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Review

Allelopathy – a natural alternative for weed control

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Abstract: Allelopathy studies the interactions among plants, fungi, algae and bacteria with the organisms living in a certain ecosystem, interactions that are mediated by the secondary metabolites produced and exuded into the environment. Consequently, allelopathy is a multidisciplinary science where ecologists, chemists, soil scientists, agronomists, biologists, plant physiologists and molecular biologists offer their skills to give an overall view of the complex interactions occurring in a certain ecosystem. As a result of these studies, applications in weed and pest management are expected in such different fields as development of new agrochemicals, cultural methods, developing of allelopathic crops with increased weed resistance, etc. The present paper will focus on the chemical aspects of allelopathy, pointing out the most recent advances in the chemicals disclosed, their mode of action and their fate in the ecosystem. Also, attention will be paid to achievements in genomics and proteomics, two emerging fields in allelopathy. Rather than being exhaustive, this paper is intended to reflect a critical vision of the current state of allelopathy and to point to future lines of research where in the authors' opinion the main advances and applications could and should be expected.

Keywords: allelopathy; pest control; sustainability; allelochemicals; mode of action; degradation; soil; genomics; proteomics

1 INTRODUCTION

Allelopathy – a controversial word that has existed within the scientific community for the last four decades – has still not been acknowledged by many scientists. According to the definition given by the International Allelopathy Society (IAS), allelopathy 'studies any process involving secondary metabolites produced by plants, algae, bacteria and fungi that influence the growth and development of agricultural and biological systems'.[1] This definition depicts the allelopathic phenomenon as a general defence mechanism present in plants that fulfils the 'economy of resources' principle – a defence metabolite is cheaper in terms of resource investment (energy, NADPH, carbon) if it can serve more than one purpose; in other words, if it can defend the plant from more than one organism. This principle also helps to explain the wide variety of biological activities that defence plant metabolites often have. In this way, the synthesis of a chemical represents an investment of energy and resources for the organism. If the benefits it gets from this investment are reasonable, evolution will keep this trait, yet the opposite is also true: if the use of resources does not benefit the organism, this adaptation may persist or it will eventually disappear. Consequently, it is likely that living organisms – plants included – will contain compounds that will defend them from more than one competitor/predator during the time course of coevolution. This is reasonable since many basic biosynthetic pathways and biochemical processes present similarities in many types of organism. Consequently, if a given compound is able to react with a protein, nucleic acid, etc., it is possible that such a process may take place in more than one species.

To begin with, allelopathy was considered as a branch of the ecological sciences, and research focused on qualitative descriptions of predominant or invasive plant species. Currently, allelopathic studies encompass the wide range of disciplines related to plant studies: ecology, biochemistry, chemistry (natural product isolation and synthesis), plant physiology (including mode-of-action studies), technical agriculture, forestry, genetic breeding, soil studies and, recently, proteomics and genomics. The increasing importance of allelopathy can also be observed by the publication of extensive reviews documenting its different aspects. What should be the key point that will make this article different and useful? A critical view coming from inside allelopathy research will give the reader (working or not in this subject) clues about where the field is going and why

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it is of interest. The authors will also give their view regarding how applications of allelopathy will impact agriculture and forestry in the twenty-first century.

Allelopathy has often been criticised as being an empirical tale built upon field observations that were brought together with no other coherence than being unexplained facts waiting for a scientist to put them into the same bag. There have been, and still are, many cases of 'suspected allelopathy' published without proper and rigorous experimentation. Not all cases where phytotoxic activities of crude extracts or pure compounds are found mean allelopathy is the reason for their existence. Many active compounds are toxic to more than one type of organism, regardless of the initial role they have in the plant. Also, not all cases of plant predominance in a certain ecosystem are due to allelopathy. However, the fact that allelopathy is a crucial mechanism of plant self-defence and attack – a chemical weapon of the plant to gain a foothold in the community – cannot be ignored. The problem is how to detect these cases and how to support them once they have been found. In other words, scientists doing research on allelopathy have claimed 'the allelopathy paradigm'. There is no need to look for something that has been already discovered and published. The only need is to put such matters under the proper spotlight. Many examples could serve this purpose, but a representative case has been selected. The success of invasive plants has been explained through the 'lack of natural enemies' hypothesis: when a plant species is introduced into a new ecosystem, predators and competitors that used to control their populations in the native ecosystem no longer exist. However, this point of view cannot always explain the lack of biocontrol strategies, cyclic autoregulation and low impact on the invader by autochthonous predators. Consequently, other mechanisms should be considered to explain field observations. Allelopathy must play a key role in these situations: release of allelochemicals by the invasive plant into a new environment should result in its predominance, as native plants/predators/soil microbiota are not adapted to them. They lack the proper defence/detoxification mechanisms developed through coevolution. Recent revisions in studies of invasive plants under the light of allelopathy have led to the 'novel weapons hypothesis' as a key factor for explaining invasiveness.[2] This supports previous views on the importance of coevolution in the manifestation of allelopathy, thus differentiating the behaviour of an invasive plant in and out of its original ecosystem.[3]

Before going on with this subject, the authors would like to provide guidelines that any reported case of allelopathy should fit. Many authors have addressed this question previously.[4] The present authors propose the following:

1. Plant predominance/distribution/frequency cannot be explained solely on the basis of physical/biotic factors.
2. The allelopathic plants (donors) should synthesise and release into the environment chemicals that must be or become bioactive.
3. Soil permanence and concentrations should be high enough to produce effects on the germination and/or growth of neighbouring plants, bacteria and/or fungi.
4. Uptake by the target plant and evidence of the detrimental/beneficial effects caused by the chemical/s.

These four points cover major aspects involved in descriptions of allelopathy: field and ecological observations (point 1), chemical elucidation of bioactive compounds and their physiological effects on targets (points 2 and 4), soil effects (chemical and microbiological, point 3) and physiology of the donor plant and release into the environment (point 2).

Chemists working in allelopathy normally use bioassay-directed search strategies to identify the allelochemicals responsible for the allelopathic activity. This is essentially an effective approach; however, it should be pointed out that not all compounds resulting from these studies are necessarily the 'true' allelopathic agents, as compounds exposed to environmental hazards (biological or chemical oxidation, polymerisation and degradation) are not considered normally to be converted into less active compounds. However, several examples show that the opposite is also true for many plant extracts.[5,6] Some authors have suggested that exposing extracts from allelopathic plants to environmental conditions prior to chemical fractionation should solve the problem. This is not always necessary, as comparison of the biological activity of the original extracts and extracts treated under a variety of conditions (sterilised and non-sterilised soils, oxidative atmospheres, light, water over several days, etc.) will provide the same information. If there are no important changes in the activity, or if the highest activities are obtained from the original extract, then it seems reasonable to proceed with the study. On the other hand, the results obtained should be taken cautiously, as active compounds could be subjected to chemical and/or biochemical changes.

Finally, for purposes of simplicity, and regardless of the fact that all aspects of allelopathy are important since they are just parts of the whole picture, this study will be organised into five parts: 1, chemistry; 2, mode of action of allelochemicals; 3, proteomic and genetic studies; 4, future trends and applications; and 5, concluding remarks. Special emphasis will be placed on aspects such as the fate of allelochemicals and soil studies, as well as crop and forest management.

2 CHEMISTRY

To date, many allelochemicals with different skeletal types have been characterised, and their phytotoxic and biological activities are being documented. The first classification split them into 14 classes according to their biosynthetic origin[7] and included most of the more commonly cited allelochemicals: simple phenolic acids, quinones, mono- and sesquiterpenes and flavonoids, among others. Basically, this classification remains. Several other reviews have attempted to update the state of the art in research on allelopathic agents, stressing their importance in agricultural applications.[8,9,10]

2.1 Phenolic compounds

These constitute a wide group of allelochemicals comprising structures with different degrees of chemical complexity: simple benzoic and cinnamic derivatives, flavonoids, polyphenols and, recently, depsides, depsidones and other aromatic compounds of lichen origin. The most recent advances in each category will be commented upon briefly.

2.1.1 Simple phenolics

Phenol derivatives ranging to furano- and piranocoumarins have often been reported as allelopathic agents.[11] Benzoic and cinnamic acids are among the most commonly referred to allelopathic agents (Fig. 1). They have been considered as primary factors of allelopathy in forest ecosystems, being responsible for successional effects[12] and difficulties in reforestation,[13] as well as autotoxicity[13] in economic plants such as asparagus[14] and coffee.[15] In the case of tea and coffee, autotoxicity has been related to the caffeine released from litter and seed senescence and decomposition.[16]

More recently, polyphenols such as ellagic, gallic and pyrogallic acids along with the flavonoid (+)-catechin (Fig. 1) isolated from the macrophyte *Myriophyllum spicatum* L. have been reported as growth inhibitors of the blue-green alga *Microcystis aeruginosa* Kütz.,[17] thus depicting the growing interest in the study of allelopathic phenomena in aquatic ecosystems. Application of these compounds in lakes, ponds and fisheries could be of interest in solving problems as diverse as algal blooms in eutrophised systems or musty odour in fishes owing to accumulation of certain microalgae in their skins.[18]

However, few authors in allelopathy acknowledge that benzoic and/or cinnamic acid derivatives are widespread in the plant kingdom and possess important structural functions, as they are key steps in the biosynthetic pathway of lignins (e.g. in their reduced forms of *p*-coumaryl, coniferyl and sinapyl alcohols) and have important roles in plant physiology.[19] Salycilic acid itself is a mediator for the development of systemic acquired (induced) resistance in a plant's defence against disease, among other functions.[20] In addition, phenolic acids in soils are subjected to many chemical and biochemical transformations that deplete their real concentrations: irreversible binding to humic acids, sorption onto soil particles, ionisation, chemical oxidation to quinones and use by bacteria as a carbon source are among the most important.[21] These processes might deplete the concentrations in the rhizosphere and soil, so many authors suggest that allelopathic effects by phenolic acids are highly unlikely[22] and the levels reached are not high enough to reproduce the levels of phytotoxicity obtained *in vitro*. Consequently, many cases of suspected allelopathy attributed to benzoic and cinnamic acids should be reviewed.

