

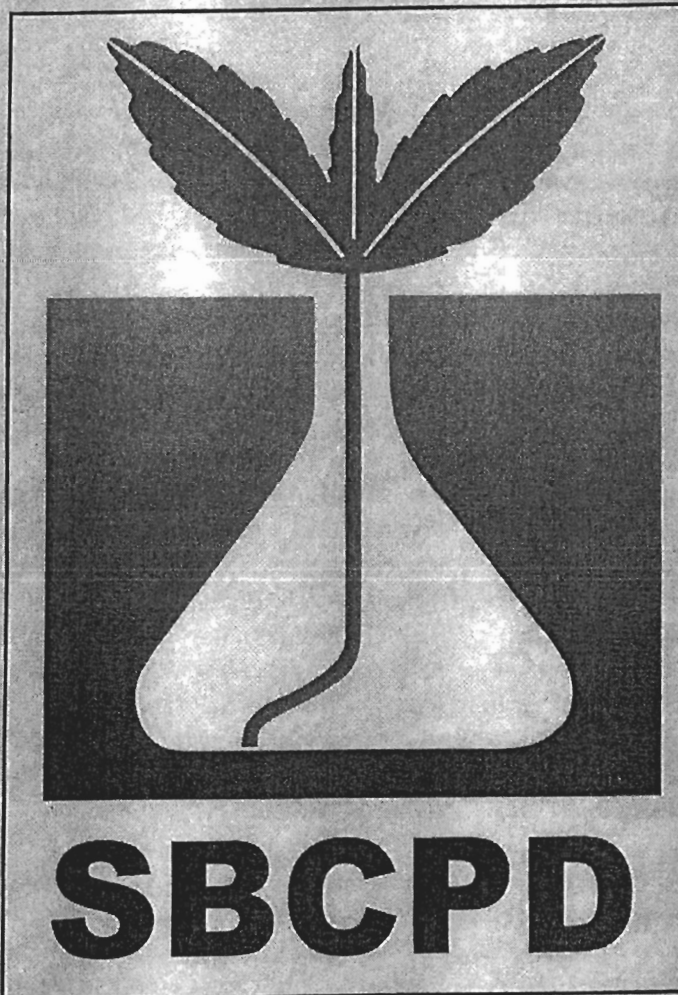
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# ALLELOPATHIC STUDIES IN SOSTAINABLE AGRICULTURE: DEVELOPMENT OF NEW AGROCHEMICALS

by

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## INTRODUCTION

Weeds may be defined as plants with little economic value and possessing the potential to colonize disturbed habitats or those modified by human activities. They have also been considered as potential sources of genetic diversity and wild relatives of crop plants, and hence must be preserved for future crop improvement programs (Hammer *et al.*, 1997).

Weeds cause a number of harms in agroecosystems. Due to their interference with crops (both competitive and allelopathic), they reduce crop yields leading to huge losses on a global scale. Allelopathic interference mechanisms are particularly difficult to separate from competition in field conditions. Plant interference can be defined as any physical or chemical mechanism that results in the reduction of plant growth over time due to the presence of other plant (Leslie A. Weston and Stephen O. Duke (2003) "Weed and Crop Allelopathy" *Critical Reviews in Plant Sciences* 22:367-389). Competition is usually described as the process whereby plants interfere with the growth of neighboring plants by utilization or competition for growth-limiting resources (light, space, nutrients and moisture). About 240 species are reported to be allelopathic and interfere with the growth and production of crops (Qasem and Foy, 2001). That provokes diminution of the crop quality, clog water ways and may cause health problems in humans. Further, the costs of weed eradication are also enormous. Piementel *et al.* (2001) have pointed out that in U.S. weeds cause about a 12% loss in crop yield and it costs nearly US\$35 billion to control them. The costs are even more in developing countries. Weeds may also harbor insects and pathogens, adding more complications to their control.

In light of these characteristics of weeds and their hazards, it becomes imperative to control them. A number of management practices are available. In the earlier times, since no synthetic chemicals were known, crop rotations, polyculture, and other management practices were tried that were low input but sustainable. With the discovery of synthetic herbicides in the early 1930s, there was a shift in the weed management practices toward high input and target-oriented ones, but the use of herbicides has provoked an increasing incidence of resistance to herbicides in weeds, so far 272 weed-resistant biotypes belonging to 172 species (98 dicots and 64 monocots) toward herbicides have been identified (Holt and LeBaron; 1990 Shaner, 1997; Heap, 2003) and new, more efficient and specific herbicides are required (Petsko, 1999). Newly commercialized herbicides have principally the same site of actions that were discovered before 1985 (Hay, 1999).

