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Entomotoxic effects of fungal lectin from *Rhizoctonia solani* towards *Spodoptera littoralis*

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ABSTRACT

The effects of the *Rhizoctonia solani* lectin (RSA) on the growth, development and survival of an economically important caterpillar in agriculture and horticulture, the cotton leafworm, *Spodoptera littoralis* were studied. The high lectin concentration present in the sclerotes of the soil pathogen *R. solani* allowed the purification of large amounts of the pure lectin for feeding experiments with cotton leafworm. Rearing of insects on a diet containing different concentrations of RSA exerted a strong effect on the larval weight gain. This effect was visible at the lowest concentration of 0.1 % RSA at day 8 and day 11. Interestingly with 1 % RSA, there was a dramatic reduction in larval weight of 89 % at the end of L6 which was followed by a high mortality rate of 82 % in the treated larvae. Furthermore, the other developmental stages of pupation and adult formation were also affected. In addition, the data demonstrated that the combination of RSA with *Bt* toxin yielded synergistic effects. For instance, 0.03 % RSA + 0.005 % *Bt* toxin caused reduced growth rate and higher mortalities. These findings suggest that RSA is an interesting tool that can be used for bioengineering insect resistance in important agronomical crops.

Introduction

Problems associated with widespread insecticide usage, together with the development of insect resistance to *Bacillus thuringiensis* (*Bt*) toxins in genetically engineered crops, have resulted in a greater interest of scientists to exploit the potential of using plant defensive proteins, such as lectins, to help in combating crop damage. Lectins are a ubiquitous group of proteins and several hundreds of these molecules have been isolated so far from plants, viruses, bacteria, fungi, invertebrates and vertebrates including mammals (Carlini & Grossi-de-Sa 2002). Plant lectins are defined as proteins possessing at least one non-catalytic domain, which binds reversibly to specific mono- or oligosaccharides (Van Damme *et al.* 2008). One of the roles attributed to plant lectins is their involvement in plant defense against pathogens and phytophagous insects (Peumans & Van Damme 1995). This protective activity is in accordance with the observation that most plant lectins are not targeted against plant carbohydrates, but preferentially bind foreign glycans (Peumans *et al.* 2000). Next to plants, it is of great interest that also mushrooms as well as other non-fruiting body forming fungi contain lectins. Although many carbohydrate-binding proteins from fungi have been reported, very little is known with respect to their physiological role (Wang *et al.* 1998).

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The *Rhizoctonia solani* agglutinin, abbreviated as RSA, is a lectin that is synthesized by the soil pathogen *R. solani* (Class: Basidiomycetes; Order: Polyporales). This plant pathogenic fungus has an asexual life cycle and survives as vegetative mycelium and sclerotia. These sclerotia enable the fungus to survive in the soil under harsh conditions. In 1987, Vranken and coworkers first reported the purification and characterisation of RSA. This lectin is a homodimeric protein composed of 15.5 kDa subunits that show high affinity for N-acetylgalactosamine (GalNAc) and more complex glycoproteins (Vranken *et al.* 1987). It was shown that high concentrations of the lectin accumulate in the sclerotes (2–3 % of the total soluble protein), whereas the lectin concentration in the mycelium is usually rather low (0.1–2 % of the total soluble protein) (Hamshou *et al.* 2007). At present, the complete RSA sequence is not known. However, judging from the N-terminal sequence of 60 amino acids, it can be predicted that RSA shows no important sequence homology to other known lectin sequences (Candy *et al.* 2001), making this lectin a potentially highly novel compound for research into innovative methods for the control of insect pests.

