activation (Liu et al. 2009; Liu et al. 2010a, b). Investigations concerning physiological/developmental apoptosis at the insect level have revealed that the *Drosophila* genome (*D. melanogaster* is a model organism to study developmentally programmed cell death in insect) encodes for single ortholog of TNF and TNF receptor family proteins, Eiger (Egr) and Wengen (Wgn), respectively. Similarly, the two Bcl-2-related proteins (in mammals, Bcl-2 family of proteins controls the mitochondrial pathway of apoptosis) in *Drosophila* are Debcl/dBorg-1/dRob-1 and Buffy/dBorg-2. Moreover, in *D. melanogaster* seven, caspases homologous to mammalian enzymes have been identified: three initiators (Dredd, Dronc and Strica) and four effectors (Drice, Dcp-1, Decay and Damm) (Cooper et al. 2009). This suggests that, at least in part, vertebrate/mammalian apoptosis proteins may be conserved (Denton et al. 2013; Jenkins et al. 2013). On the other hand, it was found that, similar to in mammals, Bcl-2 proteins and mitochondrial remodeling contribute to cell death in *Drosophila*, but their mechanisms may differ. In *Drosophila* mid-stage death in the ovary, mitochondria remodel into clusters, which are engulfed and then degraded by somatic follicle cells. This is dependent on the Bcl-2 genes Debcl and Buffy, mitochondrial remodeling genes, caspases, and autophagy genes (Jenkins et al. 2013).

Moreover, artificial diet-feeding assays have demonstrated that Con A is toxic to the grain aphid. Particularly, Con A has an inhibitory effect on fecundity (an important parameter when trying to limit the growth of an insect population) and induces an increase in the pre-reproductive period of *S. avenae*. These results were reflected in population parameter values, which showed that the addition of ConA to the diet also increased the average time of generation development ( $T$ ) and reduced the intrinsic rate of natural increase ( $r_m$ ). The results obtained here confirm earlier reports. ConA has shown deleterious effect on several aphid species such as *A. pisum*, *Macrosiphon albifrons*, *Aphis gossypii*, *Myzus persicae*, *Macrosiphon euphorbia*, and *Aulacorthum solani*. ConA affects survival, delays development durations, reduces larval weight, and increases mortality in aphid species that have ingested it (Rahbe and Febvay 1993; Rahbe et al. 1995; Sauvion et al. 1996; Gatehouse et al. 1999). Similarly, when ConA was added to the artificial diets and fed to the tara planthopper *Tarophagous prosperina*, a corrected mortality of 93 % was noted (Powell 2001). Melander et al. (2003) also showed that ConA induced the pollen beetle larvae (*Meligethes aeneus*) mortality of 60 % after feeding on anthers treated with ConA. Moreover, which perhaps should be noted here is the fact that ConA is also considered an antinutritional factor (ANF) in humans and can be toxic to non-target organisms including other insects and grazing animals. Therefore, use of phloem-specific expression

promoters (the rice sucrose synthase promoter-RSs1 or the ubiquitin promoter-*ubi*) to create transgenic plants is a potential way to solve this problem. The use of such promoters could give a higher level of expression of insecticidal proteins/Con A in phloem compared to other parts of the plant and would minimize exposure to non-target insects and other consumers of the plant material to ConA (Stoger et al. 1999, Gatehouse et al. 1996). Moreover, roasting and pressure cooking are satisfactory methods to inactivate the antinutritional properties of ConA (Sridhar and Seena 2006).

In summary, the present study shows that ConA has a marked and significant deleterious effect on the development and fecundity of *S. avenae*. This detrimental effect was associated with the death of the gut epithelial cells, resulting in changes to gut morphology and function (leading to starvation). This affected insect survival, development, and fecundity, which in turn resulted in insect death within a few days. Further studies are required to reveal the detailed mechanism of apoptosis induction by ConA/legume lectin at the insect level, especially to identify: (1) whether the link between ConA activity and apoptosis is direct or indirect; and (2) whether ConA, similarly as in mammals, induces apoptosis through the activation of the mitochondrial apoptosis pathway. Integration of the results from such research will allow us fully to explain the mechanisms behind the strong insecticidal action of this lectin.

