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Allelopathy as a new strategy for sustainable ecosystems development

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Abstract Natural products involved in plant-plant and plant-microorganism ecological interaction (Allelochemicals) are an important potential source for alternative agrochemicals and pharmaceuticals, in order to solve the many problems derived from inadequate culture practices and abuse of synthetic herbicides. Isolation, structural determination, bioassay techniques and applicability for these compounds in crop protection and pharmaceutical research are discussed, and future trends on Allelochemicals applications are examined. The new strategies for sustainable ecosystems controlled by allelochemicals offer a particular interest for the development of human bases in space, since these products can stimulate or inhibit plant germination and growth, and permit to develop crops with low residue amounts in water, facilitating wastewater treatment and recycling.

Key words:Allelochemical, herbicides, pharmaceuticals, crop protection, bioassay

Introduction

The abuse of chemicals used in crop protection has produced several problems, related to the appearance of herbicide-resistant weeds and high impact produced over human health and environment. 6 billion $ have been spent in chemicals for the protection against the 7000 weed species identified to the date, (Patterson 1985, Macías 1995) and the weed problem is far to be solved, since the efficiency of the chemical treatments for crop protection is rapidly decreasing. This loss of efficiency is mainly due to the resistance developed by weeds to the chemical treatments. Weed species evolution against herbicides has been possible since the hundreds of commercially available herbicides have just a few modes of action. The number of herbicide-resistant weed biotypes (Kropff *et al* 2000) has been increasing since the herbicide use expansion in the 1950's. In addition to this, new exigencies of law and consumers about food quality and environmental conservation complete the challenge for crop protection researchers, focused on the searching for chemicals with new modes of action, more specific interaction with weeds, low toxicity for humans and animals, and less environmental damage (Dayan *et al* 1999).

Allelopathy is defined 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). Although the term 'allelopathy' was firstly used in 1937, Pliny (I A.D.) was the first to record the inhibitory effect of Walnut Tree (*Juglans nigra*) over near plants and crops. This study of biological interactions between species is one of the most interesting alternatives in the search for alternatives in crop protection, since allelopathy researchers can take advantage of inhibitory or stimulatory effects of one plant over others present in the ecosystem, using the secondary metabolites involved as templates for new agrochemical models able to satisfy the new requirements for crop protection, including the implantation of cultivars in space.

A crop to be developed in a human base in space has to satisfy many and very exigent requirements, related to the limited amount of water available and the need for water recycling, being a low residue production very important in the optimization of such process. Being the crop production the base of the trophic pyramid, biomass yields have to be strictly controlled and optimized, and allelochemicals with crop stimulation capabilities would be the key for this optimization.

Methodology in allelopathy research

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 in allelopathic interaction is identified, it's time to isolate and characterize the substance(s) responsible for allelopathic interaction. The way the substances present in a plant are released to the environment includes volatilization from leaves, leaching from roots or aerial parts, exudation from roots, or liberation by decomposition of vegetal tissues by fungi or bacteria (Putnam 1983). 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

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study is achieved, answering to three main questions:

-Which natural products are the responsible for the bioactivity?

-What are their modes of action?

-What are their fate, role and toxicity in the ecosystem?

An accurate answer to these main questions is related to an adequate selection of starting material, an extraction method able to imitate natural conditions, and a standard method for bioactivity evaluation, able to compare between different substances and to measure effects over a wide range of targets.

The selection of the starting material (roots, aerial part, even flowers or fruits) is extremely important due to the different types of compounds present in every vegetable tissue. Most researches are related to aerial part or root exudates. The traditional extraction methods, based in soaking the plant material in water or organic solvents, extracted almost all compounds with 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.

Bioactivity evaluation

The search for new agrochemical models based on allelochemicals requires an accurate protocol for the evaluation of phytotoxicity or plant growth stimulation activity. This protocol has to satisfy several exigencies: has to be statistically reproducible and has to test compounds over a wide range of representative species with the **minimum amount** of the product used. (Duke *et al* 2000) Our research group has developed a standard bioassay for the measurement of allelopathic activity over Standard Target Species (STS) (Macías 2000). The target plants used for this bioassay include monocotyledonous and dicotyledonous commercial cultivars, representative of families of crops and weeds, and imitate natural conditions of products action. Optimum populations of seeds are immersed on buffered solutions of the compound or extract to study at different dilutions, to study the effect of concentration in biological effect. A commercial herbicide is used as internal standard and effects over germination rate and root and shoot growths are recorded. This specific bioassay can be preceded by other bioassay of general bioactivity (Wheat coleoptiles bioassay) in which the effect of compounds or extracts over vegetable undifferentiated tissues are measured (Cutler 1984) This bioassay offers reliable results over 24 h., and it is used for an initial screening that allow us to focus the phytotoxic evaluation over the more active materials. The protocol has also been developed by its efficiency in the detection of pharmacological properties. A correspondence can be observed between the results of the general bioassay protocol and the phytotoxic bioactivity assay.

