FIRST RESULTS ON THE INSECTICIDAL ACTION OF SAPONINS

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SUMMARY

In the search for new, natural insecticides, numerous scientists are currently trying to obtain useful compound from plants. A possibly interesting class of molecules are the saponins, a group of steroidal or triterpenoidal secondary plant metabolites with divergent biological activities. In this study, we investigated the activity of saponins against living caterpillars *Spodoptera littoralis)* and aphids *(Acyrthosiphon pisum)* via treatment on artificial diets containing different concentrations of saponins. We conclude that saponins have insecticidal activity, causing mortality and/or growth inhibition in the tested insects, although from our experiments the mode of action could not be identified.

Key words: Saponins, *Spodoptera littoralis*, cotton leafworm, *Acyrthosiphon pisum*, pea aphid, insecticide, survival, growth, reproduction.

INTRODUCTION

In the search for new, natural insecticides, numerous scientists are currently trying to obtain useful compound from plants. A possibly interesting class of molecules are the saponins, a group of steroidal or triterpenoidal secondary plant metabolites with divergent biological activities (Francis *et al.*, 2002).
In this project we worked with two major agriculture pest insects that represent two different manners of feeding: one with biting-chewing mouthparts and the other with piercing-sucking ones. Both insects, *Spodoptera littoralis* and *Acyrthosiphon pisum*, represent two major orders of pest insects, namely Lepidoptera (butterflies/caterpillars) and Hemiptera (aphids, whiteflies, scales, psyllids, cicadas, leafhoppers, treehoppers, planthoppers), respectively.
The cotton leafworm *S. littoralis* is considered as a major agricultural pest in many parts of the world. The caterpillars are strongly feared for their polyphagous character and many populations have developed high degrees of resistance against most groups of commercially available insecticides and even Bt. It is a very deleterious pest, capable of destroying entire crops in a matter of weeks. The wide host range includes cotton, cereals like corn, rice, wheat, millet, sorghum, sugar cane, and vegetables like tomato, melon, tobacco, lettuce, cole crops, celery, sugar and table beets, beans and peas.
Aphids are economically important insect pests that not only cause direct damage to crop plants by feeding on sap plant fluids but also often transmit devastating viral diseases. At present the control of aphids requires the use of a wide range of systemic and contact insecticides, most of which based on a neurotoxic mechanism. As a direct result of an intensive and widespread use of these chemicals insecticide-resistant aphid populations have arisen. In addition the use of these products also causes health and environmental risks. Although the loss in yields for agriculture is difficult to evaluate because it depends on species, crops, regions and countries, it was calculated that in France, for example, aphids of cereal

plants may lead to losses of 30% in wheat crops (draining of sap, depreciation of agricultural products) and up to 50% in barley (viral diseases). In this project we used the pea aphid *A. pisum* as model for the piercing-sucking insects.

MATERIALS AND METHODS

Chemicals

The saponin tested was a commercial extract powder from *Quillaja* bark (8-25% sapogenin) purchased from Sigma-Aldrich-Fluka (Bornem, Belgium). All other chemicals were of analytical grade or otherwise mentioned.

Insects

A continuous culture of *S. littoralis* larvae was maintained at standard conditions of 23±2°C, 65±5% relative humidity and a 16h photoperiod. Larvae were fed on an agar-based artificial diet as reported before (Smagghe *et al*., 2001).
The pea aphid *A. pisum* was kept under standard conditions of 23±2°C, 65±5% relative humidity and a 16h photoperiod. The aphids were synchronized and challenged to an artificial liquid diet as reported by Sadeghi *et al*. (2007).

Insecticide bioassays with *S. littoralis* and *A. Pisum*

Caterpillars from *S. littoralis* were fed with artificial diets containing different doses of saponin. For every 100 g diet [containing 25 g premix powder (purchased from Stonefly Ltd., TX, USA) and 75ml water], we added 7, 5, 3, 2 or 1 g saponin powder (7, 5, 3, 2 and 1%). The control treatment received the same diet without additional saponins. Early third stage (L3) caterpillars were placed in experimental cages (10/cage) and grown on these diets until they formed pupae or died. Three replicates were used for each concentration.
For the aphid insecticide test, liquid artificial diet containing saponin was prepared by adding a constant volume of variable saponin solutions. In treatments, saponin was added to the diet in a concentration range from 0.001% up to 7%. In the controls an equal amount of distilled water was added to the diet. First instars (0-12h old) of *A. pisum,* obtained from a synchronized population reared on *Vicia fabae* plants, were transferred at day 0 from bean onto parafilm sachets, containing 200 µl of artificial diet and saponin. We challenged 10 aphid nymphs per cage, and 3 cages were used per concentration.