It is the present authors' opinion that compounds appearing early in plant evolution – like the phenolic acids – provided the plant with an evolutionary advantage at that time. It is symptomatic that evolutionarily old plants such as certain gymnosperms (e.g. conifers) exude phenolic acids in large enough amounts to reach phytotoxic concentrations in soil.[23,24] Coevolution probably overcame this first defence barrier with the development of more sophisticated chemical weapons. Although phenolics

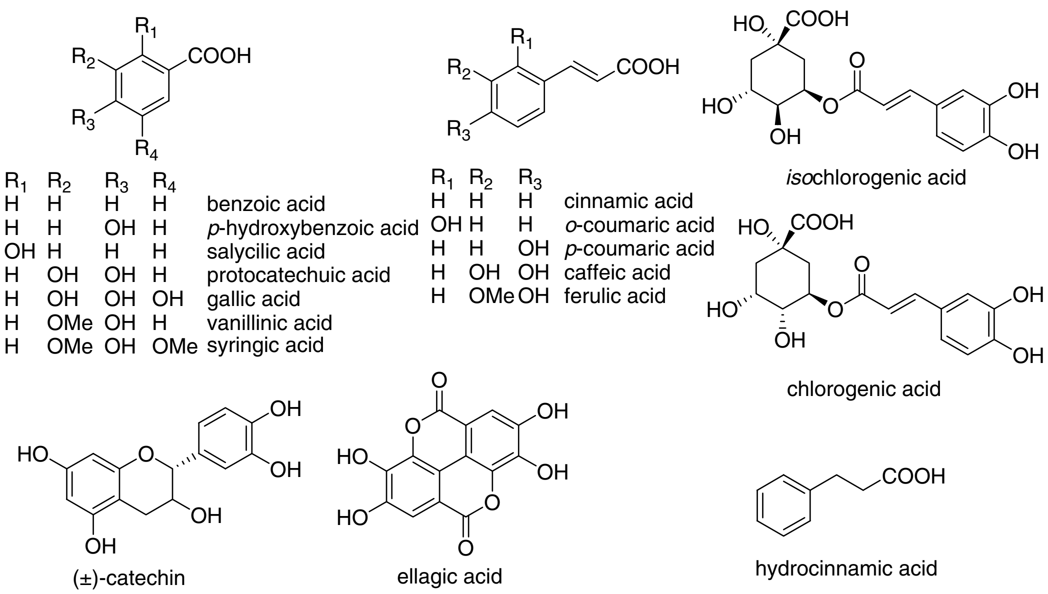

Figure 1. Common benzoic and cinnamic acid derivatives described as allelopathic agents.

are still present as part of the chemical composition of many plants, most have other important functions (structural, hormonal, etc.). A good example of functional allelopathic behaviour[25,26] due to phenolics can be seen in wheat and other Gramineae. They contain in their chemical composition hydroxamic and phenolic acids,[27] but benzoxazinoids have been finally acknowledged as being mainly responsible for the allelopathic effects.[25,26]

Quinones (Fig. 2) constitute a different case, in spite of their structural similarity to simple phenolics. Juglone (Black Walnut tree, *Juglans nigra* L.) is one of the first examples of an allelochemical to be described. Bacterial degradation does not seem to remove soil juglone completely, and consequently it has been shown to accumulate in the soil at high enough concentrations to be toxic to associated plant species.[28] Sorgoleone and related compounds[29] are the allelochemicals responsible for the allelopathic effect of sorghum. They are exuded in large amounts from root hairs as oily droplets[30,31] and accumulate in the soil. Half-life times of 10 days have been recorded, depending on the soil composition,[32] but sorgoleone can be detected up to 7 weeks later.[33] The anthraquinones emodin and physcion (alone and in their glucosidic forms) have been described as allelochemicals in *Polygonum sachalinense* Schmidt.[34]

2.1.2 Flavonoids

Flavonoids have roles associated with colour and pollination (flavones, flavonols, chalcones and catechins) and disease resistance (phytoalexins). They also have weak oestrogenic activity (isoflavones and coumestanes, e.g. coumestrol). However, few examples can be found of allelopathic flavonoids. Examples include kaempferol which has been isolated from the soils beneath *Quercus mongolica* Fisch. ex Ledeb. along with other simple phenolic acids,[35] and ceratiolin, a non-phytotoxic dihydrochalcone present in the leaves of the dominant shrub of the Florida sandhill community *Ceratiola ericoides* Michx. that easily degrades under acid or soil conditions to the phytotoxic dihydrocinnamic acid (Fig. 3).[36] However, the most prominent examples are those regarding invasive species where flavonoids have been reported as responsible: (−)-catechin in *Centaurea maculosa* Lam. (Fig. 1),[37] robinetin and other flavonoids in *Robinia pseudoacacia* L.[38] and the flavonoids isolated from mosses that show inhibition of spore germination against other moss species.[39] The most often cited allelopathic flavonoids (as free aglucones or glycosilated) are kaempferol, quercetin and naringenin (Fig. 3).[40] It seems, however, that, except in certain special cases, flavonoids are not usually allelopathic agents and exhibit other roles in the plant.

One of the latest and most enlightening examples is the case of *Centaurea maculosa* (spotted knapweed), a European native weed that is causing serious trouble in North America owing to its high invasive potential.[41] Recent publications support the belief that its invasive potential is due to the exudation of the flavan-3-ol (−)-catechin (Fig. 1).[37] This compound is exuded as a racemic mixture where only one of the two enantiomers is phytotoxic while the other has

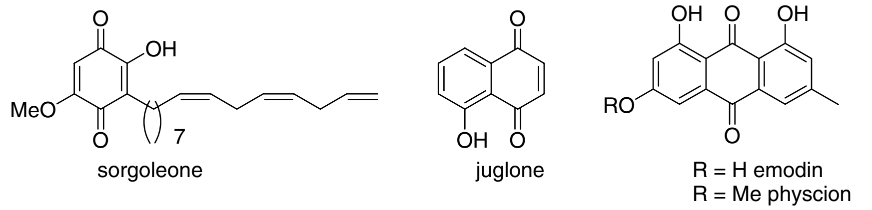

Figure 2. Benzo-, naphtho- and anthraquinones reported as allelopathic agents.

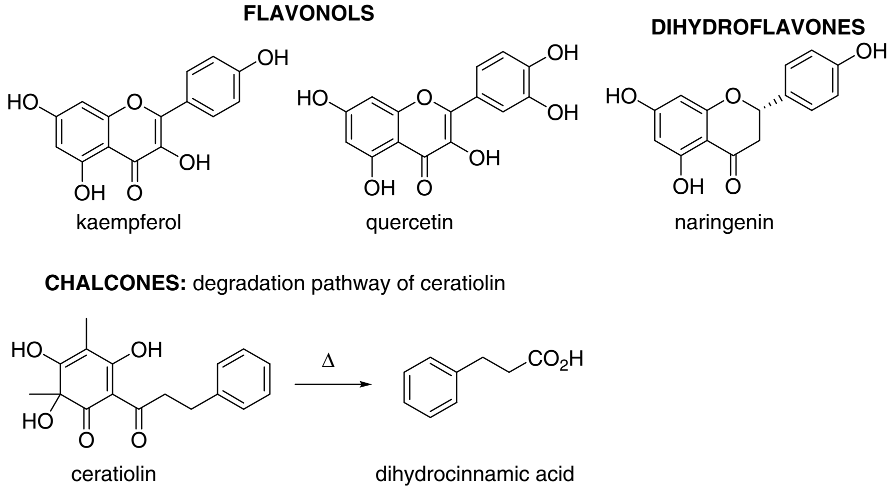

Figure 3. Some allelopathic flavonoids and thermic degradation of ceratioline to the allelochemical dihydrocinnamic acid.

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antimicrobial properties. The concentrations of (−)-catechin detected in North America soils where *C. maculosa* was found were twice those in corresponding European soils and dependent on the distance from knapweed roots.[42] Moreover, North American grasses were more susceptible to (−)-catechin than the corresponding European species.[37] Biochemical and mode-of-action studies showed that (−)-catechin is incorporated into the target plant through the roots, and it triggers a cascade of events that leads to changes in gene expression and root system death. All of these data strongly support the theory that the success of *C. maculosa* outside its native ecosystem is mainly due to the exudation of a chemical (catechin) to which native plants are not adapted.[37,43]

2.1.3 Usnic acid and lichen metabolites

Usnic acid (Fig. 4) has attracted much attention for several years past, as it is a unique and relatively abundant chemical in lichens – never reported in higher plants – with a wide array of biological activities.[44] The chemical composition of lichens is usually simple in comparison with higher plants, having a relatively low number of secondary metabolites, most of them aromatic. They can be divided into four main classes: depsides, depsidones, depsones and dibenzofurans (Fig. 4). Quinones, anthraquinones and simple phenols are also common constituents in lichens. The primary ecological roles of secondary lichen substances have been classified into four groups: protection against damaging light conditions; chemical weathering compounds; allelopathic compounds (including antibiotic compounds); and antiherbivore defence compounds.[45] Lichen allelopathy is just starting to be explored, and little has been done in this field.

