

Novel Advances with Plant Saponins as Natural Insecticides to Control Pest Insects

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ABSTRACT

Saponins are a class of secondary plant metabolites with diverse biological properties. They occur in a great number of plant species (mainly Angiosperms), both wild plants and cultivated crops. Triterpenoid saponins are mostly found in dicotyledonous species, while many of the major steroidal saponins are synthesized by monocots, such as members of the Liliaceae, Dioscoraceae and Agavaceae families. Many legumes contain saponins, such as soybeans, beans, peas, tea, spinach, sugar beet and quinoa, oats, capsicum peppers, aubergine, tomato seed, alliums and asparagus. Saponins possess clear insecticidal activities: they exert a strong and rapid-working action against a broad range of pest insects that is different from neurotoxicity. The most observed effects are increased mortality, lowered food intake, weight reduction, retardation in development and decreased reproduction. According to the main hypotheses in literature, saponins exert a repellent/deterrent activity, bear digestive problems, provoke insect moulting defects or cause cellular toxicity effects. As a consequence these interesting plant components open new strategies to protect crops in modern agriculture and horticulture with integrated pest management (IPM) programs against pest insects, either by spraying, or by selecting high-saponin varieties of commercial crops.

Keywords: crop protection, insects, insecticide (insecticidal activity), mechanism (of action), (occurrence in) plants, structural diversity

Abbreviations: 20E, 20-hydroxyecdysone; EcR, ecdysteroid receptor; FPP, farnesyl diphosphate; IPM, integrated pest management; IPP, isopentenyl diphosphate; MEP, methyl-erythritol; MVA, mevalonate

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INTRODUCTION

In agriculture and horticulture, insect pests are a very important factor of loss. As an average, they account for 20-30% of loss of production, but in some cases they provoke a total loss. In addition, many populations of already >550

species have developed resistance against most current insecticide groups, implying a high demand for novel insecticide targets. So many scientists in industry and academia are currently trying to obtain useful compound from plants as new, natural insecticides. A possibly interesting class of molecules are the saponins, a group of steroidal or triterpe-

noid secondary plant metabolites with divergent biological activities. This chapter describes the novel advances of saponins in important crops in agriculture and horticulture. To understand plant saponins, we start with the structural diversity of saponins and their occurrence in the plant kingdom. Then, we review the potencies of saponins as natural insecticides to control important pest insects with some practical examples from the lab and the field where saponins were able to reduce plant damage, and in a third part we overview the mechanisms underlying the insecticide activity. At the end we conclude with useful future perspectives to employ saponins as novel natural tactics in integrated pest management (IPM) to control pest insects, which fit in modern agriculture and horticulture.

SAPONINS IN THE PLANT KINGDOM

Biosynthesis of saponins and diversity in structure

Terpenes constitute a distinct class of natural products. With over 30,000 entities being structurally identified, they also represent the largest family of natural compounds (Sacchetti and Poulter 1997). They are derived from units of isoprene, which have the molecular formula (C₅H₈)_n. Terpene biosynthesis is, however, a complicated process mediated by two biosynthetic pathways. In eukaryotes, the mevalonate (MVA) pathway is responsible for biosynthesis of the universal C₅ building blocks of all isoprenoids. In prokaryotes, terpenes are derived from the methyl-erythritol (MEP) pathway (Eisenreich *et al.* 1998). In plants both pathways operate. The MVA pathway mainly acts in the cytoplasm, while the MEP pathway acts in the plastids. However, cross-talk between the two pathways occurs (Hemmerlin *et al.* 2003). Both pathways eventually lead to formation of isopentenyl diphosphate (IPP), as the precursor from which all other terpenes are formed via head-to-tail addition (Dubey *et al.* 2003).

The first part of the MVA pathway, until formation of farnesyl diphosphate (FPP), is exactly the same in insects and plants. In insects FPP leads to formation of juvenile

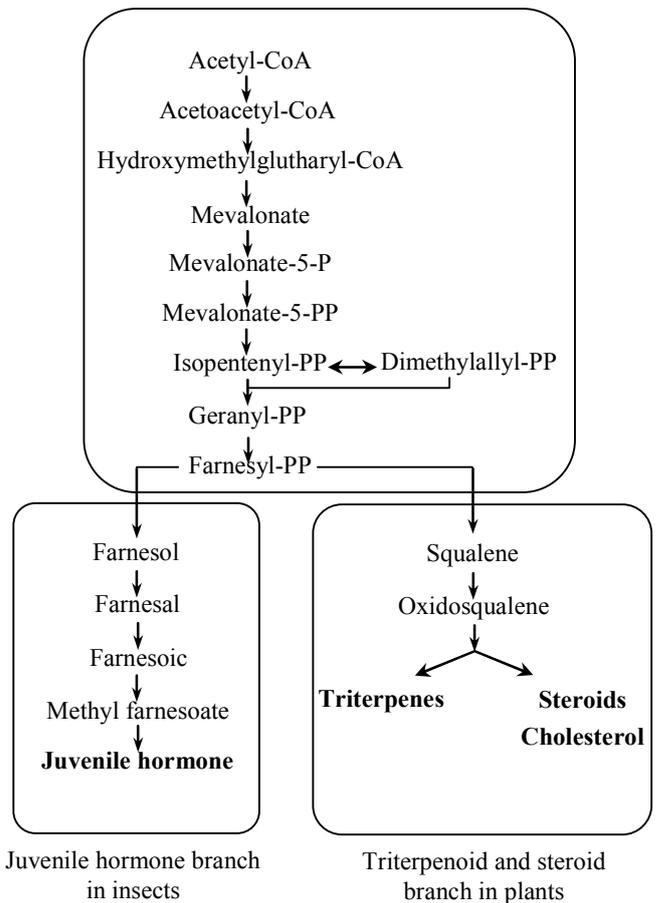


Fig. 1 Flow diagram of the MVA pathway. The pathway is exactly the same in plants and insects until formation of FPP. From there on the pathway leads to juvenile hormone synthesis in insects and to steroid and triterpene synthesis in plants.

