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Strigolactones: how far is their commercial use for agricultural purposes?

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Running title: Strigolactones in agriculture

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Abstract

Strigolactones are a class of natural and synthetic compounds that in the latest decade are exciting the scientific community not only for their intriguing biological properties, but also for the potential applications in agriculture. These latter range from the use as hormones to modify and/or manage the plant architecture, to stimulants to induce seed germination of parasitic weeds and thus control their infestation by a reduced seed bank; from "biostimulants" of plant root colonization by arbuscular mycorrhiza fungi, improving plant nutritional capabilities, to other still unknown effects on microbial soil communities. More recently, those compounds are also attracting the interest of agro-chemical companies. Despite their biological attractiveness, practical applications are still strongly hampered by the low product yields obtainable by plant root exudates, by the costs of their synthesis, by the lacking knowledge of the off-target effects, and by the not yet specified or properly identified legislation that could regulate the use of those compounds, depending on the agricultural purposes. The aim of this article is to discuss, in the light of the current knowledge, the different "scenarios" that could appear in the near future about bringing strigolactones into the practice.

Keywords: strigolactones; synthesis, legislation; agro-chemicals, non-target effects

1. Introduction

Strigolactones (SLs) are a class of natural and synthetic compounds that in the latest decade are exciting the scientific community not only for their intriguing biological properties, but also for the potential applications in agriculture. They proved to act as: hormones, involved in the modulation of the plant architecture; stimulants, inducing seed germination of parasitic weeds and thus key factor in the mechanisms of recognition of the host plant by the parasitic one; signals, helping arbuscular mycorrhizal fungi to recognize and colonize plant roots (see the successive section for exhaustive references). These different biological properties have generated further scientific and applicative interests, these latter especially for agricultural purposes, attracting more recently also the interest of agro-chemical companies. However, despite the biological attractiveness, practical applications are still strongly hampered by a number of constraints, as the low product yields obtainable by plant root exudates, the costs of their synthesis, the lacking knowledge of the off-target effects, and the not yet identified legislative requirements that could differently regulate the registration of those compounds depending on the agricultural purposes. Indeed, depending on the "type" of utilisation, SLs could follow the registration process as: (a) Plant Protection Products (PPPs), if considered as phyto-hormones or natural herbicides for the suicidal germination; (b) Plant Strengtheners, if used as compounds activating the defense mechanisms of the plant against harmful organisms; (c) Plant Biostimulants, if applied to plants to stimulate nutrient uptake or nutrient use efficiency. In the light of the current knowledge, the aim of this article is to discuss the different "scenarios" that could arise in the future when try bringing strigolactones into the practice.

2. A brief state of the art

The first SLs to be identified were strigol and strigyl acetate, isolated in 1966 as the first *Striga* germination stimulants from the root exudates of cotton (*Gossypium hirsutum* L.), a non-host of parasitic *Striga* spp. [1,2] Later on, strigol was also identified in the root exudates of real *Striga* hosts, i.e. sorghum (*Sorghum bicolor* (L.) Moench, maize (*Zea mays* L.) and common millet (*Panicum* *miliaceum* L.).[3] Since then, a number of other SLs were isolated from the root exudates of several host and non-host plant species.[4] They act as key factors in the interaction between the host and the parasite, as seeds of this latter cannot germinate, and thus cannot start the parasitic cycle in absence of this stimulus. These compounds are very powerful in inducing seed germination of root parasites, as they act at concentrations ranging between 10^{-7} and 10^{-15} M.[5,6] After germination, the parasites attach themselves to the roots of many plant species and acquire nutrients and water from them, thus causing considerable crop losses in many parts of the world. *Orobanche* spp. and *Phelipanche* spp. (broomrapes) are holoparasites and parasitize important agricultural crops around the globe such as legumes, crucifers, sunflower, hemp, tobacco and tomato. *Striga* spp. (witchweeds) are hemiparasites and cause enormous damages to cereal crops mainly in the sub-Saharan regions.[7] After having long considered SLs only for their germination stimulatory properties, an interesting question arose on why plants should exude SLs if they enable their enemies to locate them. Therefore, SLs were hypothesized to have roles other than that in parasitism recognition, most likely a positive one. Such a beneficial role was unveiled through the discovery that they induce hyphal branching and spore germination in symbiotic arbuscular mycorrhizal fungi (AMF).[8] AMF are soil borne obligate symbionts that help the plant by improving the uptake of inorganic phosphate and other minerals, and hence can sustain plant growth. These fungi penetrate and colonize plant roots, where they develop highly branched structures called arbuscules, which are the sites of nutrient exchange. The successful colonization of a host plant by AMF relies on the establishment of a network of connections between the host plant roots and the fungal hyphae, regulated by SLs. Although SLs are essential host-recognition signals for AMF, with which the majority of land plants form symbiotic associations, there are some non-hosts of AMF, such as *Arabidopsis* and white lupin (*Lupinus albus* L.), that also produce SLs. Later on, two groups independently identified SLs, or their further metabolites, as a novel class of hormones regulating plant shoot branching.[9,10] This suggested that SLs could have other unknown functions in plants, perhaps in normal growth and development. Excellent reviews on the discovery of hormonal functions of SLs have been published.[11,12] More recent efforts have been devoted to examining their effects on plant growth and development. Among other biological functions evaluated, SLs were reported to promote seed germination of some crops and weeds, to affect root architecture and plant secondary growth, and to be involved in the rhizobium-legume interactions.