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## References

- Bandyopadhyay S, Roy A, Das S (2001) Binding of garlic (*Al'm sativum*) leaf lectin to the gut receptors of homopteran pests is correlated to its insecticidal activity. *Plant Sci* 161:1025–1033
- Bussing A, Suzart K, Bergmann J, Pfuller U, Schietzel M, Schweizer K (1996) Induction of apoptosis in human lymphocytes treated with *Viscum album* L. is mediated by the mistletoe lectins. *Cancer Lett* 99:59–72
- Chen J, Liu B, Ji N, Zhou J, Bian HJ, Li CY, Chen F, Bao JK (2009) A novel sialic acid-specific lectin from *Phaseolus coccineus* seeds with potent antineoplastic and antifungal activities. *Phytomedicine* 16:352–360
- Cooper DM, Granville DJ, Lowenberger C (2009) The insect caspases. *Apoptosis* 14:247–256
- Denault JB, Salvesen GS (2003) Human caspase-7 activity and regulation by its N-terminal peptid. *J Biol Chem* 278:34042–34050
- Denton D, Aung-Htut MT, Kumar S (2013) Developmentally programmed cell death in *Drosophila*. *Biochim Biophys Acta* 1833:3499–3506
- Fitches E, Gatehouse JA (1998) A comparison of the short and long term effects of insecticidal lectins on the activities of soluble