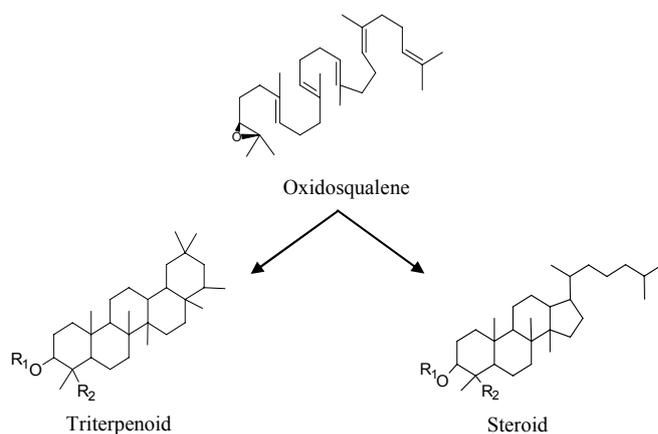


Fig. 2 Biosynthesis and occurrence of triterpenoid and steroidal saponins in economically important crops in agriculture and horticulture.

<p>Poaceae</p> <ul style="list-style-type: none"> -Avena strigosa -Avena sativa. <p>Chenopodiaceae</p> <ul style="list-style-type: none"> -Beta vulgaris -Chenopodium quinoa <p>Leguminosae</p> <ul style="list-style-type: none"> -Pisum sativum -Glycine max -Medicago sativa -Medicago truncatula -Phaseolus vulgaris <p>Theaceae</p> <ul style="list-style-type: none"> - Camellia sinensis 	<p>Poaceae</p> <ul style="list-style-type: none"> -Avena strigosa -Avena sativa -Panicum virgatum -Panicum coloratum <p>Solanaceae</p> <ul style="list-style-type: none"> -Capsicum frutescens -Solanum lycopersicum -Solanum tuberosum <p>Alliaceae</p> <ul style="list-style-type: none"> -Allium sativum -Allium nutans -Allium porrum -Allium cepa -Allium schoenoprasum
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hormone, which has (out of many other functions) anti-ecdysteroid activity. This branch of the terpene biosynthesis pathway is unique for insects (Belles *et al.* 2005). In plants FPP leads to the formation of squalene, which is the common starting point for biosynthesis of triterpenes and steroids, e.g. cholesterol (Dubey *et al.* 2003). Insects lack this branch of the pathway, which means that they can not produce endogenous cholesterol and are dependent on their environment for uptake of cholesterol and other steroids (Belles *et al.* 2005) (Fig. 1).

Saponins consist of a sugar moiety glycosidically linked to a hydrophobic aglycone which may be a triterpene or a steroid. The aglycone, or sapogenin, may contain one or more unsaturated C-C bonds. The oligosaccharide chain is normally attached at the C₃ position (monodesmosidic), but many saponins have an additional sugar moiety at the C₂₆ or C₂₈ position (bidesmosidic). The great complexity of saponin structure arises from the variability of the aglycone structure, the nature of the side chains and the position of attachment of these moieties on the aglycone (Francis *et al.* 2002). Due to this complexity, saponins are difficult to classify. Because it is no longer customary to classify compounds based on their physicochemical or biological properties, a state of the art classification based on the biosynthesis of the saponin carbon skeletons was proposed by Vincken *et al.* (2007).

Both triterpenoid and steroidal saponins originate from the C₃₀ precursor squalene. Squalene is oxidized to oxidosqualene, and this is converted to cyclic derivatives. The type of cyclase that is involved in the cyclization reaction primarily determines which skeleton is formed. Cyclization of oxidosqualene to saponins can proceed in two ways, either via the 'chair-chair-chair' or via the 'chair-boat-chair' conformation. An important difference between the two resulting skeletons lies in the stereochemistry. Triterpenes originate from the 'chair-chair-chair' conformation, while steroids arise from the 'chair-boat-chair conformation' (Vincken *et al.* 2007) (Fig. 2).

Very little is known about the enzymes and biological pathways involved in saponin synthesis from here on. Depending on cyclization, rearrangement and degradation reactions of the two mentioned cyclic derivatives of oxidosqualene, 11 carbon skeletons are found: dammaranes, tirucallanes, lupanes, hopanes, oleananes, taraxasteranes, ursanes, cycloartanes, lanostanes, cucurbitanes and steroids. These cover the main saponin skeletons (Vincken *et al.* 2007). Modification of these carbon skeletons by minor rearrangement, homologation, cleavage and degradation, will lead to subdivisions of these main classes. Functionalization of the carbon skeletons is predominantly the result of oxidation, resulting in functional groups like hydroxyl groups, carbonyl groups and carboxylic acids, which can be found at many positions in the molecules. These functional groups are often involved in mutual chemical reactions, which may lead to additional characteristic structural elements, such as ether bridges, spiroketals or lactones. Subdivisions based on these structural elements are the result of secondary type of transformation (Vincken *et al.* 2007).

Occurrence of saponins in cultivated crops in agriculture and horticulture

Saponins occur constitutively in a great number of plant species, both wild plants and cultivated crops. Triterpenes are found principally in dicotyledonous species, while many of the major steroidal saponins are synthesized by monocots, such as members of the Liliaceae, Dioscoraceae and Agavaceae families (Osbourn 2003). There is, however, no clear relationship between the plant origin and the type of saponin, nor is there evidence that specific saponins are associated with particular parts of plants (Vincken *et al.* 2007).

Triterpenoid saponins have been detected in many legumes such as soybeans, beans, peas, tea, spinach, sugar beet and quinoa. Steroidal saponins are found in oats, capsicum peppers, aubergine, tomato seed, alliums (such as leek, oni-

on and garlic) and asparagus (Francis *et al.* 2002) (Fig. 2).

Despite the sometimes negative biological actions of saponins on animals and humans, they do occur in a wide variety of crops and edible plants.

