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A Preliminary Study of the Effects of Glucosinulates and their Degradation Products in Plant Defense

Glucosinolates are an important and unique class of secondary plant compounds found in seeds, roots, stems and leaves of plants belonging to at least 11 plant families of dicotyledonous angiosperms, the Cruciferae being the most important (1,4). In addition, each plant species and genotype generally contains one predominant glucosinolate (3). This finding is important both for taxonomy and genetics of these plant families, and is also important for successfully isolating these compounds, especially for obtaining high yield and purity.

Glucosinolates are a class of molecules composed of approximately one hundred identified naturally occurring thioglucosides (5) with a common structure based on side chains (R) with varying aliphatic, aromatic and heteroaromatic carbon skeletons, all presumable derived from aminoacids by a chain-lengthening process and hydroxylation or oxidation reactions (1).

In the intact cell, the glucosinolates are separated from ß-thioglucosidase (E.C. 3.2.3.1) an enzyme generally known as myrosinase, which catalyses the hydrolysis of these compounds when the plant cell structure is damaged. The enzymatic hydrolysis of glucosinolates yields D-glucose, sulphate and a series of compounds with different side chain, such as isothiocyanates, thiocyanates and nitriles, depending on both the substrate and the reaction conditions, especially the pH. Numerous studies have demonstrated that some of such breakdown products, in addition to being responsible for the characteristic pungent flavor, are associated with some nutritive and antinutritional effects (6). In addition, these compounds, which are derived from glucosinolate hydrolysis, seem to have various biological activities. In fact, it has been reported that some of them inhibit the neoplastic effects of carcinogens (7), show antifungal and antibacterial activities (8), possess therapeutic properties against liver diseases (in fact extracts of radish roots were considered an old remedy) (13) and, finally, seem play a role in the insect and nematode attack (8,15,16).

This communication regards this last aspect, which is intriguing and of great current interest both as fundamental research aimed at a deeper understanding of the natural defense mechanism and for the practical agricultural use of glucosinolate-breakdown products as insecticides of biological origin.

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Although the molecular genetics of plants offers potentially powerful tools to induce an organ specific response via the prodution of a specific active protein as a result of insect attack (i. e. protein inhibitors), the possible utilization of certain compounds as biological insecticides to which man got rather used to during centuries, appears to be attractive and obtainable in a reasonable time. This type of research appears to be especially important if one considers environmental and consumer safety, which is receiving more and more attention today.

Over past few decades much has been written about the drawbacks of glucosinolates and their aglucone derivatives, because they are generally associated with endemic hypothyroidism and hepatossicity in human and animal nutrition. However, much less has been written about the potentially advantageous aspects that might emerge new technological uses. In this regard, glucosinolates and their enzymatic degradation products have been receiving increasing attention in the last decade as biological effectors towards phytophagous insects and fungi, although the first experiments in this field date back to the early years of this century (19).
The major part of studies involving glucosinolate and their hydrolysis products have been limited to interactions between crucifers and their potential herbivores and pathogens. For instance, it has been asserted that the feeding habits of insects and hostplant specificity are fixed by the effect of plant chemical compounds such as glucosinolates and their degradation products, which attract or repel the insects, thereby influencing their behavior. Particularly, it seems that glucosinolates function as attractans for crucifer-feeding insects and as repellents for insects that do not feed on crucifers.

The mechanism of the myrosinase-glucosinolate system in plants is very interesting because, as we have just seen, the two compounds are normally separated inside the cell. When the insect attacks the plant it generally makes a wound with its ovipositor, stilet or masticatory apparatus thereby putting glucosinolates and myrosinase in contact. Thus the hydrolysis reaction starts with the formation of toxic compounds only at the point of attack. This action has been studied only for some pathogens such as the swallowtail butterflies (20), aphids (21), some diptera (22) and some fungi (23). All these studies seem to confirm that the breakdown product or products have a real toxic effect.

In our experience we have seen a certain toxic activity of some intact glucosinolates, which is presumable due to the presence of some ß-thioglucosidases inside the insect which are able to catalyse the hydrolysis reaction. Studies aimed to clarify this effect are in progress in our laboratory.

In this regard we want to emphasize that these results are preliminary and much remains to be done to understand fully the role glucosinolates play in the defense mechanism. Nevertheless, glucosinolates appear to be very important protective compounds in the Cruciferae (24), although there are other secondary compounds, for example, cardenolides and cucurbitacins, which also seem active in plant defense mechanisms. However, glucosinolates and their breakdown products show some activity against nematode *Heterodera schachtii Schmidt*. In fact, our preliminary results indicate that these phytophages are not able to live in 0.5 % 4-methyltio-3-butenyl isothiocianate aqueous solution, which is the breakdown product of the most important glucosinolate contained in *Raphanus sativus* roots.

On the other hand, nematodes live without any problem in a 0.5 % aqueous solution of glucorafanin isothiocianate, the hydrolysis product of the main glucosinolate of *Raphanus* seeds.

This simple trial demonstrates the different activities of these two glucosinolates, although they are chemically related: glucorafanin is the oxidized form of 4-methyltio-3-butenyl glucosinulate. Taken together this preliminary results appear a resistance against nematodes quite promising.

In this regard it has been shown that growing of some cultivar of *Raphanus sativus* significantly decrease the infestation of the nematodes in the ground (25). Furthermore these results indicate the theoretical possibility of controlling only particular insects, without affecting the biological stability of the useful insects. In this regard we observed that some glucosinulates that produce high mortality in larvae of *Lobesia Botrana* appear to have no effect, at the same concentration, on the predatory mites.

We must point out that these results are still preliminary and further work must be done before practical utilization become faible. In particular, a series of problems relating to the phytotoxicity, volatility and ecological impact of the breakdown products should be studied and well evaluated.

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