3. Potential uses in agriculture

Due to the SLs effectiveness in several biological systems, scientists have tried exploiting numerous practical applications for these compounds, mainly for agricultural purposes.

3.1. Parasitic weed management

A first proposed applicative use of SLs was for parasitic weed management, the so-called "suicidal" germination. As the seeds of the parasitic weeds can survive for a very short time after germination, unless they found nearby an available host root, the idea behind this strategy is to stimulate the germination of the seeds when the host is not present, thus causing the death of the germinated seeds. This could be a long-term procedure for reducing the seed bank of the parasite. Attempts were made since long time,[13 and reference therein cited] but a number of problems hampered any real practical applications. These include: (a) the high costs to produce sufficient amount of the synthetic products; (b) the difficulties in delivering the compounds along the soil profile, and thus to reach effectively as many seeds as possible; (c) the instability of the compounds. However, the advent of new technologies (e.g. nanotechnologies, biotechnologies, advanced delivery systems) could open new applicative possibilities, and allow overcoming the problems.

3.2. Bio-fertilizers

Considering the capability of favouring the colonization of the crop roots by symbiotic fungi and that of rhizobia, from an agricultural perspective SLs could be considered as "indirect" bio-fertilizers. Indeed, one of the primary roles of AMF in the symbiotic relationship with plants is the delivery of mineral nutrients, particularly phosphate. In many areas of the world, the concentration or availability of this essential mineral nutrient in the soil is limited and this significantly affects plant growth and health. AMF can help to improve the uptake of phosphate and hence improve plant growth in these areas. In agreement with the important role of AMF in the acquisition of phosphate, root exudates produced by plants grown under phosphate limitation proved to be more stimulatory to AMF than exudates produced under adequate phosphate nutrition. [14]

3.3. Plant hormones

The initial discovery that SLs were involved in the inhibition of axillary bud outgrowth [8,9] promoted a multitude of other studies showing that SLs also play a role in defining root architecture, in particular shoot branching, secondary growth, hypocotyl elongation, root growth, nodulation and seed germination, mostly in interaction with other hormones. Their coordinated action enables the plant to respond in an appropriate manner to environmental factors such as temperature, shading, day length, and nutrient availability. [15] The exudation rate of SLs is highly sensitive to nutrient levels in the soil, with plants all exhibiting a strong increase in SL production under low phosphate conditions. [16] This strong regulation of SL biosynthesis and exudation may be the mechanism through which plants adapt their changes in shoot and root growth and architecture in response to phosphate availability. [16] Thus, a deep knowledge of those mechanisms could lead to a practical use of SLs in agriculture to regulate plant growth and shape according to the nutritional availability and the environmental characteristics, in order to increase plant fitness, and obtain its best performance in the given environment.