By these reasons, other important factor that should be considered is sustainability. New compounds must be environmentally friendly (Evans, 1999). But the term sustainable technology includes different points of view: agrochemical, environmental and economical. There is a need to discover safe compounds with new sites of action; however, this is not enough. Novel compounds should have a wide rage of activities but be targeted at one particular problem.

Plants have their own defence mechanisms and certain allelochemicals may act as natural herbicides. Allelopathy is defined (Rice, 1984) as the effect(s) of one plant (including microorganisms) on another plant(s) through the releasement of a chemical compound(s) (allelochemicals) in the environment. Both crops and weeds produce phytotoxins that could be allelochemicals providing an advantage in plant-plant competition. The knowledge of chemical relationships between plants may allow the development of new herbicides (Macias, 2001). If we mimic nature, we could get new compounds with different sites of action.

Allelopathy could be used by several ways in weed management: allelopathic cover or smother crops, allelopathic rational or companion crops, mulch or incorporation of crop residues, production of allelopathic crop cultivars with weed-suppressing potential, and as sources of natural herbicides. The two later topics will be discussed.

## ALLELOPATHIC INTERACTIONS

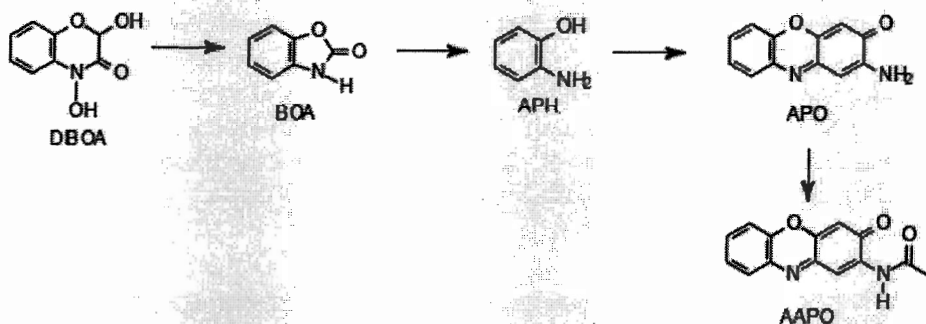
Allelopathy was defined by Rice in 1984 as 'The science that studies processes, in which secondary metabolites from plants and microorganisms are involved, affecting growth and development of biological systems' (Rice, 1984). These studies of biological interactions between species are one of the most interesting alternatives in crop protection. Allelopathic researchers can take advantage of inhibitory or stimulatory effects of one plant over others present in the ecosystem. The use of secondary metabolites involved in allelopathic interactions as templates for new agrochemical models could satisfy the requirements for crop protection.

The study of ecological relationships in the ecosystem begins with a field observation (bare zones under plants in rainforests, crops with low population of weed). Once the plant involved is identified, the influence over other plants, the study about how the substances are released to the environment, the identification of those substances responsible for allelopathic interaction, and the characterisation of degradation processes must be achieved. Traditionally, allelopathic studies were focused on evaluation of phytotoxic activity of plant residues or crude extracts (Weston, 1996). Today, the recent advances in separation and structural elucidation techniques have made a great improvement in allelopathy studies, since minimum amounts of bioactive compounds can be detected, isolated and characterized (Mallik, 2000). Thus, a more complete study is achieved, identifying the natural products responsible for the bioactivity, their modes of action and their fate, role and toxicity in the ecosystem. These imply an adequate selection of starting material, an extraction method able to imitate natural conditions, and a standard method for bioactivity evaluation.

The selection of the starting material (roots, aerial part, even flowers or fruits) is extremely important because compounds present in every vegetable tissue may be very different. In most cases, aerial part or root exudates are employed. The traditional extraction methods, based in soaking the plant material in water or organic solvents, present a lack of selectivity. As an alternative, new methods based on the imitation of natural conditions (rain simulation or root exudates recycling) offer more selectivity to find compounds related with the ecological interaction.