Integrated allelopathic studies

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, and now our studies consider stability of products as an important factor. Degradation of allelochemicals in soil by fungi or bacteria yields a wide variety of compounds, that can have an important role in the ecosystem, since their bioactivity can be different of the original compound released by the plant, and can act in synergism or antagonism with the plant compounds, depending on the participation of those bacteria or fungi in the ecological phenomenon.

A good example could be the studies that have been done with DIBOA (2,4-dihidroxy-1,4-benzoxazin-3-one). It 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*)* (Fig. 1) and it was decided to evaluate degradation kinetics, and to study biological activity of its derivatives in order to find the actual responsible compound for the allelopathic phenomenon in those cultivars and to propose new agrochemical models based on them. (Macías et al. 2003)

DIBOA degradation process

Being DIBOA Glucoside (Niemeyer 1988) the metabolite produced by plant, several processes takes place in soil and some compounds have been described to be derivatives from DIBOA yielded in biotransformation routes. Thus, 2-benzoxazolinone (BOA), 2-aminophenol (APH), 3-aminophenoxazin-2-one (APO) and 3-acetamidophenoxazin-2-one (AAPO) have been isolated in bacteria or fungi cultures.

Since 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), the most bioactive wheat varieties were selected, and soil samples of 'Astron' and 'Ritmo' wheat crop varieties were collected and inoculated with solutions of DIBOA and BOA. Samples of these solutions taken in several times were analyzed using a HPLC-DAD system. The process observed in these wheat soils was the conversion from BOA to APO.

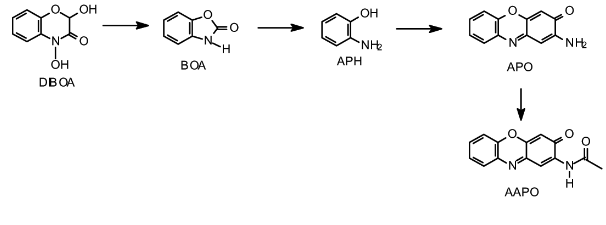

Fig. 1. Degradation process of allelochemical DIBOA

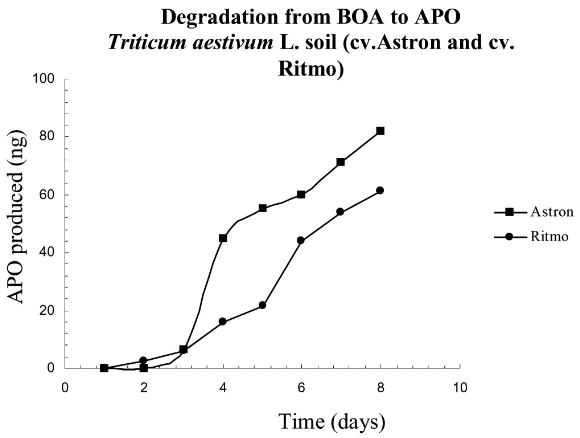

Fig. 2. Degradation process for DIBOA: APO produced vs. time.

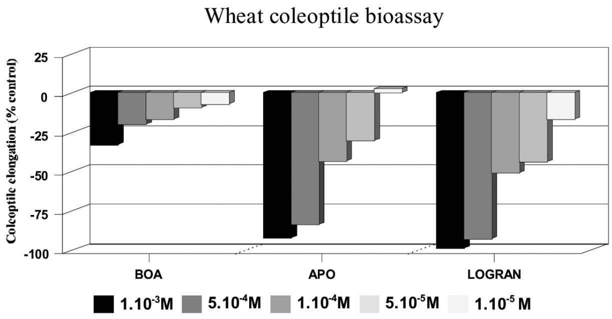

Fig. 3. Results for the general activity bioassay.