Other lichen metabolites with phytotoxic activity are the anthraquinones rhodocladonic acid and emodin. Rhodocladonic acid is a typical anthraquinone occurring in many lichen species of the family Roccellaceae,[46] while emodin (Fig. 3) is a widely distributed anthraquinone in higher plants, fungi and lichens. The phytotoxic activity of these compounds and several derivatives has been recently reported.[47] Also, the phytotoxic activities of the mixture of major orsellinates, orsellinic acid dimers (antranorin and evernic acid derivatives) and tetramers (prunastrin) isolated from the lichen *Evernia prusnatri* L. have been described (Fig. 4).[48]

In view of their wide array of biological activities, it can be hypothesised that secondary metabolite chemistry is important in determining the relative ecological success of individual lichen species within the structure and diversity of natural lichen communities. However, this question has not been fully addressed yet. In any case, the possibilities of lichen metabolites as herbicide leads remain unexplored and present a great potential. Future steps should involve the description of the fungitoxic/algicidal and/or phytotoxic activities of compounds isolated from bioassay-directed isolation procedures, SAR studies to understand the chemical clues governing their activity and suggest more active derivatives and specific mode-of-action studies.

2.2 Terpenoids

Terpenoids are secondary metabolites present in many organisms, and they have a wide range of structural

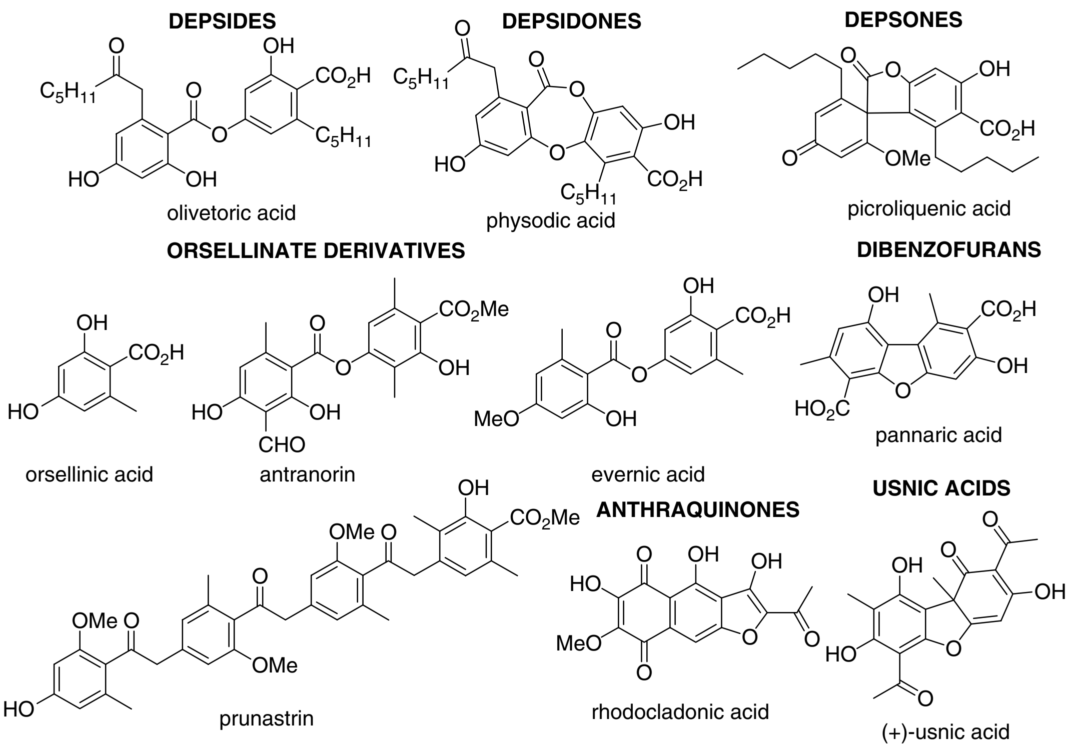

Figure 4. Lichen metabolites reported to have phytotoxic activity.

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diversity and biological activity. Their allelopathic activity has recently resulted in them being considered as possible leads in the development of new natural product based agrochemicals.[8,49] The most recent and significant examples are discussed below.

2.2.1 Monoterpenoids

Monoterpenoids are common volatiles that are major constituents of essential oils. They have been described as responsible for the allelopathic interactions in some plant communities, especially those comprising aromatic plants in arid or semi-arid zones (Fig. 5).[50,51] Inhibition of germination has been reported for several monoterpenes (e.g. camphor, pinenes, cineoles) in pure form[52] or as essential oil mixtures.[50] In spite of the fact that they are lipophilic compounds, their solubility levels (alone or with natural surfactants such as ursolic acid) are enough to exert phytotoxicity,[53] and they do not need to be dissolved or suspended in water, as they are effective using air as a carrier.[54,55] When considering their possibilities of being herbicidal lead compounds or giving rise to these, it is important to note the high structural similarity between the monoterpenes 1,4- and 1,8-cineole and the herbicide cinmethylin. Moreover, it has been demonstrated that cinmethylin acts as a proherbicide that suffers metabolic breakdown to give rise to 2-hydroxy-1,4-cineole, the true herbicide.[56]

2.2.2 Sesquiterpene lactones

Sesquiterpene lactones (SLs) constitute an interesting and well-documented group of compounds. They present a wide range of biological activities, including insecticidal,[57] antibacterial[58] and antifungal.[59] SLs are responsible for the bitter taste in the plants containing them and seem to act as antifeedants, being promising compounds in the development of new insect antifeedants.[60]

SLs have been reported as allelopathic agents in many cases[61,62] with high levels of activity,[63,64] being particularly abundant in plants of the family Compositae.[65] Many of the most noxious weeds contain SLs as allelochemicals, and their invasive potential could be directly related to their chemical content. Members of the families Centaurea, such as Russian knapweed *C. repens* L. [now *Acroptilon repens* (L.) DC] and yellow starthistle (*C. solstitialis* L.), contain phytotoxic guaianolides such as centaurepensin, solstitiolide, acroptillin and repin, or germacranolides such as cnicin, cnicin acetate, salonitenolide and salonitenolide angelate (*C. derventana* Vis & Pančić and *C. kosaninii* Hayek) (Fig. 6).[66] Many other examples of invasive noxious weeds containing SLs could be given that are beyond the scope of this review. Plants other than invasive weeds may also be considered as allelopathic. In this sense, the case of sunflower is worth noting because of the economical importance of this crop. The importance of the ecological roles of SLs and other secondary metabolites as plant defence compounds in sunflower has been reviewed.[67]

Many of the above-mentioned compounds present other biological activities related to a plant defence role. For example, a strong synergistic insecticidal effect can be observed in the larvae of *Manduca sexta* (Joh.) when typical Asteraceae SLs are coadministered

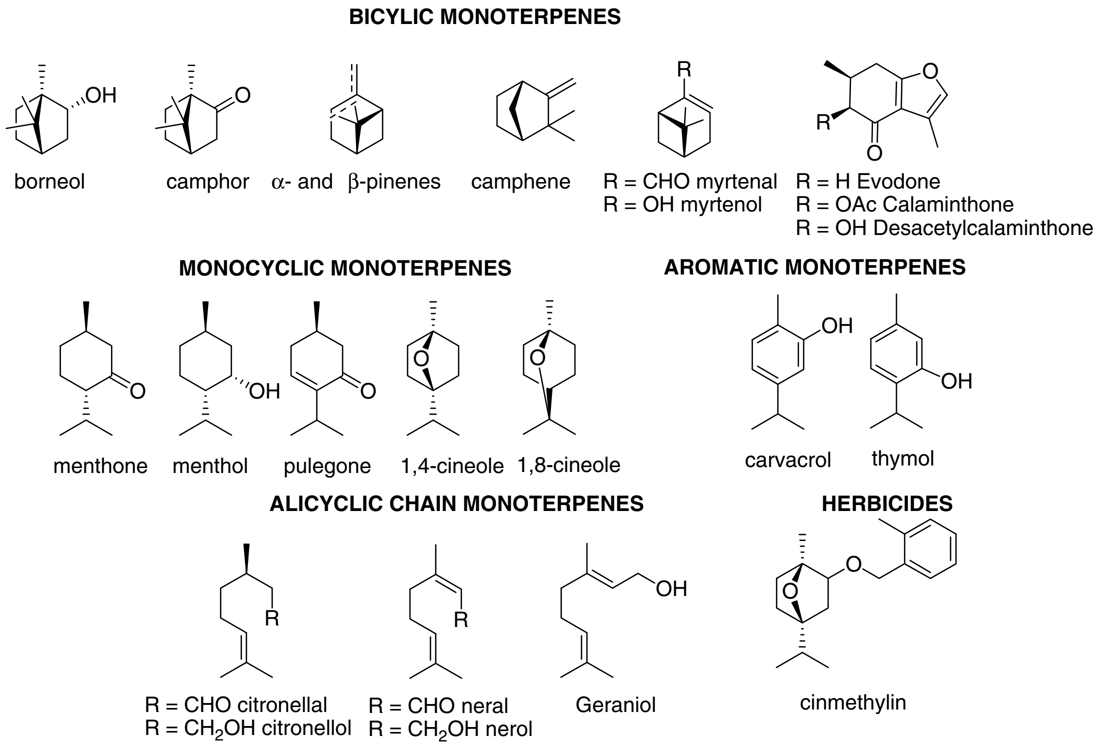

Figure 5. Typical monoterpenes present in essential oils and reported to have allelopathic and/or phytotoxic activity. Note that 1,4-cineole and 1,8-cineole present structures closely related to that of the herbicide cinmethylin.