- brush border enzymes of tomato moth larvae (*Lacanobia oleracea*). *J Insect Physiol* 44:1213–1224
- Fitches E, Woodhouse SD, Edwards JP, Gatehouse JA (2001) In vitro and in vivo binding of snowdrop In vitro and in vivo binding of snowdrop (*Galanthus nivalis* agglutinin; GNA) and jackbean (*Canavalia ensiformis*; Con A) lectins within tomato moth (*Lacanobia oleracea*) larvae; mechanisms of insecticidal action. *J Insect Physiol* 47:777–787
- Fitches E, Edwards MG, Mee C, Grishin E, Gatehouse AMR, Edwards JP, Gatehouse JA (2004) Fusion proteins containing insect-specific toxins as pest control agents: snowdrop lectin delivers fused insecticidal spider venom toxin to insect haemolymph following oral ingestion. *J Insect Physiol* 50:61–71
- Fitches E, Wiles D, Douglas AE, Hinchliffe G, Audsley N, Gatehouse JA (2008) The insecticidal activity of recombinant garlic lectins towards aphids. *J Biochem Mol Biol* 38:905–915
- Fu L, Zhou Ch, Yao S, Yu J, Liu B, Bao J (2011) Plant lectins: targeting programmed cell death pathways as antitumor agents. *Int J Biochem Cell* 43:1442–1449
- Gastman B, Wang K, Han J, Zhu Z, Huang X, Wang G, Rabinowich H, Gorelik E (2004) A novel apoptotic pathway as defined by lectin cellular initiation. *Biochem Biophys Res Commun* 316:263–271
- Gatehouse AMR, Powell K, Edmonds H (1996) Genetic engineering of rice for resistance to homopteran insect pests. In: Khush GS (ed) Proceedings of the third international rice genetics symposium. Manila, Philippines. IRRI (International Rice Research Institute), pp 189–200
- Gatehouse AMR, Dawidson GM, Stewart JN, Gatehouse LN, Kumar A, Geoghegan IE, Birch ANE, Gatehouse JA (1999) Concanavalin A inhibits development of tomato moth (*Lacanobia oleracea*) and peach-potato aphid (*Myzus persicae*) when expressed in transgenic potato plants. *Mol Breed* 5:153–165
- Habibi JE, Backus EA, Huesing JE (2000) Effects of phytohemagglutinin (PHA) on the structure of midgut epithelial cells and localization of its binding sites in western tarnished plant bug, *Lygus hesperus* Knight. *J Insect Physiol* 46:611–619
- Hamshou M, Smagghe G, Shahidi-Noghabi S, De Geyter E, Lannoo N, Van Damme EJM (2010) Insecticidal properties of *Sclerotinia sclerotiorum* agglutinin and its interaction with insect tissues and cells. *Insect Biochem Mol Biol* 40:883–890
- Hopkins TL, Harper MS (2001) Lepidopteran peritrophic membranes and the effect of dietary germ agglutinin on their formation and structure. *Arch Insect Biochem Physiol* 47:100–109
- Jaber K, Haubruge É, Francis F (2010) Development of entomotoxic molecules as control agents: illustration of some protein potential uses and limits of lectins (Review). *Biotechnol Agron Soc Environ* 14:225–241
- Jenkins VK, Timmons AK, McCall K (2013) Diversity of cell death pathways: insight from the fly ovary. *Trends Cell Biol* 23:567–574
- Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26:239–257
- Kieckhefer RW, Derr RF (1976) Rearing three species of cereal aphids on artificial diets. *J Econ Entomol* 60:663–665
- Kim M, Rao MV, Twardy DJ, Prakash M, Galili U, Gorelik E (1993) Lectin-induced apoptosis of tumor cells. *Glycobiology* 3:447–453
- Kovalenko OO, Lukash LL (2007) Induction of apoptosis in populations of mammalian cells in vitro under the influence of lectins. *Cytol Genet* 5:303–307
- Koyama Y, Katsuno Y, Miyoshi N, Hayakawa S, Mita T, Muto H, Isemura S, Aoyagi Y, Isemura M (2002) Apoptosis induction by lectin isolated from mushroom *Boletopsis leucomelas* in U937 cells. *Biosci Biotechnol Biochem* 66:784–789
- Lam SK, Ng TZ (2011) Lectins: production and practical applications. *Appl Microb Biotechnol* 89:45–55
- Levin S (1995) Commentary: a toxicologic pathologist's view of apoptosis or I used to call it necrobiosis, but now I'm singing the apoptosis blues. *Toxicol Pathol* 23:533–539
- Li H-M, Sun L, Mittapalli O, Muir WM, Xie J, Wu J, Schermerhorn BJ, Sun W, Pittendrigh BR, Murdock LL (2009) Transcriptional signatures in response to wheat germ agglutinin and starvation in *Drosophila melanogaster* larval midgut. *Insect Mol Biol* 18:21–31
- Liu Z, Liu B, Zhang ZT, Zhou TT, Bian HJ, Min MW, Liu YH, Chen J, Bao JK (2008) A mannose + binding lectin from *Sophora flavescens* induces apoptosis in HeLa cells. *Phytomedicine* 15:867–875
- Liu Z, Li CY, Bian HJ, Min MW, Chen LF, Bao FK (2009) Antiproliferative activity and apoptosis—inducing mechanism of concanavalin A on human melanoma A375 cells. *Arch Biochem Biophys* 482:1–6
- Liu ZY, Li XF, Ding XP, Zang Y (2010a) *In silico* and experimental studies of concanavalin A: insight into its antiproliferative activity and apoptotic mechanism. *Appl Biochem Biotechnol* 162:134–145
- Liu B, Bian H, Bao J (2010b) Plant lectins: potential antineoplastic drugs from bench to clinic. *Cancer Lett* 287:1–12
- Majumder P, Santanu B, Sampa D (2004) Identification of receptors responsible for binding of the mannose specific lectin to the gut epithelial membrane of the target insects. *Glycoconj J* 20:525–530
- Melander M, Ahman I, Kamnert I, Strömdahl A-C (2003) Pea lectin expressed transgenically in oilseed rape reduces growth rate of pollen beetle larvae. *Transgenic Res* 12:555–567
- Michiels K, Van Damme EJM, Smagghe G (2010) Plant-insect interactions: What can we learn from plant lectins? *Arch Insect Biochem* 4:193–212
- Miyake K, Tanaka T, McNeil P (2007) Lectin-based food poisoning: a new mechanism of protein toxicity. *PLoS One* 2:e687. doi:10.1371/journal.pone.0000687
- Porter AG, Janicke RU (1999) Emerging roles of caspase-3 in apoptosis. *Cell Death Differ* 6:99–104
- Powell KS (2001) Antimetabolic effects of plants lectins towards nymphal stages of the planthoppers *Tarophagous proserpina* and *Nilaparvata lugens*. *Entomol Exp Appl* 99:71–77
- Rahbe Y, Febvay G (1993) Protein toxicity to aphids: an in vitro test on *Acyrtosiphon pisum*. *Entomol Exp Appl* 67:149–160
- Rahbe Y, Sauvion N, Febvay G, Peumans WJ, Gatehouse AMR (1995) Toxicity of lectins and processing of ingested proteins in the pea aphid *Acyrtosiphon pisum*. *Entomol Exp Appl* 76:143–155
- Sauvion N, Rahbe Y, Peumans WJ, Van Damme EJM, Gatehouse JA, Gatehouse AMR (1996) Effects of GNA and other mannose binding lectins on development and fecundity of the peach-potato aphid *Myzus persicae*. *Entomol Exp Appl* 79:285–293
- Sauvion N, Nardon G, Febvay G, Gatehouse AMR, Rahbe Y (2004) Binding of the insecticidal lectin concanavalin A in pea aphid, *Acyrtosiphon pisum* (Harris) and induced effects on the structure of midgut epithelial cells. *J Insect Physiol* 50:1137–1150
- Shahidi-Noghabi S, Van Damme EJM, Mahdian K, Smagghe G (2010a) Entomotoxic action of *Sambucus nigra* agglutinin in *Acyrtosiphon pisum* aphids and *Spodoptera exigua* caterpillars through caspase-3-like-dependent apoptosis. *Arch Insect Biochem Physiol* 3:207–210
- Shahidi-Noghabi S, Van Damme EJM, Masatoshi I, Smagghe G (2010b) Exposure of insect midgut cells to *Sambucus nigra* L.