4. Current methods and limits in SLs production

4.1. Known natural SLs

About 20 different natural strigolactones have been isolated and characterized so far in plant root exudates, but it is easily predictable that this number is going to increase. [17] Different plant species and even different varieties of one crop species produce different SLs and/or mixtures of these signaling compounds. All the known SLs have similar structures. The core of the molecules consists of a tricyclic lactone (ABC part) connected via an enol ether bridge to a butenolide group (the D-ring, Figure 1). Among natural SLs, 5-deoxystrigol (5DS) was first isolated from root exudates of *Lotus japonicas*. [8] Due to its simplicity, 5DS was proposed to be the common precursor of other SLs. [4,18] Solanacol was first isolated from root exudates of tobacco, [19] and proved to be one of the major SLs in tomato. [20] Orobanchol and its acetate are the most common SLs in the plant kingdom. The structure of orobanchol (Figure 1), first isolated by Yokota and co-workers, [21] has been revised by Ueno and colleagues. [22] There is now a general agreement in including in the SL family compounds with modifications of the ABC core such as avenaol [23] (Figure 1), or lacking the tricyclic lactone, such as heliolactone [24] or carlactone [25] (Figure 1). [25,26] Currently there are 18 characterized SLs with a tricyclic lactone (ABC ring) and $2'R$-configured butenolide ring (D ring). All natural SLs fall into two distinct families, strigol- and orobanchol-derivatives which differ by the stereochemistry of the B-C-ring junction. The C ring of the strigol-like SLs is in the β orientation (up; 8b*S* configuration), whereas that of the orobanchol-like SLs is in α orientation (down; 8b*R* configuration).

4.2. Isolation of natural SLs

The daily production of SLs per plant is very modest. Studies carried out on young cotton plants proved that the average exudation of strigol and strigyl acetate was around 15 and 2 pg/plant/day, [27] hence the collection of the root exudates from hydroponically grown host plants requires an experimental set-up with many plants. This “natural” production is clearly not suitable for SL mass production. A few attempts have been made to evaluate the capability of cell culture suspensions of *Arabidopsis* and rice to produce SLs as cell factories. [28,29] However, although both the cell species were able to produce and release a number of different SLs to the culture media, the systems did not allow to collect large amounts of the compounds, because SLs are quickly degraded and thus cannot be accumulated into the medium. Although the systems were not further scaled up, these encouraging findings indicate that plant cell cultures could have potential for the SL mass production. The isolation of SLs from root exudates is very laborious and purification requires a careful chromatographic separation. Moreover, organic synthesis of SLs is challenging due to their complex structure and stereochemistry, making these compounds either commercially unavailable or very expensive. So far, most of our knowledge about SLs signal transduction and molecular events associated with it is mainly based on the application of a synthetic analogue. Recently, major progresses in elucidating the biosynthesis of several SLs have been obtained, and it can be expected that the genes involved in the synthesis of others will be soon identified, making metabolic engineering of SL biosynthesis feasible. SLs are synthesized from all-*trans*-β-carotene, via 9-*cis*-β-carotene and the central intermediate carlactone (Figure 1) that is considered as the precursor of the other SLs. In principle, it would then be possible to install the biochemical pathway(s) by transforming suitable microorganisms. They could be then cultured at low cost to release the bio-synthesized compounds in the growth medium. Considering the current knowledge and the recent advances in the understandings of the SL mode of action and biosynthesis, it is foreseeable that the metabolic engineering is not far away of becoming a feasible approach. This will pave the way for large-scale production of natural SLs at low cost which can be used for basic research or applied in agriculture. Moreover, the characterization of SLs transporters will allow a better understanding of their functions within crops and open up new possibilities for modulating SLs release into the soil.