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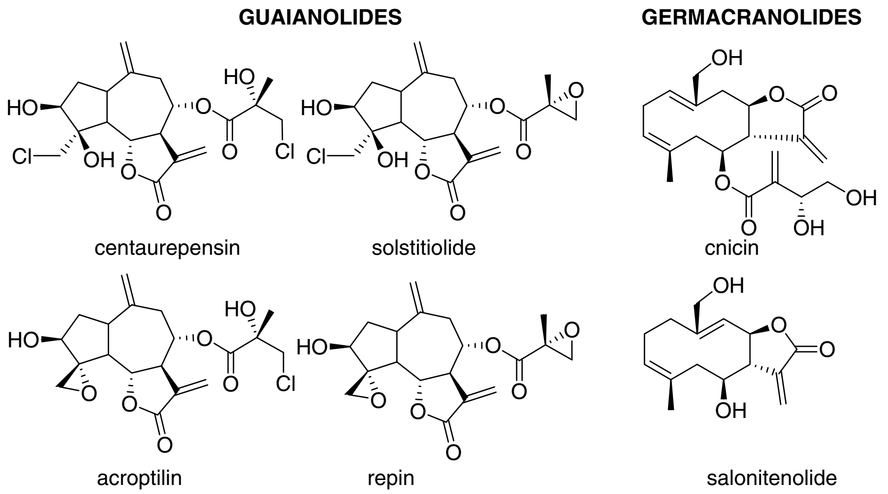

Figure 6. Some examples of SLs identified as allelopathic agents in members of *Centaurea* (now *Acroptilon*) species.

with the photooxidant polyacetylene α-terthienyl, resulting in enhanced lipid peroxidation and larval mortality.[68] However, as mentioned above, most defence activities reported for SLs are fungicidal and phytotoxic. In any case, these few examples illustrate two basic concepts:

1. They support the importance of the allelopathic factor in the invasive potential of plant species.
2. The importance of the economy principle: in order to save energy and carbon resources, plants tend to synthesise multifunctional compounds that may serve as defence against several enemies; this leads to the synthesis of compounds having a wide array of biological activities that eventually may end up in the discovery of useful compounds for mankind, as in the case of artemisinin.

The importance of lipophilicity and other physical properties in agrochemical formulation has been acknowledged,[69] as they determine an easy membrane crossing or an adequate water solubility. This has been previously demonstrated for the citotoxicity of SL, as the latter has been shown to be directly related to the size and lipophilicity of the ester side chain in 11α-13-dihydrohelenanolides,[70] but it has also been shown to occur with phytotoxic activity.[71] The model proposed fits Lipinski's rule of 5, a common and well-known rule in pharmacological studies,[72] and Tice's modification[73] for herbicides.

In summary, the bioactivity and results obtained with SLs so far make it possible to propose them as leads for future natural product based herbicide development. Efforts need to be made to obtain compounds in high amounts (through synthesis or plant extraction) at reasonable costs, perform soil stability studies and improve physical/chemical properties and formulation. However, the activities currently reported support them as possible candidates providing new modes of action. In this way, SL has been shown to be effective in controlling herbicide-resistant biotypes of several weeds.[74]

2.2.3 Diterpenes: the case of momilactones

Momilactones (Fig. 7) are pimarane diterpenes initially isolated from rice husks as growth inhibitors.[75] Diterpenes are not usually reported as allelopathic agents. Rather, their ecological role has been associated more with insecticidal, antifeedant and deterrent activity. Other diterpenes such as giberrellins act as important plant hormones involved in growth regulation.

At first, momilactones were considered as phytoalexins since their presence in plant tissues was detected and enhanced under *Pyricularia oryzae* Cavara attack,[76] UV irradiation[77] and exogenous *N*-acetylchitooligosaccharides.[78] Not until recently has their possible role as allelopathic agents been studied in depth. In a recent study, exudation of momilactone B from the roots of young rice seedlings into the environment was described in culture solution[79] and soil[80] in quantities sufficient to inhibit the growth of neighbouring plants.[81] Most of the recent work on

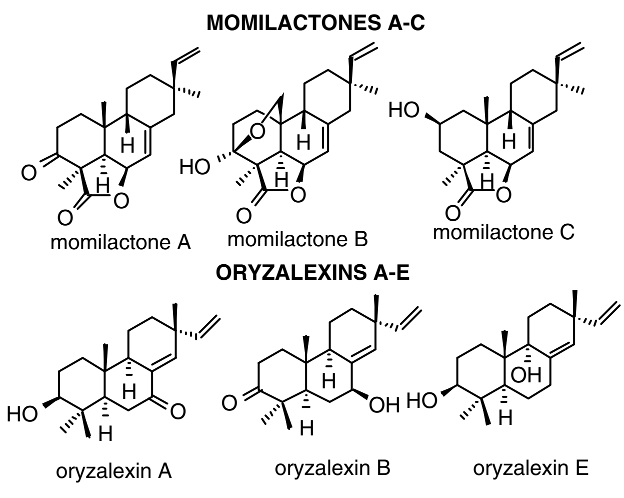

Figure 7. Rice allelochemicals: momilactones and oryzalexins. Oryzalexins and momilactones are reported as phytoalexins, whereas momilactone B is the only member of this family being identified as an allelopathic agent.

momilactones as allelopathic agents refers to momilactone B, which has been found in shoots and roots of rice plants over the entire plant life cycle, yielding the highest levels just before flowering initiation.[82] The levels of momilactone B vary among different rice cultivars and correlate well with the amounts of momilactone B found in the corresponding culture solutions.

Allelopathic compounds are usually considered as constitutive chemicals that are continuously produced and exuded into the environment. However, the definition of allelopathy[1] does not state that allelopathic compounds need to be constitutive. Consequently, a phytoalexin can be considered, at the same time, to be an allelochemical. Recently, a brilliant work by Wilderman *et al.*[83] showed that the mRNA levels encoding a specific syn-copalyl diphosphate 9β-pimara-7,15-diene synthase were upregulated in leaves subjected to conditions where phytoalexin production was stimulated, but it is constitutively expressed in roots, where momilactones are constantly synthesised and released into the soil. Differential expression of the genome is not uncommon, but this case constitutes a good example of how genomics can help to explain apparently contradictory facts: how an allelopathic agent can also be, at the same time and in the same plant, a phytoalexin, thus making a point for the 'economy of resources' principle that could be operating in diterpenoid metabolism in rice.

Many approaches to momilactone synthesis have been published as previous steps to the finally accomplished first total synthesis of momilactone A,[84] thus showing the importance of momilactones as promising leads for herbicide and fungicide development.

2.2.4 *Quassinoids*

Quassinoids are another good example of the increasing interest and work on allelochemicals. Quassinoids are decanortriterpenes known to occur in the family Simaroubaceae.[85] As in many other cases, they became of interest because of their wide spectrum of medicinal activities: anticancer,[86] antileukemic[87] and antimalarial,[88] among others. Consequently, interest in their potential applications in agriculture as insecticidal,[89] fungicidal[90] and herbicidal[91,92] agents has arisen. Their high structural complexity, owing to the presence of many chiral centres, and the low amounts usually obtained from natural sources made their total synthesis attractive, and many attempts have been made,[93] thus illustrating their importance.

The first quassinoid identified as an allelopathic agent was ailanthone, a chemical obtained from the tree-of-heaven (*Ailanthus altissima* Swingle),[94] a Chinese tree used in traditional medicine that has become an invasive species in Europe. The high invasive potential of this tree fits well with Callaway's 'novel weapons' hypothesis,[2,95] as it presents a whole array of bioactive indole alkaloids and quassinoids. Although ailanthone was originally determined to be the compound responsible for allelopathic activity, bioassay-directed isolation procedures have recently identified the quassinoids ailanthinone, chaparrine and ailanthinol B as phytotoxic quassinoids also present in the roots (Fig. 8).[96] To the authors' knowledge, neither the synthesis of ailanthone nor any of the other bitter principles of *A. altissima* has been published so far.

No structure–activity relationship (SAR) studies have been accomplished with the quassinoids obtained from the tree-of-heaven, although the *in vitro* phytotoxicity of other chaparrinone- and picrasane-type quassinoids has been tested, showing that only chaparrinones (chaparrinone, gaucarubolone and holocanthone) (Fig. 8) were phytotoxic.[92] This difference was correlated with the presence of a hemiketal bridge in the active compounds. According to this study, quassinoids appear to have a different but still unknown mode of action in plants when compared with effects in mammal cells. No further studies have been done with quassinoids regarding their phytotoxic activity.

2.2.5 *Benzoxazinoids*

Hydroxamic acids (Hx) are among the latest and more promising examples of allelochemicals isolated from plants with potential uses in agriculture as weed control agents. Hx (Fig. 9) are benzoxazines, produced by many species within Gramineae in significant amounts, which have a protective role[97] showing antifungal,[98] antimicrobial,[99] insecticidal[100] and phytotoxic[101] activities. They can also be found in members of the families Acanthaceae,[102] Ranunculaceae[103] and Scrophulariaceae.[104] Hx are stored in the plant as inactive glucosides to avoid autotoxicity.[105] Root exudation or tissue injuring by insect wounding causes their release into the environment where enzymatic and/or microbial degradation

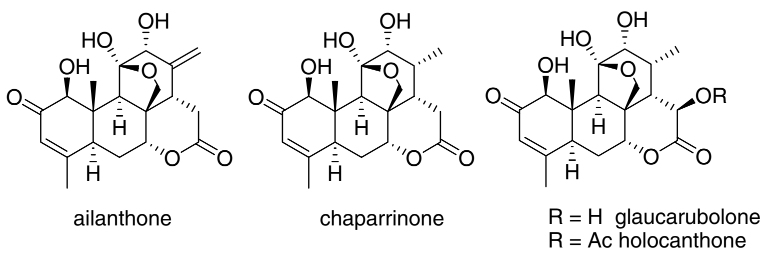

Figure 8. Structures of allelopathic/phytotoxic quassinoids.