Table 1

Monocots	*Triticum aestivum* L. (Wheat) *Allium cepa* L. (Onion)
Dicots	*Lycopersicon esculentum* Will. (tomato) *Lactuca sativa* L. (lettuce) *Lepidium sativum* L. (cress)

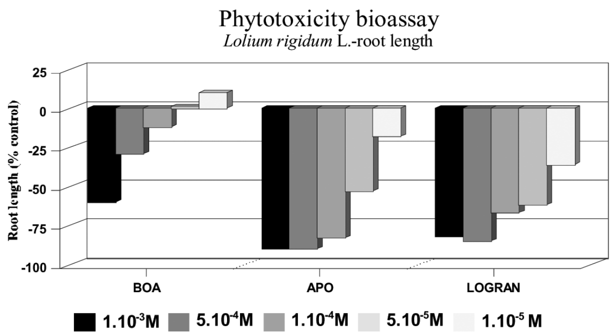

Fig. 4. Results for phytotoxic activity bioassay over *Lolium rigidum* L.

The degradation rate depends on the wheat variety (Fig. 2). Anyway, this degradation process made us to study which compounds were the responsible for the bioactivity observed in wheat for these benzohydroxamic acids and their degradation derivatives.

Due to the presence of APO after degradation step, bioactivity of BOA and APO had to be studied and compared, so that we can decide which one is the responsible for the bioactivity of DIBOA and BOA, like previous studies suggested, or APO (Gagliardo & Chilton 1992).

The wheat coleoptiles bioassay showed significant bioactivity levels for both compounds, and APO results were similar for those observed for LOGRAN©, the herbicide used as internal standard. Zero represents control, positive values stimulation, and negative values inhibition of the parameter. (Fig. 3)

The main degradation product, APO, showed excellent bioactivity levels (almost 100 % inhibition at 10^{-3} M concentration) in comparison to BOA. APO shows also a high persistence with dilution (reduction of 50% in concentration reduces bioactivity less than a 10% at $5 \cdot 10^{-4}$M).

Similar results are observed in phytotoxic activity bioassay. The species tested were the ones showed in Table 1.

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. (Fig. 4)

The inhibition behavior and the bioactivity profile are similar to the observed in general bioassay conditions, clearly inhibitory with excellent levels of phytotoxicity for APO.

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.

Taking advantage of these biological interactions is the key to control a crop as a modified ecosystem, and characterization of these microbes and their use in space crops would enhance allelochemicals properties, degradation processes, and nutrients incorporation to the crop.

The inhibition of a crop plant by its own chemicals or residues in soil (autotoxicity) is an allelopathic phenomenon detected for several crops (Tesar *et al* 1988) and its knowledge and control is needed to guarantee high yield crops at any ecosystem. Research on this matter for crops to be developed in space will offer instruments for plant growth control and for crop yield improvement.

Natural products as allelochemicals: Plants and Microorganisms

Our research group has isolated and characterized more than 400 new allelochemicals using bio-guided isolation

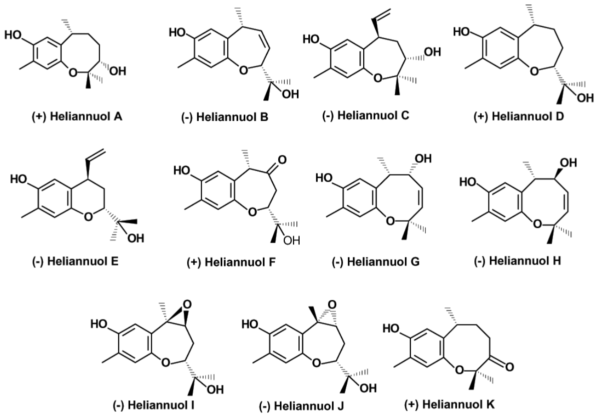

Fig. 5. Natural heliannuols from *H. annuus* L.

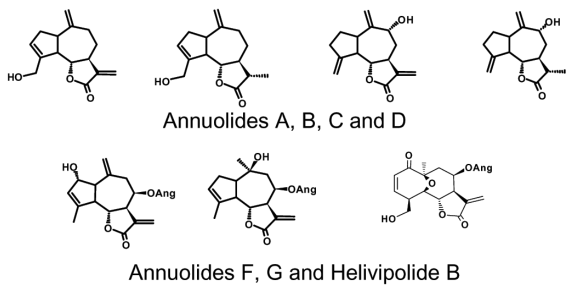

Fig. 6. Sesquiterpene lactones from sunflower.

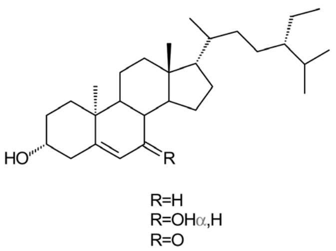

Fig. 7. Steroids from *Melilotus messanensis*.