4.3. Mechanism of action and stability

The flipside of the coin of the high activity of SLs in biological systems is their instability in soil. Strigol and its analogues are prone to hydrolysis in alkaline medium due to the high reactivity of the enol ether functionality present in these compounds, which produces an ABC-formyl lactone and 5-hydroxybutenolide (D-OH, Figure 2). The half-life of GR24 (mixture of stereoisomers), the synthetic analogue of SLs used as universal standard in most of the biological assays, at neutral pH is 10 days, while that of 5DS is about 1.5 days. The level of the formyl tricyclic lactone deriving from 5DS rapidly increased within 24 h, and then gradually decreased over time, indicating further degradation of the formyl lactone possibly by oxidation and hydrolysis. In contrast, no appreciable decrease was observed in 5DS concentration when incubated in acetone at 32 ° C for 21 d. [30] The lability of SLs in the soil is a worthwhile aspect in view of field applications as it prevents the accumulation of the chemicals into the soil, a well-known phenomenon known as DDT effect. On the other side, appropriate formulation of the chemical can partially inhibit hydrolysis. A fine-tuning between stability (to be effective) and lability (to prevent accumulation and pollution of the soil) is then the main goal of new formulated SLs-like compounds. Very recently, Kannan and Zwanenburg [31] suggested taking advantage of the lability of SLs to introduce a new concept to combat parasitic weeds by decomposing germination stimulants prior to action so that no germination of seeds can take place anymore. They used borax and thiourea in natural conditions that promote decomposition of SLs and, therefore do not allow the parasitic weeds to germinate.

5. Sustainability of synthetic production

The synthesis of SLs is by far challenging, time and money consuming, and currently not feasible for applications in agriculture. However, it should be stressed, as it was also highlighted in the recent review of Zwanenburg and Pospíšil, [32] that the total synthesis of SLs is the most reliable and recommended method for successful structure elucidation of these natural products. Naturally occurring SLs have a too complex structure for synthesis on a multi-gram scale. [33] The total synthesis of several natural SLs has been accomplished, but the synthetic pathways involves several steps, usually more than 20. In order to study the effect of SLs on various biological processes, model compounds were designed and prepared. A prerequisite is that SL analogues should have a (much) simpler structure than natural SLs, retaining at best their bioactivity. Synthetic SLs can be classified into two main categories: (a) analogues, whose structure is very similar to the canonical natural SLs; and (b) mimics, whose structure is much simpler but showing a bioactivity resembling that of SLs.

5.1. Analogues

SLs analogues plays a key role in research bioassays. GR24, [34] the SL universal standard, is produced in a multi-gram scale as a mixture of stereoisomers; chiral separation of enantiomers and enantioselective syntheses have also been proposed, [33] but higher cost of production are in this case a drawback. Within the family of GR derivatives (GR24 being the most known representative, Figure 3) the concept of designing simpler structures retaining bioactivity led to the synthesis of GR5 and GR7. This latter was used as a suicidal germinating agent against *S. asiatica*. The stimulant was applied at a 10 mg L^{-1} in boxes filled with soil, which corresponds to around 750 gr ha^{-1}. [35] The stability of the stimulants is once again a crucial factor. At pH < 7.5 the half-life of GR7 is around 100 h, at higher pH the stability rapidly decreases. The germination of *S. hermontica* seeds to GR7 and GR24 proved to be strongly influenced by pH and moisture. Worth of mention is the Nijmegen-1, that can readily be obtained from simple starting materials in a few synthetic steps and whose germinating activity is comparable to that of GR24. Nijmegen-1 has been used in suicidal germination experiments in the field. [18] It has been estimated that ca 6.25 g of stimulant is needed per ha, which means a cost of approximately 100.000 € based on the official catalogues of the companies selling the compound. Currently the high costs prevent a practical application of the SLs technology in field. In line with the concept of designing simple and accessible active molecules, a series of analogues derived from cheap and accessible ketone have been also proposed. [36] The experiments in pots showed that the compound derived from tetralone (Figure 3) gave promising results. [13] EGO10 is an indolyl-derived SL readily prepared in three steps from available reagents and it is used as plant hormone in the regulation of shoot branching. [37] In designing new analogues with germination capabilities, the replacement of an oxygen by another heteroatom led to two successful examples of such an isotheric replacement, namely imino SL analogues [38] and strigolactams. [39]