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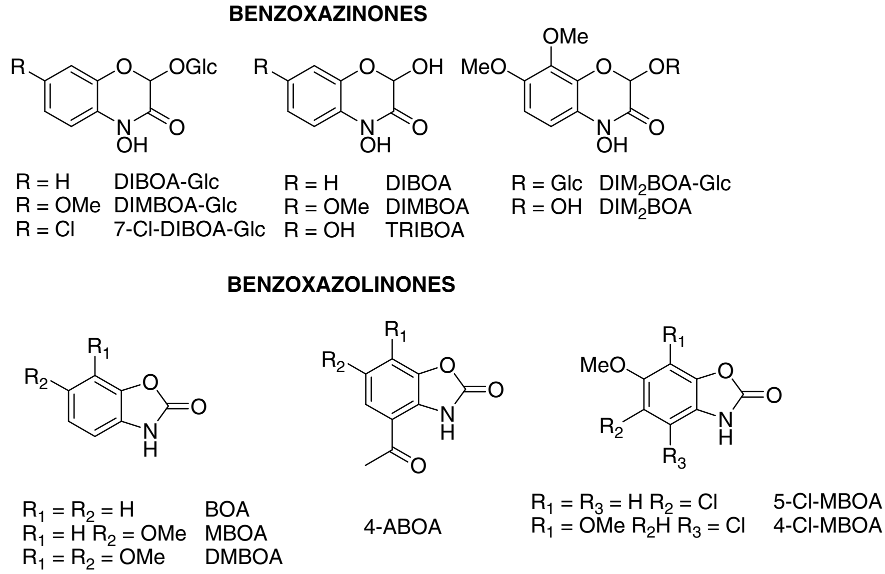

Figure 9. Allelopathic benzoxazinones and their direct degradation products, the benzoxazolinones.

breaks down the glucosidic bond and liberates the aglucones.[106] These compounds are unstable and undergo hydrolysis and ring contraction into the corresponding benzoxazolinones with short half-lifes (1 day for DIMBOA) (Fig 9).[5,107,108] Benzoxazolinones might be further transformed, either chemically or by soil microbes, into more toxic degradation products. Consequently, their chemistry has been extensively studied owing to their high degree of bioactivity and potential for agricultural applications as weed and pest controls.[109]

These compounds have attracted much attention in the past few years, and much work has been done on their phytotoxic activity, looking for:

(a) development of new models for herbicide design;
(b) allelopathic varieties of crops with high Hx content;
(c) breeding of crops to improve their chemical arsenal;
(d) genetic engineering for Hx-allelopathic trait transfer.

However, the use of benzoxazinoids as agrochemical leads is challenged by several facts concerning their safety with regard to human health and their soil stability. The arylhydroxamic group is known to be a mutagenic group, and therefore 1,4-benzoxazinoids isolated from cereals present mutagenicity.[110] In order to assess the potential dangers of using cereal hydroxamic acids in agriculture, the EU has recently financed the first international project to study allelopathy in winter wheat and their use in relation to consumer and environmental safety,[111] which has ended with positive preliminary results regarding their safety.

Regarding their soil stability, several soil degradation studies have been performed with these compounds, with interesting results. The degradation routes observed for many benzoxazinones are known to have a determining role in the allelopathic interactions modifying the overall defence capacity of the plant in a highly significant manner. Moreover, knowledge of the stability and degradation routes is necessary if the compound is going to be used as an agrochemical lead. The degradation pathways of several Hx have been extensively studied, showing the importance of the chemical structure, soil microbes and conditions (Fig. 10).[5]

Regarding their mechanism of action, it has been shown that the combination of cyclic hemiacetal and cyclic hydroxamic acid is necessary for high bioactivity, as reactions with the electrophilic ring-opened aldehyde form of the hemiacetal and with a multicentred cation generated from N–O fission are likely to occur with bionucleophiles.[109] All of this research shows the interest that these compounds have generated and their possibilities as herbicides,[112] insecticides or pest control agents.

2.2.6 *Glucosinolates*

Glucosolinates are a small group of defence compounds comprising around 120 structures and found only in plants of the families Brassicaceae, Resedaceae and Capparidaceae.[113] They are non-toxic compounds that, upon tissue disruption, are enzymatically degraded by endogenous β-thioglucosidases (myrosinases) into active compounds such as nitriles, isothiocyanates, oxazolidinethiones and thiocyanate salts.[114,115] Glucosinolates have been described as allelochemicals involved in defensive roles against insects (acting as repellents) and microorganisms, and also as volatile attractants of specialised insects such as specific butterfly species.[116]

Glucosinolates have been reported also as allelopathic agents in several cases. Glucohirsutin, hirsutin, arabin, camelinin and 4-methoxyindole-3-acetonitrile have been isolated from the roots of the Cruciferae *Rorippa sylvestris* (L.) Bess. (yellow fieldcress)[117] and

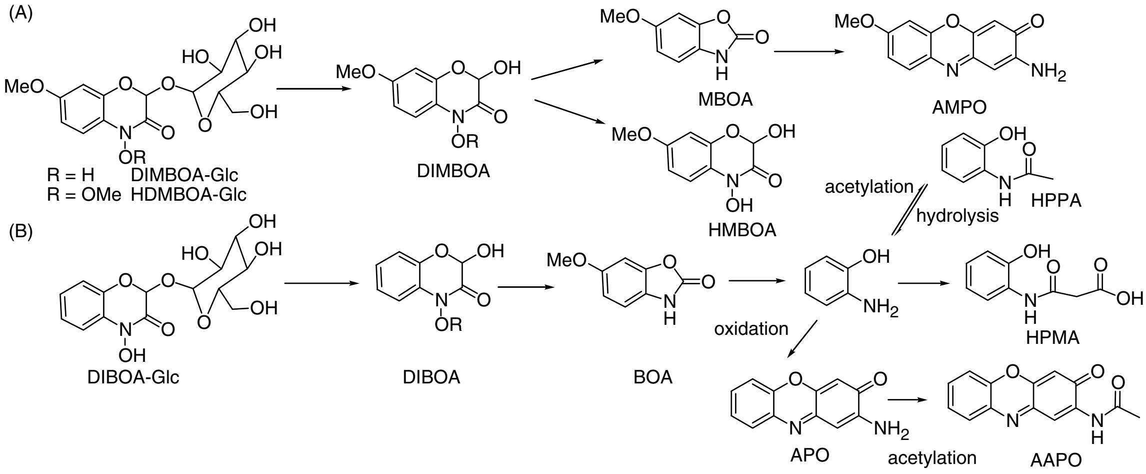

Figure 10. Degradation pathways proposed for benzoxazinoids: (a) DIMBOA derivatives; (b) DIBOA derivatives. Note how the presence of a methoxyl group in the aromatic ring may change the chemical behaviour and thus the degradation pathway, depending on the soil conditions.[5]

Rorippa indica,[118] the invasive weed *Alliaria petiolata* Bieb. (garlic mustard)[119] and other Brassicaceae[120] (Fig. 11). Seasonal variations in glucosinolate content were recorded and positively correlated with phytotoxic and allelopathic effects of *R. sylvestris*.[117] Other studies documenting the role of glucosinolates in *Brassica napus* L. contradict this,[121] possibly owing to volatilisation or degradation. The possible use of glucosinolates and glucosinolate-containing tissues as bioherbicides for weed control has been proposed.[122] However, their high volatility induces a fast disappearance from soils amended with allyl glucosinolate.[123] Although this is good for the environment, it also constitutes a problem, as the compounds do not last enough time to exert their herbicidal action.

2.3 Mode-of-action studies

Most of the arguments against the feasibility of using natural products as herbicide leads rely on the low amounts of compound usually obtained from natural sources, and their complex nature, which usually leads to expensive synthetic procedures. On the other hand, one of the main advantages that allelochemicals may offer is the discovery of new modes of action for herbicide design,[124] and this has been acknowledged and highlighted several times in the last decade.[8,125] It is well known that most herbicides target only a few molecular sites,[126] and also that no new molecular targets have been discovered in the last few years. In fact, the approximately 270 herbicides currently on the market have only 17 modes of action, with almost half of them acting on three sites: PSII, ALS and protox inhibition. Allelochemicals represent the reverse face of this coin: during the last 10–15 years, increasing attention has been paid by plant physiologists, biochemists and chemists to allelochemical–target molecular interactions. Gaining an understanding of the molecular mechanisms that underlie the macroscopic effects observed not only offers new molecular targets for herbicide design but also provides credibility to allelopathy itself. Also, it is important to point out that the new targets and modes of action discovered and disclosed are different from those already described for herbicides,[127] thus giving new ideas and molecular targets for new products.[124] In the following sections, an attempt will be made to give an overview of the state of the art in mode-of-action studies on the compounds mentioned previously.

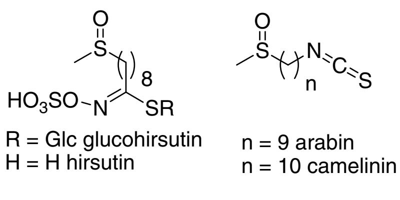

Figure 11. Allelopathic glucosinolates and isotyiocyanates.

2.3.1 Hydroxamic acids

Much of the work on their mode of action has been performed on the benzoxazolinones BOA and MBOA, as they are likely to be involved in the allelopathic effect of the plants because of the short half-life of their parent compounds DIBOA and DIMBOA. Recent studies show that BOA significantly delays cell cycle progression in lettuce meristems and decreases the mitotic index.[128] Detoxification responses to this allelochemical proceed predominantly through *O*-glucosylation and carbamate glucoside formation.[105] Biochemical studies show that transcriptional responses of *Arabidopsis* treated exogenously with BOA involve gene expression encoding protein families related to cell rescue and defence, many of them being most likely involved with detoxification pathways.[129] However, MBOA – which only differs from BOA by the presence of a methoxyl group in the aromatic ring – seems to inhibit germination through inhibition of α-amylase activity.[130] Time course experiments with DIMBOA showed an enhanced activity of horseradish peroxidase catalysed NADH oxidation.[131] Experiments were carried out within a timeframe in which DIMBOA had not yet degraded, but because of its short half-life[107]

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it is not likely to be directly involved in the allelopathic action of the plant. Currently, no definitive indications of the mode of action of these compounds have been disclosed, nor whether clues regarding the different families of compounds (benzoxazines, benzoxazolinones, amines, etc.) suggest a common mode of action.