Saponins in cereals and grasses

Cereals and grasses appear to be generally deficient in saponins, with the exception of some grasses, like switchgrass (*Panicum virgatum*), kleingrass (*Panicum coloratum*), and oats (*Avena* spp.) (Patamalai *et al.* 1990; Lee *et al.* 2001; Osbourn 2003). Members of the genus *Avena* synthesize two different families of saponins, the steroidal avenacosides and the oleanane type triterpenoid avenacins. The distribution of these two classes of saponins is mutually exclusive; avenacosides accumulate in the leaves and avenacins in the roots. The major oat root saponin avenacin A-1 is esterified with N-methyl antranilic acid and fluoresces under ultra-violet light. This property is extremely unusual amongst saponins and has proven to be a valuable tool for the dissection of triterpenoid biosynthesis. In addition, all four isolated avenacins have an antifungal action (Osbourn 2003). The wide interest in oat-root saponins can be explained by the resistance of oat roots to the fungus *Gaeumannomyces graminis*, which causes "take-all" disease in wheat and barley. Indirect evidence for a role for avenacins as a determinant for the resistance of oats to *G. graminis* has been provided by Papadopoulou *et al.* (1999). The role of saponins in protecting plants against fungal attack was investigated by means of saponin-deficient oats (*sad* mutants). The study showed that all 10 independent *sad* mutants were clearly more susceptible to fungal infection than the wild-type line. Also, a mutant with partial reduction in avenacins content gave only limited disease symptoms when inoculated with *G. graminis*. *Sad* mutants that lacked detectable levels of avenacins were substantially more susceptible to disease (Papadopoulou *et al.* 1999).

As already mentioned, most of the grasses and cereals do not produce any saponins. This may be because they are naturally lacking in these secondary metabolites, or because saponins have been counter selected for during breeding of cultivated cereals. The isolation of additional genes for saponin biosynthesis will help to shed light on this.

Saponins in other economically important crops

Alliaceae

Many of the *Allium* species, from the Alliaceae family, contain steroidal saponins. A lot of the research is focused on the saponins in garlic (*Allium sativum*), because of their health benefits. These saponins are responsible for the cholesterol lowering functions of garlic. They also have antifungal activity (Matsuura 2001).

Saponins also occur in leek (*Allium porrum*), onion (*Allium cepa*) and chives (*Allium schoenoprasum*). They are detected in flowers as well as in bulbs. In *Allium nutans*, the concentration of saponins was determined to amount to 4% of the dry matter, which makes this species a good source of steroidal saponins for commercial use (Akhov *et al.* 1999).

Chenopodiaceae

The Chenopodiaceae, with 1500 species and 100 genera, are well represented in triterpenoid saponins, with oleanolic acid as the predominant aglycone. Two important crops from this family containing saponins are *Beta vulgaris* (sugar beet) and *Chenopodium quinoa*. Saponins have been identified in the roots and leaves of sugar beet, but in spite of the economic importance of this plant, not much information is published on the sugar beet saponins (Massiot *et al.* 1994). More attention has been given to saponins of quinoa, a highly nutritious Andean food plant. All parts of the plant are utilized. Seeds in particular are of interest because of their high protein quality and content. The seeds require special treatment before usage for nutritional purposes because of the bitter tasting saponins in the outer layers

of the seed coat. There are also varieties that do not have the bitter taste ('sweet' variants), but even those seeds need to be washed before usage, because of the known and unknown biological effects of other saponin derivatives (Dini *et al.* 2002).

Leguminosae

Oleanolic saponins are present in many edible legumes like soybeans (*Glycine max*), beans (*Phaseolus vulgaris*), peas (*Pisum sativum*), etc. Because of the health-promoting properties of soyasaponins (saponins that were first identified in soybeans), a lot of information is available on these secondary metabolites. Whole soybeans contain approximately 4-6% saponins on a weight basis; in seeds 0.6% up to 6.5% of the dry weight consists of triterpenoid saponins, depending on the variety, cultivation year, location grown and degree of maturity (Berhow *et al.* 2006). When comparing hulls, germ and cotyledons from soybeans, there are much more saponins in the germ than in the other parts of the bean. The investigation of Berhow and co-workers also shows that there can be a considerable variation in saponin concentration in soybeans from the same farmer in two consecutive years. There are nearly twice as much saponins in the soybeans from the first year compared with those of the second year. This may be due to different drought conditions in both years. Analysis of four varieties of soybeans grown on the same farm in one year, showed a lower degree of saponin concentration variation (Berhow *et al.* 2006).

Most of the research on saponins in peas (*P. sativum*) has focused on their potential insecticidal action. Extracts from *P. sativum* have previously been shown to exert utility to control insect pests (Taylor *et al.* 2004). The content of saponins in some species is high, for example the saponin content of unprocessed cultivars of pigeon peas ranged from 2164 to 3494 mg per 100 g on dry matter basis. These quantities decrease significantly when the peas are cooked or dehulled (Duhan *et al.* 2001).

Other economically important members of the *Leguminosae* family are *Medicago sativa* (alfalfa) and *Medicago truncatula*. The two crops contain similar concentrations of saponins, constituting about 0.15-0.22% of the dry weight. Saponins isolated from *M. sativa* are of great interest because of their activity against microbiota, fungi and insects (Kapusta *et al.* 2005).

Solanaceae

A lot of solanaceous plants contain saponins that have diverse biological and pharmacological activities. Two steroidal saponins have recently been purified from cayenne pepper (*Capsicum frutescens*) (Kohara *et al.* 2007), and saponins have also been described in tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum*). In potatoes, the content of saponins varies from 105-228 mg/kg fresh weight (Turakainen *et al.* 2004).

Theaceae

The best known genus of this family is *Camellia*, which includes the plant whose leaves are used to produce tea (*Camellia sinensis*). Triterpenoid saponins (oleanane type) are present in seeds, roots and flowers of the tea plant. In the seeds the content of saponins even amounts to 10% of the dry weight. Tea saponins have diverse physiological effects, and a lot of effort is done to identify all the saponins present in the tea plant (Kohata *et al.* 2004).