Due to the high requirements for actual allelopathy research, isolation, identification and bioactivity evaluation are not enough to propose a natural product as an agrochemical model. Some aspects as the stability of products are important factors to be taken into account. These allelochemicals can suffer degradation in soil by fungi or bacteria to a wide variety of compounds. These degradation products can have important roles in the ecosystem, since their bioactivity can differ from that of original compounds released by the plant. These bioactivities can be lower or ever higher. Other factors as synergism or antagonism between compounds exudated and/or generated by bacteria or fungi have to study.

An example of the complexity and possibilities of these studies could be the allelopathic interactions of wheat and many cereals. DIBOA (2,4-dihidroxy-1,4-benzoxazin-3-one) is an allelochemical produced by many important commercial cultivars including wheat, maize and rice, with phytotoxic bioactivity. Several degradation pathways for this compound has been reported, (Yue *et al* 1998 & Zikmundová *et al* 2002) (Figure 1). Some compounds have been described to be derivatives from DIBOA. Thus, 2-benzoxazinone (BOA), 2-aminophenol (APH), 3-aminophenoxazin-2-one (APO) and 3-acetamidophenoxazin-2-one (AAPO) have been isolated in bacteria or fungi cultures. We evaluated degradation kinetics, and biological activities of DIBOA and its derivatives in order to find those compounds responsible for the allelopathic phenomenon.

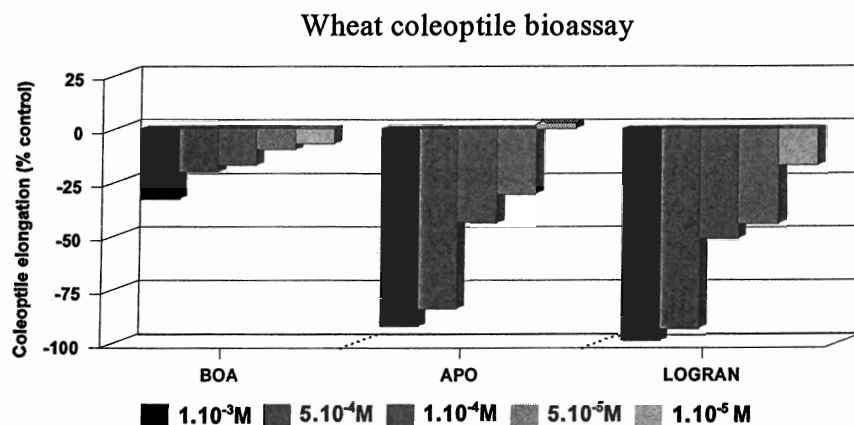


**Figure 1.** Degradation process of allelochemical DIBOA.

Degradation process to take place depends on bacteria population in soil, and this population can change with the variety of the culture in study (wheat in this case). We selected bioactive wheat varieties, and collected soil samples that were inoculated with solutions of DIBOA and BOA. They were analyzed after different time incubation using a HPLC-DAD system and the conversion from BOA to APO was observed. The degradation rate depended on the wheat variety. Anyway, the process is so quick that neither DIBOA nor BOA can be the only responsible for the allelopathic bioactivity observed in wheat for these benzohydroxamic acids and derivatives. (Macías *et al* 2004)

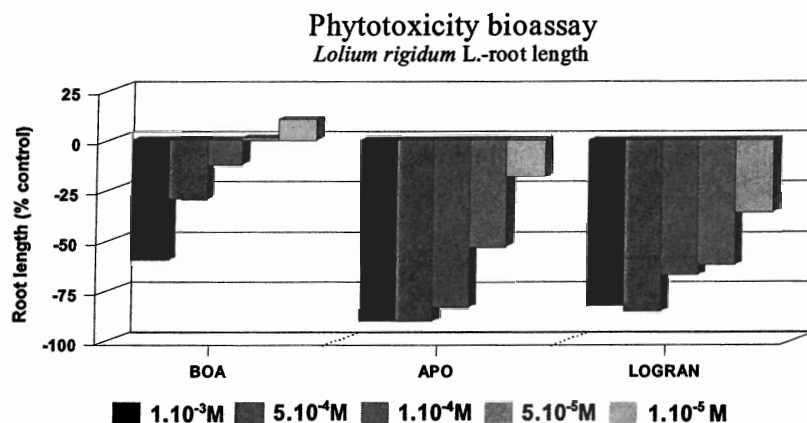
The high concentration of APO after degradation step suggested that APO could be one of the most responsible compound of the allelopathic activity. Bioactivity of BOA and APO was studied and compared and we observed that in the wheat coleoptile bioassay both compounds showed significantly levels of bioactivity. APO bioactivity presented similar profiles for those observed for LOGRAN<sup>®</sup>, a herbicide used as internal standard. In the graphics, (Figure 2), zero represents control, positive values stimulation, and negative values inhibition of the parameter. The main degradation product, APO, showed excellent bioactivity levels (almost 100 % inhibition at  $10^{-3}$  M concentration) in comparison to BOA.