5.2. Mimics

The so-called "SL mimics" are compounds lacking the ABC scaffold but retaining the D-ring connected to an additional group by means of an ether or ester functionality. The term "mimics" comes from the observation that they mimic SLs activity. Due to their simpler structures retaining high activity, they can be considered promising candidates for agricultural applications. One group of mimics with seed germination stimulatory activity shows an aryloxy substituent at C-5 (Figure 3), and were named debranones (furanones showing debranching activity) because the main activity profile is the inhibition of shoot branching. [40,41] Seeds of *Striga hermonthica* respond modestly to debranones, whereas *p*-chlorophenoxy induced the highest activity. The second group of SL mimics has an aroyloxy substituent at C-5 of the D-ring.[42] These SL mimics are moderately active as germination stimulant towards *S. hermonthica* seeds, but remarkably active in the case of *Orobanche cernua* and *Phelipanche ramosa* seeds. The inhibition of shoot branching by these aroyloxy SL mimics has not been tested, yet, but experiments are ongoing. A SL mimic having a S-aryl at C-2' and an extra methyl group at C-3' has been reported to be surprisingly active.[43] Interestingly, the same authors synthesized a compound named AR36 consisting of a D-ring connected to an acyclic unsaturated moiety (Figure 3). It proved to be highly active as shoot branching inhibitor in pea but, at the same time, it did not induce the seed germination of various root parasitic weeds. This is a nice example of how a suitable molecular design can help in separating beneficial from detrimental effects.[44] The search for new efficient and selective biological active compounds for field use can be also addressed by testing libraries of available compounds. In this respect, a yeast-based high throughput screening protocol was developed, leading to potential candidates mimicking SLs activity.[45] However, until more information about synthesis feasibility, production costs, toxicity and persistence in the soil are available, their potential use as new agents for applications in agriculture remains questionable.

5.3. Inhibitors

Due to the role of SLs as multifunctional molecules, the search for simple agonists or antagonists may also play a role in both basic research and agricultural applications. Given that most of the enzymes involved in the biosynthesis of SLs are known, biosynthesis inhibitors have been identified and successfully applied.[46] However, the search for perception inhibitors is still in its infancy. To date, all the SLs agonists identified show a D-ring or derivative, with the only exception of the cotylimide (CTL) compounds,[45,46] whose structure does not involve a D-ring. The identification of suitable inhibitors may allow a fine control and tuning on SLs effects.

5.4. Effect of synthetic strigolactones on the rhizosphere, threats of environmental effects, stability and possible effect of SL degradation products

After the identification of SL as phytohormones, an intense scientific activity has provided insights on the multiple plant traits that are controlled by the hormonal action of SLs. In general, SLs contribute to plant adaptation in poor soils and many scientific papers have proposed the use of SLs in agricultural soils with the aim to increase crop productivity. However, the SL impact on indigenous soil microbial community is unknown. A further implementation in the SLs "story" would be to ensure that the use of SLs to enhance crop performance is safe for the soil life. Biodegradability of lead compounds through studies on molecules stability in aqueous medium at different pH, their photo stability and the identification of by products will be highly desirable. The proven lability of SLs assures minimal SL persistence in soil and prevent SL accumulation. However, whether SL hydrolysis products influence soil microorganisms (structure, abundance and function of the soil microbial communities) needs still to be investigated.

6. Off-target effects

Due to the availability of only modicum amounts of SLs, the studies of off-targets effects have received only limited attention. Among them, some tests considered the use of synthetic GR24 at concentrations up to 8.5 10^{-5} M, which proved to have an inhibitory activity of the radial growth of some phytopathogenic fungi, among which *Fusarium oxysporum*, *Sclerotinia sclerotiorum* and *Botrytis cinerea*, [47] associated to an increase of hyphal branching. However, the concentrations that were found to be active were far higher compared to the "physiological" amounts of natural SLs produced and excreted by roots. More complete and exhaustive bioassays on a number of off-target organisms would absolutely be necessary in view of any SL practical applications (see above). To perform those bioassays very large amounts of compounds would be necessary, and the high costs have probably made this kind of biotic evaluations not economically affordable yet, despite the scientific interests.

7. Regulatory aspects

The discovery of new natural molecules for agriculture uses generates new scientific, methodological and regulatory issues. From this latter point of view, the first step to understand how the future use of a new molecule could be regulated is to identify its features. Considering the biological properties of SLs above described, different scenarios could arise for the future regulation of their use in agriculture. Indeed, these chemicals may be configured as plant protection products, or as plant strengtheners, or as biostimulants, and subsequently they would be subjected to different registration procedures. They are summarized in table 1, and discussed in the successive sections.