To date different saponin powder and solutions from tea are present at the marketplace to be used as effective natural insecticides in the Asian region (China); a good example is "Liquid Tea Saponin" from Hangzhou Choisun Tea Sci-Tech Co. Saponins could be applied exogenic by spraying them on fields. On the other hand, Ltd. Farmers employ the natural saponin content of plants by selecting high-saponin varieties (<http://choisuntea.en.alibaba.com/>).

POTENCY OF SAPONINS TO REDUCE INSECT DAMAGE

Data from the laboratory and the field

Although little is known about the exact functions of saponins, it is generally accepted that they play an important role in plant defence. Plants need to protect themselves against herbivory and diseases; they can be eaten by vertebrate herbivores or molluscs, or suffer from viral, bacterial or fungal infections. Saponins are known to have noticeable effects on each of these groups; but those have been described elsewhere (for a review, see Francis *et al.* 2002; Sparg *et al.* 2004; Tava and Avato 2006). Here, we focus on the most important group of invertebrate plant herbivores: the insects.

Plant compounds with a negative influence on insects have been used by man long before the compounds themselves were even discovered. Farmers have been trying to breed crops with high resistance against insect pests for centuries, mostly without a notion of the cause of this resistance. This breeding practice could have led to an increase in saponin level in economically important plants. On the other hand, the bitter taste of saponins might have made them undesirable in food, and so they may also have been eliminated from other commercial crops. Since scientists started to pay attention a few decades ago, many studies have been carried out to clarify the biological activities and ecological role of saponins, and it has become clear that they are a trigger of plant resistance against insects, thought not the only one.

Alfalfa (*Medicago sativa*)

Alfalfa has a long history as a feeding crop for animals. Among scientists, it is also known for its saponins, and it is probably the most studied of all saponin-containing crops. The plant has a wide assortment of cultivars, each with their own specific characteristics. This makes it even more interesting for scientific research.

Pedersen *et al.* (1976) compared six alfalfa cultivars selected for low and high saponin concentration. They were tested for seven agronomic traits and resistance to six diseases, five insects, and two nematodes. High saponin concentration was correlated with resistance to pea aphids (*Acyrtosiphon pisum*), but it had no appreciable effect on the other insects or the nematodes. For the diseases, high saponin concentration retarded the *in vitro* growth of southern blight (*Sclerotium rolfsii*), but had very little effect on any of the other disease organisms studied.

Better indications for the effect of saponins were found by Golawska *et al.* (2006). They noticed that on an alfalfa line with high saponin content, the development, survival and reproduction of pea aphids were disturbed or reduced compared to a low-saponin line. They used an EPG-test (electrical penetration graphs method) to determine the duration and number of aphid feeding activities, and found that aphids on the high-saponin line show more and longer "potential drops" (attempts to feed) during the first hour. Later on, there was a prolonged penetration of epidermis and mesophyll (fewer probes of longer duration) and a significant reduction in phloem sap ingestion (still on the high-saponin line). The investigators also performed an analysis of the active alfalfa saponins. They isolated two compounds occurring in higher doses in the high-saponin line: zanhic acid tridesmoside and 3-GlcA,28-AraRhaXyl medicagenic acid (two oleanane saponins). When added to artificial diet, these two compounds caused the same effects, implying that they contain insecticidal activities.

To determine the effects of 'pure' saponins, numerous laboratory-tests were carried out. In most cases insects were fed with (treated) leaves or artificial diets containing saponins. Horber *et al.* (1974) tested saponins extracted from 'DuPuits' (DP) and 'Lahontan' alfalfa cultivars on the potato leafhopper (*Empoasca fabae*) and the pea aphid (*A.*

pisum). First instar leafhopper nymphs were fed a crude-saponin mixture from DP or a commercially available saponin from *Yucca* sp., added to artificial diet. Leafhoppers fed with 5% and 1% *Yucca* saponin all died after 2 and 3 days, respectively, and with 0.1% after 10 days. Adding 0.01% saponins had no effect. The rapid mortality can be explained by a strong non-preference of the leafhoppers for diets containing saponins, or by toxic effects of the saponins. Applying the crude-saponin mixture from alfalfa (DP) gave similar results: all nymphs on the 5% and 1% diet died after 1 day. On the 0.1% concentration there was 70% mortality after 3 days, but the mortality curve levelled conspicuously after the 5th day at 85% mortality. This could mean that 15% of the leafhopper population is less reluctant to eat saponins, or that the damaging effects of saponins are somehow neutralised some time after ingestion (time-dependent decomposition or detoxification).

Pea aphid nymphs (first or second instar) were also reared on an artificial diet containing *Yucca* saponins in the same concentrations as above. The results were very similar, apart from the lower threshold for aphids compared to leafhoppers: 0.01% saponin gave 50% mortality after 4 days. Additional experiments were set up with 0.1% of the extracted alfalfa saponins for each of the isolated fractions. The activity of these fractions was found to be very diverse, with clear differences not only between the fractions but also between corresponding fractions from the two cultivars. Presence or absence of mediagenic acid seemed to explain most of the differences in biological activity of 'DuPuits' and 'Lahontan' cultivars.

Szczepanik *et al.* (2001) tested total saponins from alfalfa on larvae of the Colorado potato beetle (*Leptinotarsa decemlineata*) by dipping potato leaves for 5 s in aqueous solutions at various concentrations of the extracted saponins. They observed a reduced feed intake, growth rate and survival. Again, the activity of the compounds was closely correlated with the dose. At a concentration of 0.5% the larvae hardly fed and died after 4-6 days. Lower doses (0.01-0.001%) only vaguely decreased feeding rates, but still caused significant growth inhibition, an extension of the larval stage with approximately 4 days and about 80% mortality (Fig. 3). Also, the surviving larvae generally did not reach the same body weight as the controls despite their comparative high food consumption. This suggests that the assimilation of the food ingested is lowered due to the saponins.