2.3.2 Phenolic compounds

These constitute the most extensively studied and well-known group of allelochemicals with regard to their mode of action. The literature available is more detailed than for any other group of allelopathic compounds, and shows them as the least specific compounds in their action. In an excellent revision, Einhellig[11] summarized the state of the art, suggesting that simple phenolic acids, coumarins and tannins appear to have similar modes of action that affect the growth of plants and microbes through multiple physiological effects that confer on them a general toxicity.

Owing to their aromatic nature, most of them interfere with processes where charge flux (electron or cations) is present, e.g. photosynthetic processes, electron deflectors, radical scavengers, inhibitors or competitors of the PSII system, ion membrane transport and permeability. An attempt will be made to give a bird's eye view on what is known to date about their molecular mechanism of action and the physiological responses induced by their presence.

2.3.2.1 Benzoic and cinnamic acid derivatives. Benzoic and cinnamic acid derivatives are among the most studied and well-known allelochemicals, probably owing to their wide distribution. They do not seem to present a sole target; rather they seem to affect many physiological processes. They appear to act at three levels: plasma membrane functions, enzymatic processes and energy-related systems. Their first effect appears to be on plasma membrane functions, reducing transmembrane electrochemical potential.[132] The extent of the depolarisation depends on the lipophilia of the compounds and their ability to dissolve in the membrane lipids, and causes a loss of cations and anions from the tonoplasm, which correlates with the inhibition of ion uptake reported.[133,134] Lipid peroxidation caused by free radical formation has been reported in the case of plants treated with cinnamic or benzoic acids.[135] Einhellig[11] suggested that sulfhydryl group decrease observed in the membranes owing to oxidation or crosslinking causes changes in the membrane architecture that will affect proton pumps, channel proteins and/or membrane transporters. Effects on membrane proteins induce a cascade of events inside the cell that ends up in cell death. As a consequence, first effects on this chain of effects should be noted in water balance. Treatment with phenolic acids (*p*-coumaric, caffeic, hydrocinnamic, salicylic, *p*-hyroxybenzoic, gallic, chlorogenic acids, hydroquinone, vanillin) (Fig. 2) causes loss of turgor, reductions in leaf water potential and water stress, as indicated by changes in tissue carbon isotope ratio.[136]

Phenolic acids have also been reported to inhibit a variety of enzymatic processes, including those affecting the levels of the phytohormone IAA through interaction with the IAA oxidase, which may either increase the enzymatic activity (increasing IAA decarboxylation) or inhibiting the oxidase degradation.[137,138] The effects depend on the allelochemical and the dose. Many other enzymes can be affected by phenolic acids, such as catalase, maltase, invertase, and phosphatase, among others.[139] However, they are unlikely to be primary targets, even though their failure adds to the overall effect. Also, the expression of medium to low molecular weight proteins and the melting point of DNA in soya treated with *m*-hydroxy and *p*-hydroxyphenylacetic acids are affected. These compouds have been related to soil sickness syndrome in soya monocultures.[140]

Finally, energy-related systems such as respiration and photosynthesis are also affected by phenolic acids. This has been proven in respiration of plants and microorganisms, especially mycorrhyzal fungi[141] from soils of phenolic-acid-producing species such as spruces.[142] Oxygen uptake is inhibited by benzoic and cinnamic acids in isolated mitochondria;[143] however, effects of phenolic acids on overall respiratory metabolism can be either stimulatory or inhibitory, depending on the concentration, the concentrations needed for inhibition being too high for a primary site of action. The effects observed in mitochondria seem to be related to alteration of the inner membrane,[143] and it has been suggested that blockage of electron transport in the b/c_1 cytochrome complex may exist.[11]

Photosynthesis is affected, probably owing to action on stomata and chlorophyll reduction.[133] Reduction of chlorophyll by phenolic acids seems to be caused through targeting of Mg-chelatase[144] and enhancement of the chlorophyllase and Mg-dechelatase activities, both of which are responsible for the chlorophyll degradative pathway.[145]

In summary, phenolic acids cause a wide variety of physiological effects that affect multiple targets at various concentrations. Phenolic acids are old compounds that appeared early in the evolution in plants such as conifers and brackens. Their defensive effect was probably overcome by development of new chemicals, and this could be the reason why many authors have doubts about their role as allelopathic agents. However, they clearly have this role in several plants, and their success in avoiding development of resistance could rely on the number of molecular targets they can affect. In spite of this, the concentrations needed to exert their phytotoxic action are clearly higher than those of the herbicides actually used, so their applicability in agriculture is limited.

2.3.2.2 Flavonoids. Flavonoids are known to act as potent antioxidant agents.[146] *In vitro*, flavonoids can bind electrophiles, inactivate oxygen radicals, prevent lipid peroxidation and inhibit DNA oxidation. However, in cell cultures they increase the rate of apoptosis and inhibit cell proliferation and angiogenesis. *In vivo* they can induce the activities of protective enzymes (conjugating enzymes such as glutathione transferases and glucuronosyl transferases) of the intestine and the liver. They have also been reported to induce apoptosis in animal cells[147] and to stimulate a mitochondrial Ca^{2+} uniporter channel that allows mitochondria to buffer local cytosolic calcium changes, which in turn modulates a variety of related phenomena, from respiratory rates to apoptosis.[148] Whether these activities are connected with their role as allelochemicals is not yet known. If so, it is surprising that (−)-catechin generates reactive oxygen species that result in cell death, which suggests a different mode of action in this particular case.[37] Flavonoids block mitochondrial oxygen uptake[149] and CO_2-dependent oxygen evolution in chloroplasts.[150] Their action has been related to membrane effects that might lead either to electron transport inhibition or ATP generation blockage.[149] In fact, flavonoids are known to mimic ATP and bind nucleotide-binding proteins, the structural requirements for such a binding being described to be polar linking hydroxyl groups at C-3, C-4′ and C-5′.[151] However, flavonoids have also been reported to present a protective effect, preventing membrane lipid oxidation and helping to keep membrane integrity and fluidity, mainly on account of their hydrophilic properties, the degree of oligomerisation and the number of hydroxyl groups in the molecule through interactions with the polar head of phospholipids at the water–lipid interface.[152]

In any case, further work is needed with flavonoids to determine their molecular targets and to check whether or not they present a common action related to membrane integrity and functionality in plant and animal cells. In any case, their applicability as antioxidant supplements and chemopreventive factors in diet has been acknowledged. Their possible use as agrochemicals is still under discussion and/or development.

2.3.3 Quinones

Quinones are a small group of allelochemicals. However, juglone is one of the first reported allelochemicals. It has been reported to inhibit oxygen uptake and react with SH groups of cysteine, glutathione and bovin serum albumin, decreasing the content of SH groups in barley and bean seedling extracts.[153] Juglone has also been reported to stimulate plasma membrane Protox (protoporphyrinogen oxidase) activity, but not in etioplast Protox,[154] and inhibit *p*-hydroxyphenylpyruvate dioxigenase (HPPD).[155]

Sorgoleone is another allelochemical reported from sorghum exudates that has attracted much attention in recent years. Sorgoleone has been reported to cause bleaching, inhibit photosynthesis and reduce oxygen evolution.[156] Lately it has been shown that sorgoleone presents two different primary modes of action. One is acting as a competitive inhibitor of the atrazine-binding site at PSII,[157] and it has been suggested that sorgoleone acts as a competitor of plastoquinone at the binding site of the D1 protein at PSII.[158] The second molecular target has been shown to be HPPD, in a similar way to sulcotrione and other commercial herbicides, resulting in inhibition of plastoquinone biosynthesis.[155] The existence of two different, though complementary, modes of action will make difficult the development of resistance through evolution. This has been pointed out by other authors as an advantage in the development of new herbicides.[11]

The mode of action of the lichen anthraquinones emodin and rhodochladonic acid analogues has also been studied. Emodin is a widely distributed metabolite in lichen, fungi and higher plants, and has been reported to be a nucleotide-binding site-directed inhibitor and an inhibitor of the protein kinase CK2[159] through substrate competition with ATP.[160] Emodin analogues with a hydroxyl-ended side chain caused selective bleaching in monocotyledons, whereas those with a methyl-ended side chain did not affect chlorophyll content. However, these effects do not seem to be related to light-dependent membrane leakage.[47]

Rhodocladonic acid is a poorly investigated lichen metabolite, and its mode of action remains to be understood. In this case, alkyloxy and hydroxyalkyloxy analogues did not affect the chlorophyll or carotenoid contents, although an increase in PSII activity was observed in both series.[47]

2.3.4 Terpenes

Knowledge of the structures and occurrence of phytotoxic/allelopathic terpenoids is greater than that of their mode of action. However, some papers reviewing this subject have been published recently.[161,162] An attempt will be made to summarize the main modes of action described so far, grouped by families of compounds.

2.3.4.1 Essential oils and their volatile monoterpenes. Compounds such as camphor and cineoles are known to inhibit cell mitosis, but whether or not this is a primary target site is still unknown for most of the allelochemicals cited.[162] The mode of action of cineoles and the cineole-like herbicide cinmethylin has recently been disclosed, with interesting findings. In spite of the similarities in their structures, they present different modes of action. 1,8-Cineol presents a variety of effects such as swollen root tips,[163] inhibition of mitochondrial respiration,[164] mitosis blockage[165] and inhibition of DNA synthesis.[166] 1,4-Cineol does not inhibit mitosis, even though it is itself a powerful growth inhibitor, but instead targets asparagine synthase,[56] being a unique mode of action that opens the door for new herbicide development. Cinmethylin [2-(2-methylphenoxy)-1,4-cineole] presents the same symptoms *in vivo*, but is unable to inhibit asparagine

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synthetase *in vitro*. It has been proposed that the ether bond of cinmethylin suffers enzymatic breakdown to give *cis*-2-hydroxy-1,4-cineole, a more potent inhibitor of asparagine synthetase, this being the real herbicide. This is is not an uncommon situation.[133,135]

Finally, diacetyl piquerol A is a potent growth inhibitor[167] that inhibits H^+-ATPase plasma membrane and tonoplast membrane activity.[168] However, no further work has been performed with these compounds to determine their primary mode of action.