7.1. Plant Protection Products (PPPs)

Considering that SLs can be translocated within plant tissues, and that they act at very low concentrations, these two typical features could allow their registration as phyto-hormones. Furthermore, although SLs do not directly kill the seeds of the parasitic weeds, in case of use for the suicidal germination (see above), they would probably face the registration procedures required for natural herbicides. In both cases, the regulatory procedure at the EU level for their inclusion in the legislation would be that defined by the Regulation (EC) No 1107/2009 [49] concerning the placing on the market of PPPs, setting rules for the assessment and the authorization of active substances, safeners and synergists, adjuvants and co-formulants. The EC evaluates each active substance for safety before it can reach the market in a PPP. Indeed, besides general or specific beneficial effects against organisms harmful for plants (including other plants), active substances, and their residues in food should not have any harmful effects on human and animal health, and any unacceptable effects on the environment and non-target species. These requirements are defined in Annex II, points 3.6, 3.7 and 3.8, respectively, of the regulation above cited, and also in the Regulation (EU) 544/2011, [50] which establishes the procedures to evaluate the impact of compounds on human health, the toxicological and ecotoxicological criteria, the fate and behaviour of active substances in the environment.

For the approval of an active substance, the producer must submit an application to a Member State, under the payment of a fee and together with a complete dossier demonstrating that the active substance fulfils the criteria required for approval. Under the EU rules, it takes approximately 2.5 to

3.5 years from the date of application to the publication of a Regulation approving a new active substance, and this time varies greatly depending on the complexity and completeness of the dossier. PPPs contain at least one approved active substance and, before any of them can be placed on the market or used, it must be authorized by the Member State(s) concerned, according to the rules and procedures for authorization provided by the cited Regulation. [49]

The zonal system of authorization operating in the EU countries divides Europe into 3 zones (North, Central and South), identified on the basis of specific agro-climatic characteristics of the various countries. Member States assess applications on behalf of other countries in their zone and sometimes on behalf of all zones. As required by Regulation (EU) 545/2011, [51] implementing Regulation (EC) No 1107/2009 as regards the data requirements for PPPs, compulsory data include, among others, agronomic efficacy, toxicological and ecotoxicological data, and residues studies (see Table 2 for a more exhaustive list). As far as it is known, none of the required and necessary tests for acute and chronic effects on aquatic (e.g. algae, *Daphnia* and fish) or terrestrial organisms (e.g. earthworms), have ever been performed for SLs. The procedures for registering and authorizing the use of PPPs are long and laborious, and require the support of many experts; moreover, they are very expensive, due to the high amount of information that the producer must provide to the competent authorities for the assessment and the subsequent authorization of the product. Costs can range from a few hundreds of thousands of euros for low impact products, to a few millions of euros for new complex products.

7.2. Plant Strengtheners (PSs)

Considering the unusual mechanism of action against seeds, SLs could also be used as PSs, which by definition are compounds intended to protect plants against harmful organisms by activating the defence mechanisms of the plant, but also to defend plants against non-parasitic impairments. As PSs are borderline products between fertilizers and PPPs, there are no clear European laws that regulate the registration procedures for those products. Currently those products have different names in different countries, and their use is disciplined under the Reg. (EC) No. 1107/2009 concerning the placing on the market of PPPs, or in the context of the Reg. (EC) No. 2003/2003 relating to fertilisers. [52] Moreover, some Member States issued national legislation to allow the use of these products in agriculture, and in some others countries these products are even under the laws regulating cosmetics and food additives. [53] In cooperation with the Member States the EC Services elaborated a working document on "Data requirements for plant strengtheners with low risk profile" (SANCO/1003/2001 rev. 3), [54] very useful for defining the authorization procedure of PSs. According to it, for PSs with a low risk for humans, animals and the environment, a minimum dossier is required for the first assessment of the product. If the first examination deems it appropriate, further information may be required case by case. However a revision of the Regulation (EC) No. 2003/2003 on fertilizers is in progress, with the objective to extend its scope to other fertilizers including plant biostimulants (see below), and it is desirable that PSs will be included in this latter category.