Szczepanik *et al.* (2004) also tested total saponins from three other *Medicago* species (*M. arabica*, *M. hybrida* and *M. murex*) on Colorado potato beetle larvae. At 0.5%, the results were similar to those for alfalfa: drastically reduced food intake and body weight, extended larval stage and high mortality. At a 0.1% dose, however, there was almost no effect, and the larval development was similar to that of the control group.

Hussein *et al.* (2005) tested the effect of saponin from alfalfa roots on the hairy rose beetle, *Tropinota squalida*. Adults fed with 750 ppm showed higher mortality ($\pm 10\%$),

reduced fecundity (40% less eggs) and hatchability. The rate of diet consumption was not affected. Second instar larvae fed one time with 75 ppm saponin suffered 40% extra mortality, they developed slower and formed smaller pupae. Only half of them became adults and they all died without progeny.

Dried alfalfa also affects growth and development of the European corn borer *Ostrinia nubilalis* (Nozzorillo *et al.* 1997). On a diet with 10% saponin, all larvae died within 9 days; lower concentrations of 0.5% and 1.6% caused 53% and 60% larval mortality, respectively. Larval growth and weight of the adults decreased with decreasing saponin concentration, while the time to pupation increased.

Adel *et al.* (2000) also reported on similar results with alfalfa saponins in the cotton leafworm *Spodoptera littoralis*: increased mortality, prolongation of development and reduced fecundity. At a dose of 10 ppm, the population growth is reduced to 10% of the controls. Although the treated larvae initially consumed less food, their feeding period lasted longer, and at the end (just before pupation) they reached the same weight as the control larvae. However, they did have a lower pupal weight. It is suggested that saponins slow down the passage of food through the gut, perhaps by reducing digestibility, and this could secondarily influence the food uptake.

Other plants

Apart from alfalfa, lots of other plants contain saponins that have been isolated and tested. A number of interesting studies is listed below (not exhaustive).

In 1970, Shany *et al.* tested lucerne saponins against *Tribolium castaneum* (red flour beetle) and observed strong growth inhibition. Sapogenins were found to be more active than the saponins they originated from. The strongest growth-inhibiting activity was exerted by mediagenic acid (insect growth was <20% of controls at a 0.1% concentration) and to a lesser extent by another unidentified sapogenin; the isolated soyasapogenols had no effect. When cholesterol was added to the diet, however, the negative influence of the saponins was abolished. The same effect was found for the other plant sterols tested (β -sitosterol, stigmasterol and campesterol).

Harmatha *et al.* (1987) saw that dried flowers and leaves from leek *Allium porrum* cause mortality and ecdysial failures in larvae of the leek-moth *Acrolepiopsis assectella*. Artificial diet with 0.4% flower extract caused 97% mortality and 19% ecdysial failure, and the effects were dose-dependent. The main active compound was aginosid, a steroidal saponin, which caused 56% mortality and 19% ecdysial failure at a 0.09% concentration; but it was probably supported by other substances. In this study as well, the effects could be counteracted by addition of cholesterol or β -sitosterol to the diet (22% mortality and 6% ecdysial failure left after addition of 0.1% cholesterol to the aginosid diet).

Nawrot *et al.* (1991) tested 14 plant allelochemicals as

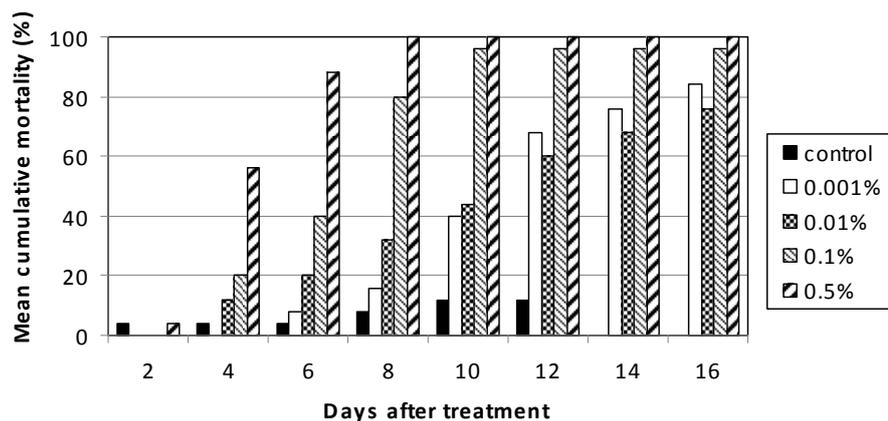


Fig. 3 Effect of different concentrations of saponins from alfalfa roots on survival of Colorado potato beetle larvae. In the control groups, measurements were stopped after 12 day because the mature larvae moved into the sand for pupation. (Redrafted from Szczepanik *et al.* 2001).

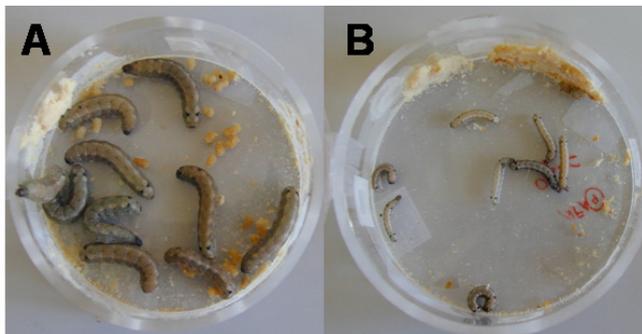


Fig. 4 Feeding of saponins at 3% in the diet caused a strong inhibition of larval growth in *S. littoralis* (B) compared to controls (A). The photos were taken at 11 days of treatment.

antifeedants and larval growth inhibitors against the variegated cutworm, *Peridroma saucia*, and the bertha armyworm, *Mamestra configurata*, in a cabbage leaf disc choice test. Aginosid again proved to be a strong deterrent to *P. saucia* larvae with >50% deterrence at 5.7 µg/cm². In a no-choice bioassay with neonate larvae, aginosid (1.0 µmol/g fresh weight) caused mortality and significant growth inhibition for both *P. saucia* and *M. configurata*. Topical application to fourth instar larvae of *P. saucia* with aginosid also significantly inhibited growth.