APO showed also a high persistence with dilution (reduction of 50% in concentration reduces bioactivity less than a 10% at  $5 \cdot 10^{-4}M$ ).



**Figure 2.** Results for the general activity bioassay.

Similar results are observed in phytotoxic activity bioassay. The species tested were *Triticum aestivum*, *Allium cepa*, *Lycopersicon esculentum*, *Lactuca sativa* and *Lepidium sativum*. In addition, two species of weeds-*Avena fatua* L. and *Lolium rigidum* L. were introduced in the bioassay. The effects over root length of *L. rigidum* can be shown below (Figure 3). The inhibition behavior and the bioactivity profile is similar to the observed in general bioassay conditions, clearly inhibitory with excellent levels of phytotoxicity for APO.



**Figure 3.** Results for phytotoxicactivity bioassay over *Lolium rigidum* L.

These data allow us to conclude APO to be the real responsible for the bioactivity observed in plants with high amounts of DIBOA, and suggest a collaborative interaction between the plant and soil microorganisms population.

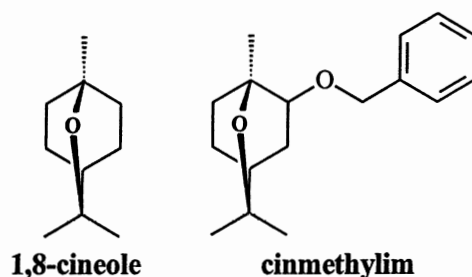
Moreover, the allelopathic phenomenon is more complex. A wide variety of toxic chemicals has been identify as produced by ungi and bacteria in rhizosphere of wheat. It has been reported that various extracts of these fungi and bacteria affect the elongation of roots and shoot of wheat and maize.(Ma Ruixia, 1997)

In agroecosystems crops, weeds, trees, and microbes constitute the biotic components, which not only interact among them but also with the abiotic environment. The allelopathic interactions among various biotic components have a great potential in improving crop production, genetic diversity, maintaining ecosystem stability, nutrient conservation, and above all in management of weeds and pests (Altieri and Doll, 1978; Putnam and Duke, 1978; Leather 1983b; Purvis, 1990; Einhellig, 1996; Swanton and Murphy, 1996; Weston, 1996; Kohli et al., 1998a; Anaya, 1999; Chou, 1999; Singh et al., 2001).

Unlike weeds, crops have a great potential in managing weeds in a number of ways. They can directly interfere with the weedy species by releasing chemicals into the environment. Batish et al. (2001a) have listed 35 crops affecting the weeds. This property can be capitalized for sustainable weed management. Further, the residues of crops like rye, sunflower, wheat, and barley, etc. could also be extremely useful in suppressing weeds (Leather, 1983b; Liebl and Worsham, 1983; Barnes and Putnam, 1986; Weston, 1996; Batish et al., 2001a). The allelopathic property of cover, smother, and green manure crops or crops grown in rotation could form another strategy worth exploiting for weed management (Leather, 1987; Jordán, 1993; Weston, 1996; Anaya, 1999; Chou, 1999; Singh et al., 2001; Batish et al., 2002a). Unfortunately, modern highyielding cultivars are devoid of this potential because of excessive selection pressure toward the qualitative and quantitative enhancement of yield (Lovett et al., 1982). Foley (1999) suggested that wild relatives of crop cultivars should be identified, and genes lost during cultivation should be reintroduced through molecular genetic techniques or conventional methods.