7.3. Plant Biostimulants (PBs)

PBs are defined as products whose function, when applied to plants or the rhizosphere, is to stimulate natural processes to benefit nutrient uptake, nutrient use efficiency, tolerance to abiotic stress, and crop quality, independently of its nutrient content and not including products with declared and specific plant health function. A recent survey about the regulation of such materials in different countries indicated a considerable discrepancy in the form of placing on the market of such products. For instance, some countries do not foresee any authorization for the placing on the market of PBs, while most require the submission of a detailed dossier including the toxicological and ecotoxicological risk assessment, demonstration of agronomic effectiveness and analytical methods for the characterization. For their inclusion in the new European Regulation, PBs will be subject to an assessment procedure upon the submission of a dossier, to be evaluated by a third party. So in the next future, if registered as PBs at the EU level, SLs would be subjected to an assessment procedure performed by an evaluator body. Stakeholders will submit a dossier identifying the active substance and justifying its effectiveness and harmlessness. However, the procedure for the regulation of a new type of PBs is generally easier than the one for PPPs. Therefore, considerable attention is put in avoiding that a company tries to circumvent the strict rules for the authorization of PPPs, requiring the inclusion of the same product in the law for fertilizers. Pending the entry into force of the new European Regulation for fertilizers, the placing on the market of PBs will remain subject to national legislations, which differ from country to country.

8. Conclusions

The road leading to the practical use of SLs in agriculture seems to be still very long and paved by several barriers that could slow down this process. Conversely, the advent of novel technologies and biotechnologies, the increasing interest and investments by agrochemical companies, and the powerful and supportive results obtained by the scientific community could have very positive effects in shortening the registration procedures. A further encouraging stream toward the registration of SLs for agricultural purposes could be the possible use of these compounds in other applicative fields, e.g. the medical and pharmacological ones, which historically are able to attract many more attentions and research funds. Indeed, some SLs where recently preliminarily and positively evaluated for their anticancer properties. [55]

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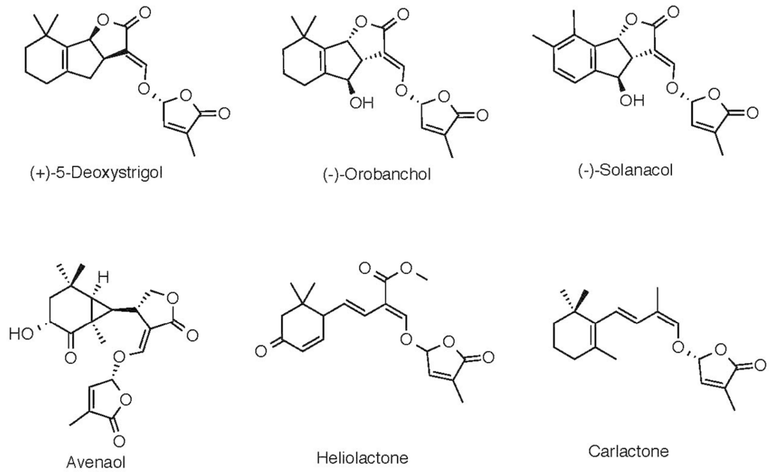

Figure 1
190x253mm (200 x 200 DPI)