Westcott *et al.* (1992) tested the effect of 22 secondary plant compounds, including commercial saponins, on the migratory grasshopper, *Melanoplus sanguinipes*. Although they used up to 4% dry weight, they found no effect on survival after five days. Moreover, even if about one third of the tested compounds did affect survival rates, the grasshoppers generally proved themselves remarkably resistant against these unnaturally high concentrations of plant compounds. This fits well with the species' generalist lifestyle and important pest status.

Soulé *et al.* (2000) tested the spirostanoic (steroidal) saponin luciamin (isolated from *Solanum laxum*) on aphids (greenbug/wheat aphid, *Schizaphis graminum*). Survival rates decreased with 55%, but in contrast to earlier mentioned experiments, this decrease was the same for all saponin concentrations (50-500 µM), suggesting a deterrent rather than a toxic activity.

Taylor *et al.* (2004) tested extracts from field pies (*P. sativum*) on the rice weevil (*Sitophilus oryzae*). They isolated dehydrosoyasaponin I (an oleanane saponin), which proved to be an active antifeedant, and three lysolecithins that were inactive alone but enhanced the insecticidal activity of dehydrosoyasaponin I in mixtures.

Recent experiments of De Geyter *et al.* (2007) with *Quillaja* bark saponins (oleanane type) on pea aphids (*A. pisum*) and cotton leafworm caterpillars (*S. littoralis*) confirmed the insecticidal activities. Third instar *S. littoralis* larvae were fed on artificial diets containing saponins up to adult formation. In treatments with 3 to 7% saponins in the diet, there was ≥70% mortality. Hence, a remarkable retardation in development was observed with significant differences in weight from day 1 onwards, and the different larval stages lasted longer (Fig. 4A, 4B). The surviving larvae developed into apparently normal adults, but with slightly reduced weight. In the bioassay with first-instar nymphs of *A. pisum*, total mortality was scored within 2 days of feeding with saponin at ≥0.3%. At lower concentration (0.2% and 0.1%) the aphid nymphs lasted longer, but after 5 days they also had mostly perished (≥70% mortality). The remaining few were smaller compared to controls, and none of them developed into adults.

In the field

In laboratory experiments, the effects of saponins are clear: reduced feed intake, inhibition of growth and increased

mortality. But even if these effects are found reproducibly in artificial set-ups, this is no guarantee for their use under practical field conditions.

As mentioned before, Pedersen *et al.* (1976) compared six alfalfa cultivars, and according to their findings a high saponin concentration makes the plant resistant to pea aphids, but not to any other insect. Yábar *et al.* (2002) compared two varieties of quinoa (*Chenopodium quinoa*), Blanca de Junín and Amarilla de Maranganí, who differ only in their content of saponins late in the season. Although the overall abundance of insects on the two varieties was very similar, late in the season more insects were found on Amarilla (the high-saponin variety). And Burgess and Chapman (2005), who investigated the relation between the diet choice of invertebrate herbivores (mostly insects) and the amounts of protein, alkaloids, saponins and cyanogenic glycosides in the plants, found no correlation between invertebrate damage and any of the compounds tested.

Influence from insects on plant saponin content

Saponins in plants can affect the development and survival of insects, but the presence of insects can also have an influence on the saponin content of plants.

Agrell *et al.* (2004) investigated the herbivore-induced responses of alfalfa: plants damaged by *S. littoralis* larvae showed increased saponin levels (84%). Additionally, *S. littoralis* larvae avoided to feed on leaves that had been damaged 5 to 7 days ago (Agrell *et al.* 2003), indicating that the saponin concentration peaks after about a week. When given no choice, the larvae did feed on these leaves, but their weight was lower than that of larvae fed with undamaged alfalfa plants, although survival and development time were largely unaffected. No effect was observed at 1 and 9 days after damage; so the herbivore-induced defensive response of alfalfa seems to be very short-term.

Bede *et al.* (2006) observed the opposite effect: herbivory by the beet armyworm *Spodoptera exigua* decreases transcript levels of key regulatory genes in the early steps of the terpenoid biosynthesis pathways in *M. truncatula*. An insect salivary factor, possibly glucose oxidase (GOX) may be involved (adding GOX to mechanically wounded leaves triggers the reaction). So in this case, it appears that the insects 'intentionally' lower the defence reactions of the plant to make it easier for them to feed on it.

Exploitation of plant saponins by insects for own defence

Obviously, defensive plant compounds like saponins are meant to keep herbivore insects away. But, unfortunately for the plant, the system sometimes fails as some insects manage to obtain resistance against these compounds. Some species go even further and use the toxic compounds of their host plants for their own defence.

Morton and Vencl (1998) discovered that the faecal shields which larvae of the leaf beetles *Neolema sexpunctata* and *Lema trilinea* carry on their backs, act as a chemical defence shield against predators. Although the active compounds in the shields are not the exact same ones as found in the host plants of both species, the beetles are dependent on dietary sources for their biosynthesis, as shields of larvae of both species reared on lettuce failed to deter ants.

Prieto *et al.* (2007) found furostanol (steroidal) saponins in hemolymph extracts from sawfly larvae of the tribe Phymatocerini, which exude a droplet of deterrent hemolymph upon attack by a predator, and in samples of the toxic plants (in the orders Liliales and Ranunculales) they are specialised on. One compound was found in a 65- to 200-fold higher concentration in the hemolymph, suggesting that it is selectively sequestered.