RESULTS

Insecticide bioassays with *S. littoralis*

When starting with third-instars up to adult formation, *Quillaja* bark saponin caused ≥70% mortality (including 40-50% pupal mortality) in the treatments with 3 to 7% saponins in the diet. In the treatment with 1-2% saponins, survival rates did not differ significantly from the controls.
Although mortality rates were low during the first week(s), there was a remarkable retardation in the development of the treated animals, with significant differences in weight from the first day. During the whole experiment, the untreated caterpillars gained weight faster than any other group. The controls reached their maximal

weight on day 10 and then lost weight in preparation of pupation. The 1% and 2% treatments followed closely behind the controls, as these caterpillars reached their maximal weight on day 11 and 12, respectively. In contrast, the 3%, 5% and 7% treated larvae did not start pupation until day 17. As so, the different larval stages lasted longer. Still, the surviving larvae developed into apparently normal adults, but with slightly reduced weight.

Insecticide bioassays with *A. Pisum*

In the bioassay with first-instar nymphs of *A. pisum*, total mortality was scored after 2-3 days with saponin concentrations of ≥0.3%. At 0.1%-0.2% concentrations the nymphs lasted longer, but after 5 days most of them also had perished (≥70% mortality); the remaining few were visibly smaller compared to the controls and none of them developed into adults. Adding 0.01% saponins had no effect.

DISCUSSION

We investigated the activity of saponins against insects in order to evaluate their use as new, natural insecticides. In the experiments with insects saponins clearly show insecticidal activity, causing mortality and growth inhibition in both caterpillars and aphids.

It was of interest in the current experiments that the effects in both pest insects were qualitatively similar; however, the concentrations required to obtain these effects were different. In the experiments with *A. pisum*, 0.1% saponin killed all aphids, whereas with *Spodoptera* some caterpillars were still able to develop into apparently normal adults on food containing 7% saponin.

To explain the insecticidal activity of saponins, different hypotheses on the mode of action have been drawn so far. Saponins may pose a repellent or deterrent activity (Sylwia *et al*., 2006; Szczepanik *et al*., 2001). If the test insects refuse to eat food containing saponins, they will die after a few days, even if the saponins do not affect the insect's metabolism. In our experiments, caterpillars fed with food containing 3-7% saponins ate less than the controls, but is was not possible to conclude whether this was the consequence of their slower development or the reason. For the aphids, it was not possible to detect whether they had eaten from the food or not, but we do know that some aphids stayed alive for 5 days on diet containing 0.1% saponin. Other tests had demonstrated that aphids cannot survive a period of starvation for 5 days. As a consequence, we expect that the aphids did feed on the diet containing saponin; however it should be mentioned that the treated aphids were smaller than the controls. As so, it can be hypothesized that these aphids feed on the diet, but that they consume lower amounts, though enough to survive but not to grow properly.

In addition, saponins may also affect food uptake by slowing down the passage of food in the insect's gut, perhaps due to a reduction of the digestibility (Adel *et al*., 2000). This can secondarily influence food uptake, and as a consequence the nutrient uptake and growth.

Another possible mode of action is a blockage of the uptake of sterols. Insects cannot biosynthesize sterol structures by themselves, but they do need them for the synthesis of steroids like ecdysteroids and the insect moulting hormone 20-hydroxyecdysone (20E). This implies an obligatory uptake via their food; cholesterol or phytosterols provided by plant material can act as precursors for herbivorous insects (Belles *et al*., 2005). It is presumed that by forming indigestible bind-

ings with the sterols in the food, saponins can prevent their absorption, thus suspending the biosynthesis of ecdysteroids (Harmatha, 2000). This could cause disturbances in insect moulting and ecdysis.
Steroidal saponins have a steroid structure and show structural similarity to ecdysteroids, like the insect moulting hormone 20E. As so on the cellular level, it has been suggested that saponins may interact as agonists or antagonists with the receptor site for 20E, the ecdysteroid receptor complex (EcR), causing ecdysial problems (Dinan *et al.*, 2001). However, so far, there is no convincing evidence supporting this hypothesis.
Another possibility is that saponins are toxic because of their membrane-permeabilising abilities. Saponins increase the permeability of plasma membranes, and they are known to cause lysis of erythrocytes *in vitro* (Francis *et al.*, 2002). As so a high dose of saponins in the gut might lead to a disruption of the cell membrane and cell lysis of the intestine mucosal cells.
Taken all together we may conclude that saponins possess insecticidal activity, causing mortality and/or growth inhibition in the insects tested, the cotton leafworm *S. littoralis* caterpillars and the pea aphid *A. pisum*. However, the mechanism(s) of action underlying the insecticidal effects remains unclear, and we envisage further research to understand better and identify the targets of saponins in insects.

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