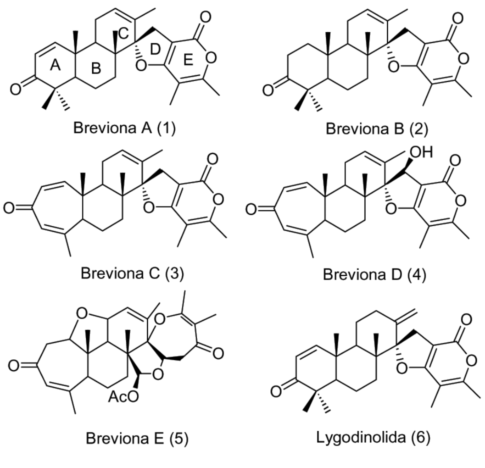

Fig. 8. Spiroditerpenoids from *P. Brevicompactum*.

methodologies, focusing its work in plants in sunflower, and extending it to wheat (see Integrated allelopathic studies above), rice and other plants.

Sunflower (*Helianthus annuus* L.) is a plant with a high commercial interest, and it is known to have a high level of secondary metabolites production. Bioactivity evaluation of crude extracts from aerial part at different growth stages was achieved, and stimulatory bioactivity was observed at the first growth stage for *L. sativa* germination. (Macías *et al* 1995)

Taking advantage of these stimulatory effects, and isolating allelochemicals responsible for that behavior, a strategy for sustainable crop improvement would be generated. We believe this kind of effects to be very useful for space crop implementation, since the effect can be provoked with low amounts of compounds and low residue generation. In fact, agricultural byproducts or their extracts could be used with this purpose.

We will make a brief discussion of two important families of new compounds isolated from sunflower: heliannuols and guaianolides.

Heliannuols are phenolic sesquiterpenes with a molecular structure described as a benzenic ring with a fused heterocycle of six, seven or eight members (Fig. 5). They were isolated from intermediate polarity fractions of *H. annuus* cv. Peredovick® leaves extracts. Heliannuols A and C showed high percentage of inhibition of *L. sativa* germination. (Macías *et al* 1999a)

Heliannuols have a high interest as potential templates for the development of new herbicide models, and our current efforts are directed to develop a synthetic procedure at a large scale for all the members of the family. Several synthesis procedures, racemic and asymmetric have been reported, and synthetic analogues are being evaluated from the point of view of their bioactivity.

Several sesquiterpene lactones with guaiane skeleton have been isolated from sunflower, with a high interest for their potential phytotoxic and pharmacological bioactivities. (Fig. 6)

Some steroids (Fig. 7) isolated from 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* 1999)

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. (Fig. 8)

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 on allelopathy

Between the many accessible fields for allelopathy researchers, some of them have special interest and relevance. The study of ecological interactions at the sea, in the search for new bioactive agents useful in crop protection and medicine, is attracting higher interest in connection to chemical studies of several types of marine organisms (soft corals, algae and phytoplankton).

Plant-insect interaction is also growing in interest and applicability, since these biochemical relationships can offer interesting alternatives in the field of antifeedant compounds as low toxicity insect protectors for crops. Plant-animal interactions offer analogous possibilities. The possibility to develop transgenic organisms with allelopathic capabilities would eliminate the need for compounds administration.

Studies of compound distribution into the vegetable organism and between the ecosystem members on low gravity conditions are very important to the development of useful crops in space, aimed to constitute the base of human and animal feeding. The positive effects of allelochemicals for plants (germination and growth stimulants) are a reliable alternative to imitate natural earth conditions in space, since new techniques for implementation of complete ecosystems are needed for this purpose.

Microbial degradation studies like the one showed above, with quantification and time course evaluation, are getting a high importance. These methodologies, in addition to ecotoxicological evaluations and genetic implementation of allelopathic properties, are the key to advance in allelopathy knowledge for the near future.

Conclusions

A crop in earth or in space is a modified ecosystem, and its biological interactions must be exhaustively known, in order to avoid undesired processes, as autotoxicity, that may harm crop production.

Allelopathy may help in several ways:

- The knowledge of autotoxic effects would avoid unexpected harvest losses.
- In order to construct a sustainable ecosystem the relationships between allelochemicals, microorganisms and degradation processes must be known.
- The high specificity and low doses needed for allelochemicals to control crop behavior facilitates wastewaters treatments and vegetable resources management.
- The election of plant species, plant residues or allelochemicals with germination and growth stimulation capabilities will be crucial to assure a success in space crop establishment.

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