Examples of use against insects

Dead-end cropping

The diamondback moth *Plutella xylostella* is the most serious insect pest of Brassicaceae worldwide (Talekar and Shelton 1993). According to Idris and Grafius (1996) females lay more eggs on *Brassica* crops like broccoli and cabbage than on wild Brassicaceae. Larval survival is generally higher and developmental time shorter on the crops. There is no survival on *Barbarea vulgaris*. The percentage of parasitism by *Diadegma insulare* was also higher on crops than on the wild species. Idris and Grafius (1996) concluded that choosing the appropriate *Brassica* cultivar (some suffered higher diamondback moth infestation than others) and planting wild Brassicaceae in or around the field (especially *B. vulgaris* and *B. kaber*) could seriously reduce the numbers of *P. xylostella* on the crops, and provide a reservoir for insecticide-susceptible diamondback moth and *D. insulare*. Continuing on these promising results Shelton and Nault (2004) found that *P. xylostella* preferentially lays eggs on the glossy-type *Barbarea vulgaris* when compared with broccoli and cabbage. But, as mentioned before, *P. xylostella* larvae do not develop on this plant. This creates an even more interesting situation for insect pest management: planting *Barbarea vulgaris* near fields with commercial crucifers would not only protect the crops against *P. xylostella* (since they prefer *B. vulgaris* for oviposition), but also reduce populations of this important pest species as the larvae do not survive.

Shinoda *et al.* (2002) account the non-survival of the larvae on *B. vulgaris* to an oleanane saponin with strong feeding-deterrent activity. Topical application of 0.18 µg/mm² of the isolated compound on cabbage leaves reduced consumption by third instar larvae to less than 11% of the control treatment, and consequently all larvae died. Agerbirk *et al.* (2003) compared two different types of *B. vulgaris* (the G-type and the P-type) of which one is resistant to *P. xylostella* (G-type) while the other is not. They too conclude that this resistance is due to an oleanane saponin that could be isolated only from the G-type; however, this saponin is different to the one found by Shinoda *et al.* (2002).

Vector control

Wiesman and Chapagain (2006) reported a strong correlation between saponin content and mortality for *Aedes aegypti* (yellow fever mosquito) larvae exposed to extracts of *Balanites aegyptiaca*. 0.0014% (v/w) of the most active fraction proved to be sufficient to kill 50% of the larvae before formation of adults. *A. aegypti* is the main vector responsible for the transmission of dengue fever and yellow fever. *B. aegyptiaca* grows widely in the mostly poor areas where these diseases pose a serious problem, and extraction of the active fraction is relatively easy (unpure mixtures of saponins work well in most cases). Therefore saponins, as a cheap way to reduce the mosquito population, could play an important role in controlling the diseases.

Pelah *et al.* (2002) also found high mortality of *A. aegypti* and *Culex pipiens* (northern house mosquito, vector of the western Nile virus) mosquito larvae when exposed to serial concentrations of *Quillaja* bark saponins. Third or fourth instars placed in water cups with dissolved saponins were killed rapidly, with 100% mortality within 1 day at 800 ppm for *A. aegypti* larvae and within 5 days at 1000 ppm for *C. pipiens* larvae.

Protection of stored products

Pemonge *et al.* (1997) tried to reduce destruction of stored products by Coleopteran pest species, *Tribolium castaneum* and *Acanthoscelides obtectus* (bean weevil), with powdered leaves/seeds and extracts from *Trigonella foenum-graecum*. Both topical application and addition to the diet caused adult and larval mortality, growth inhibition and decreased

fecundity.

MECHANISMS UNDERLYING THE ACTION AGAINST INSECTS

Saponins are known to have various biological properties. They have membrane-permeabilising, haemolytic, antioxidant, anti-inflammatory, immunostimulant and anticarcinogenic activities, they affect feed intake, growth and reproduction in animals, and they can be used as fungicides, molluscicides and pesticides, as well as against some bacteria and viruses (Francis *et al.* 2002; Sparg *et al.* 2004; Avato *et al.* 2006; Tava and Avato 2006). In this part we will discuss the activity of saponins against insects.

Saponins give rise to increased mortality levels, lowered food intake, weight reduction, retardation in development, disturbances in development and decreased reproduction in pest insects. The mechanism underlying these actions is, however, still largely unknown, but it is likely that saponins have multiple activities. The main hypotheses are that saponins could either make the food less attractive to eat (repellent/deterrent activity), bear digestive problems, cause moulting defects or have toxic effects on cells.

Repellent or deterrent activity

Numerous of the above mentioned studies reported a lower food intake of insects fed on saponin-containing food (by measuring the area of leaves/amount of artificial diet consumed), although in nearly all cases the test insects made at least some attempts to feed on the plants/diets before rejecting them (Adel *et al.* 2000; Szczepanik *et al.* 2001; Shinoda *et al.* 2002; Agerbirk *et al.* 2003; Agrell *et al.* 2003; Szczepanik *et al.* 2004; Taylor *et al.* 2004; Golawska *et al.* 2006). Here in all these cases, the reduction of food intake was dose-dependent. However, two exceptions were reported: Hussein *et al.* (2005) found that the rate of diet consumption was not affected (in spite of high mortality rates), and Soulé *et al.* (2000) saw an equal decrease in survival for all concentrations of saponins tested.

Slowing down the passage of food through the gut

According to Ishaaya (1986) saponins slow down the passage of food through the insect gut. Perhaps they reduce the digestibility of the food by inhibiting the secretion of digestive enzymes (proteases) (Ishaaya and Birk 1965; Golawska *et al.* 2006) or by formation of sparingly digestible saponin-protein complexes (Potter *et al.* 1993). An obstruction of alimentary contents in the gut would limit or inhibit food uptake. Starvation, as well as disturbance of the digestion and assimilation processes, could reduce the insect growth rate. Adel *et al.* (2000) supported this hypothesis because in their experiment the treated larvae lost more weight than the controls just before pupation (gut purge).

Blocking sterol uptake

Insects are not capable of synthesizing sterol structures by themselves (Belles *et al.* 2005), but they do need them for the synthesis of steroids like cholesterol and the insect moulting hormone 20-hydroxyecdysone (20E). That means they have to gain them from their food; cholesterol or phytosterols from plants act here as precursors (Belles *et al.* 2005). Shany *et al.* (1970) suggested that saponins can block sterol uptake. Saponins can form insoluble complexes with sterols, thereby preventing their absorption. If all cholesterol in the food is bound to saponins, the insects cannot utilise it. Moreover, if larvae feed on a saponin-rich food, the ingested saponins may complex even cholesterol in their body, and thus suspend the biosynthesis of ecdysteroids. This could cause a disturbance of ecdysis (ecdysial failures) (Harmatha *et al.* 1987; Harmatha 2000). It was observed that the effects of the saponins could be countered by adding a surplus of cholesterol or plant sterols to the diet

(Shany *et al.* 1970; Harmatha *et al.* 1987).