In summary, little work has been done with monoterpenes, and much of this field remains unexplored, providing great opportunities for the discovery of new molecular targets and herbicide development. Moreover, their relatively simple structures, which should allow easy and inexpensive synthesis, along with their wide distribution and abundance in nature as essential oils, are advantages for their use as leads.

2.3.4.2 Sesquiterpenes. In spite of the high number of sesquiterpenes, especially sesquiterpene lactones, described as allelopathic or phytotoxic compounds, little is known regarding their molecular targets and modes of action. The modes of action of sesquiterpene lactones (SLs) have been extensively studied in animal cells owing to their anticancer properties. SLs seem to induce apoptosis in both normal and cancer cells,[169] and this seems to be a general behaviour in SLs; the pseudoguaianolide helenalin inhibits human telomerase.[170] Most of these activities have been attributed to their differential reactivity towards sulfhydryl-containing biomolecules such as cysteine and glutathione through α,β-unsaturated carbonyl systems, such as those in the lactone moiety or cyclopentenones.[171] Activities on important sulfhydryl-containing enzymes such as phosphofructokinase, glycogen synthase and inosine monophosphate dehydrogenase have also been reported.[171] Some of this knowledge can be translated to the allelopathic/phytotoxic action of SLs, especially that regarding their reactivity towards SH-containing biomolecules. However, little is known about their mode of action in plants.

Dehydrozaluzanin C (DHZ) (Fig. 12) causes separation of the plasma membrane from the cell wall and electrolyte leakage that is not light dependent, but its mode of action does not seem to be related to lipid peroxidation or radical production.[172] It also reacts with reduced glutathione, which fully reverses the growth inhibitory effects and electrolyte leakage. The DHZ phytotoxic effect can also be partially reversed by addition of the aminoacids histidine (his) and glycine (gly), resulting in atypical dose–response curves that show this is not the primary target site. Isozaluzanin C (the dihydroderivative of DHZ) is a much weaker phytotoxin, lacking the cyclopentenone ring. It does not cause any membrane leakage, but its phytotoxic effect can be reversed by equimolecular amounts of reduced glutathione, probably through the Michael addition reaction. The authors suggest that the leakage effect is caused by the cyclopentenone ring, while the reversal effect observed with the aminoacids is due to a second mode of action that proceeds through the lactone ring.

Artemisinin (Fig. 13) has been mentioned before in this paper as an atypical sesquiterpene lactone bearing an endoperoxide moiety and without the common double bond in the lactone ring. It has strong antimalarial activity[173] and a powerful inhibitory effect on plant growth.[174,175] Both seem to be related to the endoperoxide moiety, as deoxyartemisinin remains inactive. The presence of a plastid in *Plasmodium* spp. with much in common with the plastids in plants has been adduced as a reason for such a similar behaviour.[161,176] In any case, the primary mode of action is not yet fully understood, even though a plethora of physiological effects have been described for its action: leakage of proteins in duckweed but not in other species,[177,178] inhibition of peroxidase synthesis[177] and respiration;[179] other symptoms were stimulation of root oxygen uptake, inhibition of mitosis and induction of abnormal mitotic figures that were also present in all phytotoxic artemisinin analogues, allowing the authors to propose a common, yet unknown, mode of action for all artemisinin-like SLs.[177]

Sesquiterpenes are also involved in the chemical recognition between parasitic plants and their hosts.[180]

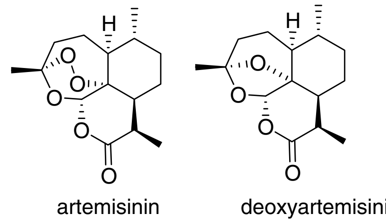

Figure 13. Artemisinin: active principle obtained from the trichomes of *Artemisia annua*.[206]

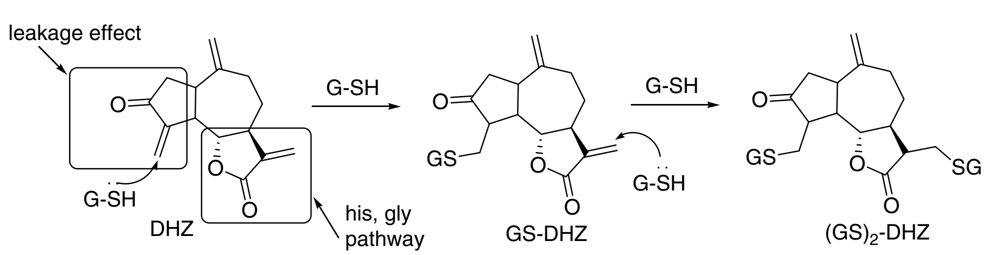

Figure 12. DHZ reacts with reduced glutathione (GSH) in a Michael reaction. The cyclopentenone moiety is more reactive than the unsaturated lactone ring and reacts first.[172]

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Regarding the mode of action of SLs on witchweed and broomrape, a hormonal mode of action has been proposed to explain dihydroparthenolide stimulatory activity.[181] However, the molecular receptors for these compounds and for the strigolactones have yet to be determined. Recent work by Reizelman *et al.*[182] reported the synthesis of biotin-containing strigolactone analogues that linked to a binding protein in the membrane fractions of *Striga hermonthica* (Del.) Benth. seeds, thus being the first attempt to isolate and characterise the molecular target of the germination inductors of a parasitic plant.

To the present authors' knowledge, no other sesquiterpenes have been studied for their mode of action.

2.3.4.3 Diterpenes. Quassinoids are the only diterpenes whose mode action has been studied. Chaparrinones were shown to be potent plant growth inhibitors, causing bleaching and inhibiting mitosis at different levels.[92] However, in spite of their reported effect on the plasma membrane NADH oxidase activity[183] of animal cells, they did not show any effect on plant cells.

In summary, much effort is currently being invested by scientists working on allelopathy to elucidate the mode of action of allelochemicals. This effort is not evenly distributed among the different skeletal types. In fact, there is a large amount of literature available on certain classes of compound (e.g. phenolics), and little is known about others (e.g. terpenes). The main reason for this situation is that most phenolics are much more readily available in large amounts (many can be purchased from chemical companies or they can be isolated in reasonable amounts from their original sources) than other types of compound. This is a crucial point, as the isolation of secondary metabolites is expensive and time consuming. Often, it does not produce the amounts of compound necessary to complete the study. In spite of all these difficulties and disadvantages, new target sites are expected to be discovered during the coming years that will allow the development of new herbicides to control herbicide-resistant biotypes of weeds. The authors are strongly confident that spectacular advances and applications can be foreseen in this particular area.

3 PROTEOMICS AND GENOMICS

Proteomics and genomics represent one of the more recent advances in biological and biochemical sciences. The characterisation of protein profiles, the changes induced by biotic and xenobiotic factors, the characterisation of the genes involved in each biosynthesis pathway, the up- and downregulation of their expression as a function of the cellular environment – all of these can give a 'photo-finish' of the state of the cells or the organism at a given moment under given circumstances and provide insights into their specific response towards specific stimuli.

3.1 Proteomics

As in other research fields, the use of proteomics has lagged behind genomics as a tool to study the effects on plant metabolism by different stress stimuli. To date, few reports regarding the effects of allelochemical stress on protein expression have been published.[184] The use of two-dimensional gel electrophoresis (2D-PAGE) and gel scan densitometry analysis to detect differences in the cytoplasmatic protein pattern expression of bean and tomato caused by aqueous extracts obtained from the dry leaves of tropical allelopathic plants (*Acacia sedillense, Lantana camara* L. and *Callicarpa acuminata*) has been recorded, showing modifications in the protein content.[185] In the case of *C. acuminata,* microsequence analysis showed increased expression of proteins with a high degree of similarity to an α-amylase inhibitor-like and a glutathione-*S*-transferase (GST) protein in bean and tomato roots respectively.[186] Both proteins have been related to general and non-specific detoxification and defence responses, as they have previously been involved in other stress responses.[187] Similar studies found a different number of *de novo* stress proteins induced for kidney bean and maize treated with aqueous extracts of the allelopathic trees *Acacia nilotica* Del. and *Eucalyptus rostrata* Schlecht.[188] However, studies have not gone far beyond this point, and much more work is needed in this area to characterise the metabolic response of targeted plant cells towards the presence of allelochemicals. To the present authors' knowledge, data regarding specific protein expression responses to pure allelochemical stress have not been addressed.

3.2 Genomics

On the other hand, genetic studies have been conducted for more than a decade on important economic crops with allelopathic traits, addressing in particular the genetic variation and inheritance of allelopathy. The advantages of crops with an enhanced allelopathic character are obvious: an increased resistance to weeds and pests, lower inputs in herbicide dosing and lower yield losses due to weed infestation, among others. The advances in this area would be comparable to the discovery of the herbicides or the development of herbicide-resistant crops. In this field, the greatest efforts and progress have been achieved in rice[189] and wheat,[190] two of the most economically important crops in the world. With regard to gene manipulation, two main alternatives are available for enhancing the allelopathic traits in allelopathic crops: classical genetic breeding and genetic engineering.