#### NATURAL PRODUCTS AS HERBICIDES

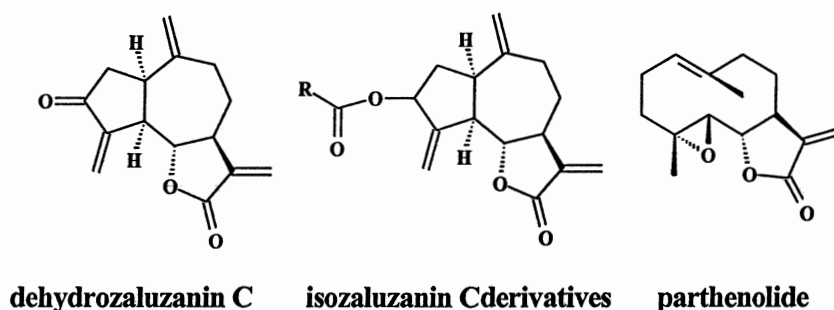
The use of natural products as a reservoir of bioactive compounds has been extensively used in medicine, being the discovery and use of antibiotics just an example. The ethnobotanical approach focusing the studies on those plants traditionally used in medical practices throughout the world evidences the importance of nature as a source of new drugs. This point of view has been used only recently in agronomic studies. During the last two decades the search of herbicides and pesticides from natural sources has raised. However, only a small number of compounds have been marketed as herbicides. Cinmethylin, a derivative of the allelopathic monoterpene 1,8-cineole, and glufosinate, the synthetic version of the fungal metabolite phosphinothricin, are the most commonly mentioned examples. However, it is important to mention that no matter the structural similarities between cinmethylin and 1,8-cineole (Fig. 4) are, the design and development of this product was not made on a natural-product based strategy. Still, it is also noteworthy to point out that this coincidence illustrates the great possibilities of this approach, in spite of the small amounts of compound obtained usually and their structural complexity, that are still challenging their applicability as herbicides



**Figure 4.** Natural product 1,8-cineole and commercial compound cinmethylin.

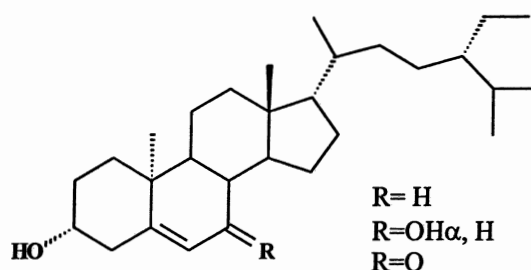


guaianolides and other germacranolides like parthenolide (13) (Macías, 1999b) have been reported (Figure 6). In particular, the mode of action of dehydrozaluzanin C, not totally well established yet, represents a new target site that makes this compound suitable as lead for further development.



**Figure 6.** Sesquiterpene lactones with phytotoxic properties.

Some steroids (Figure 7) isolated from small sweetclover (*Melilotus messanensis*) are active plant growth promoters, stimulating germination and growth of *Hordeum vulgare*. Administration of these compounds to crops would produce positive effects in crop production. Their main advantage offered by these compounds is the low dose needed for bioactivity, since effects could be observed in aqueous solution at  $10^{-7}$ M. (Macías *et al* 1999a).



**Figure 7.** Steroids from *Melilotus messanensis*.

Microorganisms can be considered as a source of new allelochemicals being researched at this time, and their phytotoxic and pharmacological activities are creating a growing interest. Between the many compounds isolated from *Penicillium brevicompactum* Dierckx, Breviones have attracted our interest for its chemical structure and biological properties.

These mixed biogenesis allelochemicals have a diterpenoid moiety joined to a poliketide ring through a spiranic bond. Our current efforts in this matter are directed to develop a synthesis procedure for these molecules, and introducing them and their synthetic analogs in structure-activity relationships studies. Preliminary results at this point suggest higher bioactivities in breviones and related structures with a seven membered ring on the diterpenoid moiety (breviones C, D and E).

## ***FUTURE TRENDS***



All of these examples constitute promising compounds that could be used as lead for the development of new herbicides and to illustrate the idea that nature constitutes a rich source of possible and marketable solutions to solve the actual agriculture problems. We have to maintain the efforts at new phytotoxic natural products isolation from fungi and allelopathic plants. Once they have been identified, it is necessary to elucidate their modes of action and to carry out structure-activity relationship (SAR) studies to design new, biorational herbicides.

The application of allelopathic knowledge will help us in the development of new environmentally friendly agricultural practices.

One important possibility in the future will be the determination and cloning of the genes responsible of the phytotoxins production and its incorporation into commercial crops.

#### **Acknowledgements**

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biotransformation by *Chaetosphaeria* sp., an endophytic fungus from *Aphelandra tetragona*.  
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