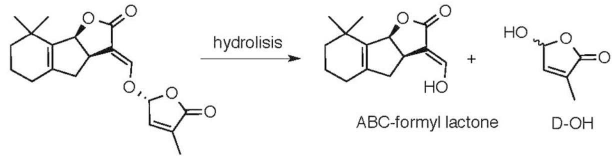

Figure 2
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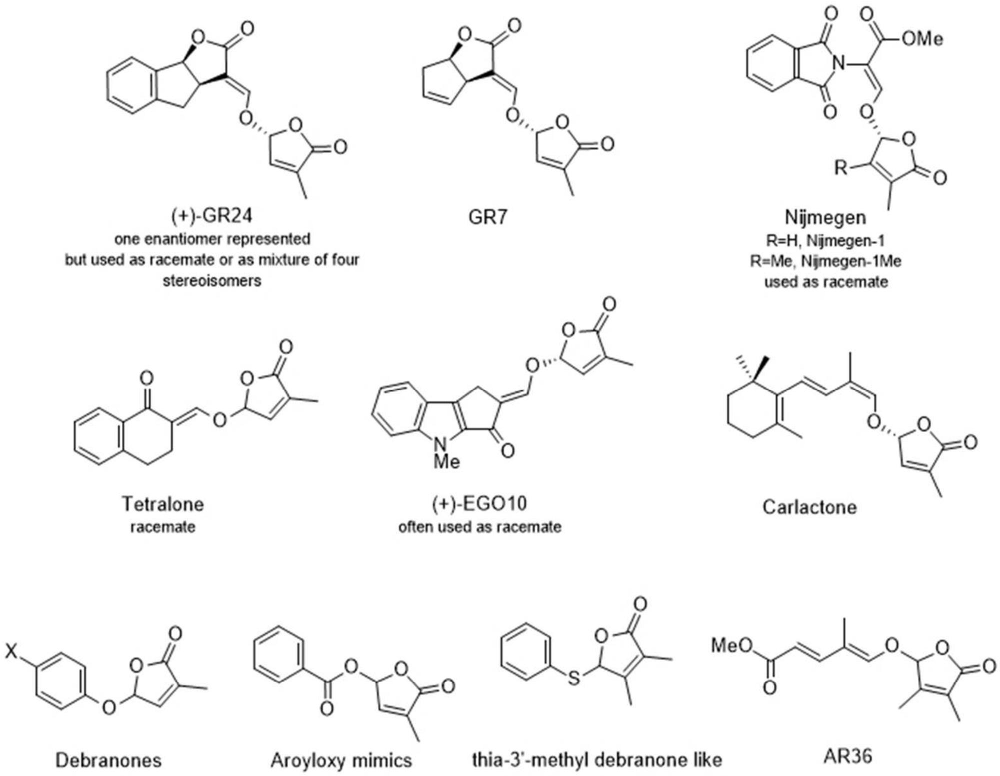

Figure 3
239x186mm (72 x 72 DPI)

Table 1. Categories of products in which the SLs could fall, depending on the supposed use in agriculture

Acronym	Type of Product	Definition	Regulation	Possible use of the SL
PPPs	Plant Protection Products	Products that: - protect plants or plant products against pests/diseases, before or after harvest; - influence the life processes of plants (such as substances influencing their growth, excluding nutrients); - preserve plant products; - destroy or prevent growth of undesired plants or parts of plants.	EC 1107/2009	As phyto-hormones or for suicidal germination
PSs	Plant Strengtheners	Compounds (including microorganisms) intended to protect plants against harmful organisms by activating the defence mechanisms of the plant, but also to defend plants against non-parasitic impairments.	EC 1107/2009, or EC 2003/2003, or national legislations	Favour AM fungi growth
PBs	Plant Biostimulants	Products able to stimulate natural processes to benefit nutrient uptake, nutrient use efficiency, tolerance to abiotic stress, and crop quality, independently of its nutrient content and not including products with declared and specific plant health functions.	A new European Regulation for fertilizers is expected. Currently, regulated at a national level	Direct or indirect (through AM fungi) influence of plant fitness

Table 2. Information required by EC for the registration of PPSs, as explained in the text

- Agronomic efficacy (different trials on different crops) [a]
- Chemical-physical characteristics
- Toxicological data (short, medium and long term) [b]
    - acute oral toxicity
    - acute inhalation toxicity
    - acute dermal toxicity
    - skin irritation
    - eye irritation
    - skin sensitisation
    - dermal absorption
- Data on exposure [c]
    - assessment of exposure of operators, workers, residents and bystanders
- Eco-toxicological data
    - tests on birds and on terrestrial vertebrates other than birds
    - tests on aquatic organisms (e.g. algae, fish, aquatic invertebrates)
    - tests on bees
    - tests on arthropods
    - tests on soil non-target micro-organisms
    - tests on soil macro organisms
- Determination of analytical methods
- Residues studies (different trials on different crops) [d]
- Environmental fate and behaviour (in soil, in water, in air)
- Classification and labelling

[a] The number of trials to be conducted depends mainly on factors such as the properties of the active substance(s) contained, climatic differences, the range of agricultural practices, the uniformity of the crops, the mode of application, the type of harmful organism and the type of PPPs.

[b] For proper evaluation of the toxicity of preparations sufficient information on acute toxicity, irritation and sensitisation of the active substance are needed.

[c] The risks for those using plant protection products depend on the physical, chemical and toxicological properties of the PPP as well as on the route, the degree and duration of exposure.

[d] The number of trials to be conducted depends normally on factors such as climatic differences existing between production areas, differences in production methods, seasons of production, type of formulations, etc.