- agglutinins I and II causes cell death via caspase-dependent apoptosis. *J Insect Physiol* 56:1101–1107
- Singh K, Kaur M, Rup MK, Singh J (2008) Effects of plant lectin from cobra lily, *Arisaema Curvatum* Kunth on development of melon fruitfly, *Bactrocera cucurbitae* (Coq.). *J Environ Biol* 29:911–916
- Sprawka I, Goławska S, Goławski A, Czerniewicz P, Sytykiewicz H (2012) Antimetabolic effect of phytohemagglutinin to the grain aphid *Sitobion avenae* Fabricius. *Acta Biol Hung* 63:342–353
- Sprawka I, Goławska S, Parzych T, Goławski A, Czerniewicz P, Sytykiewicz H (2013) Induction of apoptosis in the grain aphid *Sitobion avenae* (Hemiptera: Aphididae) under the influence of phytohemagglutinin PHA. *Appl Entomol Zool*. doi:10.1007/s13355-013-0214-2
- Sridhar KR, Seena S (2006) Nutritional and antinutritional significance of four unconventional legumes of the genus *Canavalia*—a comparative study. *Food Chem* 99:267–288
- Statsoft Inc. (2011) Statistica (Data Analysis System), version 9. [www.statsoft.com](http://www.statsoft.com)
- Stoger E, Williams S, Christou P, Down RE, Gatehouse JA (1999) Expression of the insecticidal lectin from snowdrop (*Galanthus nivalis* agglutinin; GNA) in transgenic wheat plants: effects on predation by the grain aphid *Sitobion avenae*. *Mol Breed* 5:65–73
- Sumithra P, Britto CP, Krishan M (2010) Modes of cell death in the pupal perivisceral fat body tissue of the silkworm *Bombyx mori* L. *Cell Tissue Res* 339:349–358
- Tilly JL (1993) Ovarian follicular atresia: a model to study the mechanisms of physiological cell death. *Endocrine J* 1:67–72
- Van Damme EJM, Lannoo N, Peumans WJ (2008) Plant lectins. *Adv Bot Res* 48:107–209
- Vandenborre G, Smaghe G, Van Damme EJM (2011) Plant lectins as defense protein against phytophagous insect. *Phytochemistry* 72:1538–1550
- Vandenborre G, Van Damme EJM, Smaghe G. (2009) Natural products: plant lectins as important tools in controlling pest insects. In: Ishaaya I, Horowitz AR (eds) Biorational control of arthropod pests. doi:10.1007/978-90-481-2316-2\_7
- Wyatt I, White PF (1977) Simple estimation of intrinsic increase rates for aphids and tetranychid mites. *J Appl Ecol* 1:757–776
- Wyllie AH, Kerr JFR, Curie AR (1980) Cell death: the significance of apoptosis. *Int Rev Cytol* 68:251–300
- Zhuang HM, Wang KF, Miyata T, Wu JW, Wu G, Xie LH (2011) Identification and expression of caspase-1 gene under heat stress in insecticide-susceptible and-resistant *Plutella xylostella* (Lepidoptera: Plutellidae). *Mol Biol Rep* 38:2529–2539