Because of the high lectin concentration in the sclerotes, it has been suggested that RSA could play a role as a storage protein and could be involved in the defense of the fungus against predators (Kellens & Peumans 1990). This hypothesis was put forward based on some striking similarities with plant lectins that have a dual role. Indeed, for many highly abundant plant lectins, it was shown that they combine a role in storage with a role in plant defense whenever the plant is under attack by predators (Peumans & Van Damme 1995). Indeed, many plant lectins have been shown to have toxic effects towards insects (Van Damme 2008; Vandenborre *et al.* 2009). Using experiments in which purified lectins were added to an artificial diet or transgenic plants were used to overexpress a lectin gene it was clearly shown that carbohydrate-binding proteins interfere with the growth and reproduction of insects from different orders. Although evidence shows that the carbohydrate-binding activity of plant lectins is necessary for their insecticidal activity, the mode of action of lectins in insects remains enigmatic. Over the last decade lectins particularly those binding mannose, have received significant attention, predominantly *Galanthus nivalis* agglutinin (GNA) (Sauvion *et al.* 1996; Down *et al.* 1996; Couty *et al.* 2001). Since then the potential use of mannose-binding lectins in plant protection against several insects has been investigated in detail (Van Damme 2008; Vandenborre *et al.* 2009). In addition, some reports have shown that the combination of two different insecticidal proteins in a single system provides an effective insect control and also reduces the potential for development of resistant insects. For instance, the combination of GNA with *Bt* toxin resulted in synergistic effects (Maqbool *et al.* 2001; Zhang *et al.* 2007).

The present paper reports the effects of RSA delivered via artificial diet on the survival and growth of the cotton leafworm *Spodoptera littoralis* (Order: Lepidoptera; Family: Noctuidae). This polyphagous noctuid species is an economically important caterpillar in agriculture and horticulture, damaging at least 87 economically important plant species belonging to 40 families distributed worldwide (Smagghe & Degheele 1994; Sadek 2001).

Materials and methods

Isolation of RSA

Rhizoctonia solani strain AG 1-1B was grown on autoclaved wheat grains. To produce large quantities of sclerotia, 25 g of wheat kernels and 60 ml of water were mixed in 250 ml Erlenmeyer flasks. After autoclaving, small pieces of approximately 1 cm^2 agar covered with mycelium from a 5-d old culture of *R. solani* grown on potato dextrose agar were added and the fungal cultures were incubated in a growth chamber at a temperature of 25–27 °C. After 4–5 weeks the sclerotia were harvested and used for lectin extraction. Sclerotia were lyophilized and ground to a fine powder using a coffee mill. The dry powder was extracted in phosphate buffered saline (PBS, 25 ml g^{-1} dry^{-1} weight material) for approximately 12 h at room temperature. Then the mixture was centrifuged at 3000 g for 10 min and remaining debris removed by passing the supernatant through filter paper (Whatmann 3MM). Affinity chromatography was performed on a galactose column equilibrated with PBS. After loading the extract, the affinity column was washed with PBS until the absorbance of the effluent at 280 nm was <0.1. Subsequently, the lectin bound to the column was eluted with 20 mM 1,3-diaminopropane (DAP). The lectin fractions obtained after the first affinity chromatography were brought to pH 7.0 and run on the galactose column for a second time. The RSA preparation obtained after the second affinity chromatography was loaded on an anion exchange chromatography column of Q Fast Flow, equilibrated with DAP. After washing with DAP the lectin was eluted using 0.1 M Tris–HCl (pH 7.0) containing 0.5 M NaCl. If necessary, these chromatography steps on the galactose and Q Fast Flow columns were repeated in order to obtain high-purity lectin preparations. Finally, the lectin fractions were dialyzed against water and lyophilized. The purity of the lectin was analyzed by SDS-PAGE.

Insects

An established colony of the cotton leafworm *Spodoptera littoralis* was reared under standard conditions of 23–25 °C, 60–70 % relative humidity and a 16:8 (light:dark) photoperiod in the Laboratory of Agrozoology at Ghent University as described before (Lemeire *et al.* 2008). Larvae were fed on artificial diet (Stonefly Heliothis diet, Ward's Natural Science, Rochester, NY), an artificial diet for Lepidopteran larval insects that can be prepared by adding cold water. Under these conditions, the duration of each of the first five larval instars is about three days each, whereas the sixth and last larval stage (L6) takes approximately six days. Larval instars were determined on the basis of their respective head capsule width.

Effects of RSA feeding on insect survival, growth and development

Newborn (0–6 h) first instar larvae of *Spodoptera littoralis* were selected from the continuous stock colony and RSA was fed using Stonefly Heliothis diet. Based on previous range finding tests, RSA was mixed at three concentrations of 0.1, 0.5 and