Antagonistic or competitive activity on the ecdysteroid reporter complex

(Steroidal) saponins have a steroid structure and show structural similarity to ecdysteroids, like the insect moulting hormone 20E. Some other secondary plant compounds with a similar structure are known to perform an antagonistic or competitive activity on the receptor site for 20E, i.e. the ecdysteroid reporter (EcR) (Dinan *et al.* 2001). Likewise, saponins could exert a similar mode of action and disturb ecdysis. There are few, if any, indications supporting this hypothesis. Digitonin and aginosid, two steroidal saponins, were tested for their direct effect on the ecdysone receptor in a *Drosophila melanogaster* B-II assay by Harmatha and Dinan (1997), together with two additional leek flower saponins and some aglycones; but none of the compounds showed significant agonistic or antagonistic effects on EcR. In another B_{II} cell bioassay for ecdysteroid agonist and antagonist activities (Dinan *et al.* 2001) there were no saponins showing agonist activity but few showed principal antagonistic activity on the EcR complex.

Membrane-permeabilising abilities

On the cellular level, saponins might be toxic because of their membrane-permeabilising abilities. Saponins are known to cause lysis of erythrocytes *in vitro*. The primary action of membranolytic saponins upon the cell is to cause a general increase in permeability of plasma membranes. They can interact with and permeabilize the small intestine mucosal cells of animals, leading to a marked reduction in their ability to transport nutrients (Francis *et al.* 2002). This is suspected to be result of their bipolar structure. The lipophilic component of the saponin could be easily integrated into the lipid fraction of the plasma membrane. The hydrophilic glycosidic portion that follows the lipid fraction presumably irreversibly disorders the plasma membrane and disrupts its integrity. It is of interest to mention that this membrane-permeabilising ability of saponins can also have beneficial effects on animals (and humans): small quantities of dietary saponins may assist in the absorption of nutrients, drugs and toxins by increasing the permeability of the small intestine mucosa (Chuke 1967; Oakenfull *et al.* 1979). However, so far there are no indications for beneficial effects on insects.

Other aspects

Apart from working on the insect gut saponins can also affect the microflora living in there. For most herbivore insects (invertebrates) the digestion of leaf material is mediated by symbiotic microorganisms that reside in the hindgut (Waterman 1993). Any compound that kills off a reasonable amount of these supporting bacteria could undermine the insect's digestive capabilities.

Also, under natural conditions the situation might be a bit more complicated than in the laboratory. Oleszek *et al.* (1992) tried to quantify the biological activity of some saponins from alfalfa by measuring their inhibitory activity against the fungus *Trichoderma viride*. Initially they found little or no growth inhibition in a laboratory test. However, when the main compound (zahnac acid tridesmoside, an oleane saponine) was then hydrolysed in alkaline or acidic solution, it degraded easily into its monodesmoside, and the antifungal activity increased considerably. So when this rather inactive compound is eaten by an animal, the hydrolytic conditions in the gut will cause it to break down and it will become an active compound, which could influence the microflora and/or various digestion and absorption processes. This makes clear that laboratory tests are not overly useful to determine a compound's activity, unless real life circumstances are taken into account.

Adel *et al.* (2000) tested the activity of a number of

saponins and sapogenins on the cotton leafworm *S. littoralis* and learned that the activity of glycosylated saponins depends on their sugar moiety. So it is likely that glycosylated saponins exert insecticidal activity only when they are hydrolyzed (i.e. the sugar component is cleaved off) by insect gut glycosidases, liberating an active aglycone. Yet glycosylation may be useful because it renders the apolar aglycones water-soluble and thereby facilitates their ingestion. These results are confirmed by Shany *et al.* (1970) and Avato *et al.* (2006), who found that sapogenins are more active than saponins against red flour beetles and several bacteria, respectively.

After having listed the potential mechanisms underlying the insecticidal action of saponins postulated in literature, it appears that there is no simple answer to the question how exactly saponins perform their insecticidal activity. The variable effects observed support the notion that probably multiple activities are involved. We believe that the mechanism is likely to depend on the type of saponin, the concentration/dose exposed, and the species and development stage of the insect.

CONCLUSIONS AND FUTURE PERSPECTIVES

Saponins have potency to be used as natural insecticides. They exert a strong insecticidal activity against a broad range of insect types and stages. Already at sublethal concentrations, saponins lower the food intake of the insects, thereby reducing the damage done to the crops. Another advantage is their immediate impact on insects. This is important not only for protecting plants against insect pest damage, but even more so to prevent transmission of insect-mediated diseases.

Saponins could be applied exogenic, by spraying them on fields. At this moment, saponin powder and solutions are already commercially utilized as natural insecticides in the Asian region (China), for example "Liquid Tea Saponin" from Hangzhou Choisun Tea Sci-Tech Co. Ltd. Farmers could also employ the natural saponin content of plants by selecting high-saponin varieties of commercial plant species. Perhaps, if the side effects (for example bitter taste) do not overrule the advantages, the presence of saponins might be a trait to be selected for in plant breeding programs.

Further investigations may also be focusing on investigating the detailed insecticide mode of action of saponins, for a better understanding of their structure-activity relationship and specificity. Also in the context of modern agriculture and horticulture with IPM to control pest insects, it is essential to obtain more insights about the selectivity of saponins and their effects on relationships with natural insect enemies and the ecosystem.

ACKNOWLEDGEMENTS

The authors acknowledge support from the Special Research Fund of Ghent University (BOF-2005; project 01101405) and the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT, Brussels; project 040093).

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