With regard to genetic breeding techniques, the greatest need for proper advances in enhancing allelopathic traits relies on the availability of appropriate molecular markers related to the allelopathic character, e.g. the genes encoding the synthesis of allelochemicals. This will give the breeders a powerful, reliable and easy-to-use tool to quantify the improvements in enhancing the allelopathic character of new varieties.

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Otherwise, breeders are forced to test the allelopathic potential of new varieties through bioassay with adult crop plants or with their corresponding extracts. This method is slow, expensive and time consuming. Specific allelopathy-aided breeding programmes have led to the creation of highly allelopathic rice varieties.[191] However, the results do not seem to justify the huge efforts made, from a practical point of view, even though the study of these varieties will make easier the identification of genes involved in allelopathy.

In this way, some advances have been achieved in the identification of quantitative trait loci (QTL) associated with wheat[192] and rice[193,194,195] allelopathic character. Genes encoding hydroxamic acid biosynthesis have not yet been identified in the case of wheat, and the chemical nature of allelochemicals responsible for the allelopathic activity in rice is still unclear, even though there are some advances in the identification of genes encoding momilactone synthesis.[196]

Sorgoleone is another case study, as it accounts for much of the phytotoxicity of sorghum. The sorgoleone biosynthetic pathway has been recently disclosed,[197] and work is in progress to identify the genes involved.[198] Efforts to enhance gene expression of the sorgoleone pathway in *Sorghum* spp. are in progress.[199]

In spite of the high number of allelochemicals known, little or nothing is known about the genes encoding their biosynthetic pathways, probably because many of these compounds are of weed origin. Genomic maps of economically important crops are still not available and are urgently needed. Important advances in this area are expected in the coming years, as increased efforts and economic and scientific resources are being invested.

During the time course of breeding programmes, research has focused on specific, commercially attractive traits (e.g. colour, size of the fruit, grain yield, etc.). It is likely that, during this process (started at the beginning of mankind's history), domesticated plants lost part of their resistance with regard to their wild relatives, even though this fact is still unclear.[200] Nevertheless, germ plasm recovery programmes are under way to set up libraries to preserve old varieties of important crops. Research programmes to identify ancient varieties of wheat in the Middle East can pursue this objective. This will give the breeders the opportunity to gain access to wheat varieties with enhanced allelopathic characters.

The alternative to classical breeding is the development of genetically engineered crops.[201] Little has been done with the objective of increasing the allelopathic potential of crops using transgenes. Recently, it has been suggested that one of three possible strategies to reduce herbicide applications is through genetic engineering[202] and enhancement of crop competition using allelopathic traits.[203,204] Irrespective of ethical considerations, there is no doubt that overexpression of genes encoding the biosynthetic pathway of allelochemicals in a crop will result in an enhancement of aggressive and defence characteristics towards weeds and, probably, other pests. Also, the insertion of foreign genes encoding the biosynthetic pathway of phytotoxins into a given crop will result in highly allelopathic varieties. The source of these genes can be the same crop, another plant or other organisms (probably microorganisms). The direct consequence will be a reduction in the amounts of agrochemicals needed. However, besides the technical problems, issues such as avoidance of autotoxicity, translocation of the allelochemicals to the rhizosphere and metabolic imbalances need to be resolved in the new crops. Advances in determining and cloning relevant genes involved in monoterpene synthesis in the essential-oil-producing plant model peppermint has provided strategies for increasing the monoterpene biosynthesis.[205]

Also, following the herbicide-resistant transgenic crop strategy, Duke[203] suggested as another alternative the development of pathogen-resistant transgenic crops and the use of these pathogens as mycoherbicides.

4 FUTURE TRENDS AND PROSPECTS

Allelopathy cannot be considered any longer as a nascent science, yet a long road remains ahead. Curiously, the main weakness pointed out by many critical voices has shown the path for the best achievements and the next steps and developments. The authors envision allelopathy's primary future trends in some very specific topics and subjects:

1. Much has been done in the chemistry of allelopathy during the last three decades. More still needs to be done. The identification and knowledge of the molecules responsible for allelopathy is the keystone. Any other point of view will cause allelopathy to remain conjectural, out of testable arguments. However, it must be pointed out again that not all phytotoxic compounds isolated from a plant need to be allelopathic, as they might exert other defensive or internal roles. Consequently, the chemistry should be closely connected with the apropriate ecological field studies and bioassays, supporting the allelopathic role of the compounds. Also, underexplored fields like aquatic (marine and freshwater) allelopathy, fungi and lichen allelopathy are promising areas where much research is needed and where important discoveries with practical applications are expected.
2. Mode-of-action studies: the understanding of how allelopathy actually works requires knowledge at the molecular level of how allelochemicals exert their effects. Modern herbicide research has achieved great successes through target-oriented herbicide development. It is extremely important to know the reaction/s involved in the allelochemical action, and the architecture of the putative binding sites in proteins, enzymes or DNA. For such work,

two approaches can be applied that represent two directions of the same highway: SAR studies to determine the structural requirements needed for activity, and isolation and characterisation of the molecular targets and their binding sites. In any case, knowledge of the mode of action is needed. Important developments and applications can be expected in a short time from the results of mode-of-action studies with allelopathic compounds. However, for this type of work, development of more accurate and case-related bioassays is needed, as the allelopathy phenomenon is extremely specific in each situation. For mode of action studies, allelopathy scientists should take advantage of all the work already developed for herbicide studies.

3. Genomics seems to be sometimes like the philosopher's stone: the magical source for any knowledge and richness. However, the great potential of this technology cannot be neglected. Genomics, along with proteomics and metabonomics, is still awaiting application in allelopathy. It is true that some really good work has begun, but many allelopathy scientists are still waiting for the development of technological tools that will further the scope of their studies. In this sense, much work remains to be done in developing appropriate procedures in proteomics and metabonomics, and the three of them – genomics, proteomics, and metabolomics – are thought to constitute a field where much effort should be spent in the next decades. Conversely, the main advances and applications of allelopathy are expected in this field, but they will necessarily be proportional to the effort and resources invested, which are still low. Also, ethical and environmental concerns arise from the development and use of genetically modified organisms (GMOs). Public opinion regards their use as controversial, especially in Europe. Determination of the genes encoding allelopathic traits, description of the biosynthetic pathways, and transfer of biosynthetic pathways from one plant/microorganism/organism to another, enhancing the overexpression of allelopathic genes, through genetic engineering and/or classical breeding, are some of the fields that are being developed.
4. However, the key point will be the development of real applications of allelopathy in agriculture and forest management. The development of new herbicides with new modes of action that will help to fight the problem of weed resistance to herbicides is expected. To date, the only herbicide structurally based on a natural product (bialaphos) that has been succesfully marketed is glufosinate. Cinmethylin cannot be considered as such, since its development was not based on the cineole structure. Other predictable applications come from the side of genomics: classic approach or obtaining GMO's to enhance allelopathic traits in crops. Also, new agricultural practices using allelopathy to maintain weeds at a reasonable level that do not adversely affect crop yields are expected: such practices should not rely so heavily on herbicide use, thus alleviating environmental concerns, and would help developing countries effectively to control their weed problems.

5 CONCLUDING REMARKS

The authors believe that allelopathy should be understood as a general mechanism by which plants interact with the surrounding organisms in their ecosystem. This interaction usually leads to an equilibrium where distribution and relative abundance of each member of the community utilise their own adaptations to face adverse environmental situations: seed germination power, seed viability, growth rate, physical defences, ... and allelopathy. When a plant is taken out of its own niche, often other members of the community are not prepared for the new chemical weapons, and the new plant then becomes invasive. Consequently, when trying to explain the structure of an ecosystem, allelopathy cannot be the only reason for plant distribution patterns, but should be considered as an actor with an important role.

To reach this goal, there is a need for more accurate studies overcoming the philosophy of phytotoxic extracts = allelopathy. Extracting massive amounts of extracts with organic solvents is useful to establish the chemical composition of a plant and a previous step to metabonomics. However, it does not necessarily reflect allelopathic potential, even though most of the important allelochemicals (juglone, sorgoleone, SLs, phenolics, hydroxamic acids, etc.) have been isolated and characterised by this means. Ideal conditions involve non-aggressive extraction methods (whole plant, lixiviation, rain simulation). Compartmentalisation of phytotoxic compounds is a general trend (trichomes, vacuoles in surface tissues of leaves, flowers and roots); localisation of allelochemicals in the plant should provide clues to their allelopathic role.

In any case, all phytotoxic compounds are useless from the allelopathic point of view if they are not exuded into their environment. Consequently, the allelopathic potential should be measured as a function of the amount released to the surroundings, as their concentration inside the plant cannot be considered as conclusive.

Another point to be discussed is that allelopathy is usually considered with regard to the producer plant, but few studies are available in which the uptake and/or biotransformation/detoxification of the allelochemical are measured. Considerations of the selection of appropriate target plants for each study (ecological or just phytotoxicity) should be introduced into research design prior to its completion.

Finally, it is important to point out that the compound released is not necessarily the one reaching the target plant. Compounds may be subjected to chemical and biochemical transformations and processes that limit their availability or change their chemical and biological properties. Consequently, soil degradation studies under sterile and non-sterile conditions should be done, the evolution of the allelochemicals should be measured and their time curves and half-lifetimes described, and their conversion/degradation products should be characterised, as well as the biological activities of the resulting compounds. This will provide a more accurate picture of the actual effects observed and the probabilities of these compounds being real signal transducers of the allelopathic effect.

As a final remark, the authors wish to highlight the great change that allelopathy has experienced in the last 3–4 decades, from not being considered as a science to the present, with a solid body of knowledge coming from all the different fields involved in plant sciences: ecology, chemistry, biochemistry, genomics and soil studies. From this change, practical solutions and developments are expected during this decade, the most predictable of which include the development of new herbicides, the development of allelopathic crops with significantly decreased herbicide applications and the discovery of new modes of action and transgenic crops.

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