



## Review article

## Cover crops, hormones and herbicides: Priming an integrated weed management strategy

Maxwel C. Oliveira<sup>a,b</sup>, O. Adewale Osipitan<sup>c,1</sup>, Kevin Begcy<sup>d,1</sup>, Rodrigo Werle<sup>b,\*</sup><sup>a</sup> Department of Agronomy, Western São Paulo University, Presidente Prudente, São Paulo, 19067, Brazil<sup>b</sup> Department of Agronomy, University of Wisconsin-Madison, Madison, WI 53706, United States<sup>c</sup> Department of Plant Sciences, University of California-Davis, Davis, CA, 95616, United States<sup>d</sup> Environmental Horticulture Department, University of Florida, Gainesville, FL 32611, United States

## ARTICLE INFO

## ABSTRACT

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Herbicide weed resistance has been a major issue of conventional global row crop agriculture for decades. Still current strategies and novel technologies available to address weed resistance are mainly herbicide-based. Thus, there is a need for innovative means of integrated weed management strategies. Our approach proposed herein integrates cover crops, plant hormones and pre-emergence (PRE) herbicides as part of weed management programs. Plant hormones such as gibberellic acid ( $GA_3$ ) and abscisic acid (ABA) have the potential to induce seed germination and seed dormancy, respectively. Prior to crop emergence, plant hormones are tank mixed with PRE herbicides and sprayed to cover crop residue. Two strategies are proposed (1) PRE herbicides +  $GA_3$  and (2) PRE herbicide + ABA. The hormones provide different results;  $GA_3$  is likely to stimulate a more uniform weed seed germination, thus enhancing efficacy of PRE herbicides. Conversely, ABA could promote weed seed dormancy, reducing selection pressure and weed infestations until crop canopy closure. Much research is needed to understand the impact of hormones on weed and crop species, optimize products and rates, and compatibility of hormones with herbicides and cover crops. If successful, this approach could open a new opportunity for agricultural business, enhance farming sustainability by reducing dependence on herbicides and minimizing agronomic, economic and environmental issues related to weed resistance.

## 1. Current challenges in weed management

There are over 500 unique cases of herbicide-resistant weeds worldwide [1]. Herbicide weed resistance has been a concern since the introduction of synthetic herbicides for weed management. First reported cases of herbicide resistance (HR) date back from 1950s with a population of climbing dayflower (*Commelina diffusa* Burm. f.) in Hawaii [2] and wild carrot (*Daucus carota* L.) in Canada [3] insensitive to 2,4-D application. In the early 1990s, 47 weed species were documented to have biotypes resistant to one or multiple herbicide site of actions (SOAs) in the United States [4]. The introduction of glyphosate resistant (GR) crops combined with over-reliance on glyphosate post-emergence (POST) reduced herbicide diversity in soybean (*Glycine max* (L.) Merr.) and cotton (*Gossypium hirsutum* L.), which slowed down HR to various SOA but increased glyphosate (EPSPS-inhibitor) resistance [5]. HR represents evolution at its finest [6,7]. For example, waterhemp

(*Amaranthus tuberculatus* (Moq.) Sauer) and Palmer amaranth (*Amaranthus palmeri* S. Watson) are native from midwest and southwest United States and represent the most troublesome weeds in these regions, respectively [8,9]. The combination of species biology [10,11], including genetic diversity (dioecy and high fecundity) [12], small seed size (adapted to no-tillage), extended seed emergence window and competitiveness combined with overreliance on POST herbicides for weed control strongly contributed for adaptation of *A. tuberculatus* and *A. palmeri* to cropping systems in the United States. These two *Amaranthus* species have evolved resistance to eight herbicide SOA [1]; nonetheless, current weed management approaches are still heavily based on herbicides. For instance, the introduction of novel transgenic auxin-resistant crops is likely to increase reliance on the synthetic auxin herbicides 2,4-D and dicamba in POST applications. Also, current recommendations for control of herbicide resistant weeds include herbicide mixtures and the use of pre-emergence (PRE) followed by layered

\* Corresponding author.

E-mail addresses: [maxweloliveira@unoeste.br](mailto:maxweloliveira@unoeste.br) (M.C. Oliveira), [waleos08@yahoo.com](mailto:waleos08@yahoo.com) (O.A. Osipitan), [kbegcy.padilla@ufl.edu](mailto:kbegcy.padilla@ufl.edu) (K. Begcy), [rwerle@wisc.com](mailto:rwerle@wisc.com) (R. Werle).<sup>1</sup> Equal contribution.

POST herbicides (addition of chemicals with soil residual activity to the POST application) with multiple SOAs [13,14].

Synthetic herbicides will continue to be the foundation for weed control in conventional cropping systems. Herbicides are cost effective and provide flexibility for weed management. Moreover, the herbicide industry is again revamping the search for new herbicide SOAs [15]. New herbicide SOAs are needed to increase effective chemical diversity and thus reduce selection pressure on weeds. However, herbicides alone might fail to address current and future weed management challenges which are 1) metabolic resistance [16,17] and 2) multiple herbicide resistance [18]. Metabolic resistance is a major threat for weed management as weeds could evolve resistance to herbicides not commercially available or even discovered yet [6]. Also, metabolic resistance is contributing for rapid evolution of multiple herbicide resistance in weeds. Weed populations with more than four stacked resistance mechanisms have been reported [19,20]. Understanding of complex processes associated with metabolic HR is at its infancy and represents a new frontier in Plant and Weed Science research [21,22]. Therefore, the constantly evolving herbicide resistance arising from rapid adaptation in weed populations combined with the shortage of new SOAs accentuates the need for innovative, integrated and diversified means of management to reduce and postpone further selection of resistance.

Weed management practices are becoming increasingly sophisticated with the use of machine learning and geospatial innovations such as remote sensing and geographic information system technologies [23]. Technologies such as unmanned aerial vehicles (UAV) and robots are being developed for weed management [24]. Although robots are still limited to small scale farming systems, UAV and weed mapping are available and could optimize weed management decisions [25]. While technologies advance to aid weed management, herbicides are and will continue to be fundamental components of weed management in conventional agricultural settings worldwide. Therefore, herein we propose an approach which integrates herbicides with cover crops and selected plant hormones in an attempt to manage weeds while at the soil seedbank. This approach could lead to a reduction of herbicide-resistant weed selection pressure while enhancing overall weed management and agricultural sustainability.

## 2. Cover crops as a weed management strategy

The adoption of cover crops is gaining more attention because of the growing efforts to minimize and manage herbicide resistant weed species, and to ensure sustainable agricultural practices, particularly in no-tillage cropping systems [26–28]. Weed suppression is one of many benefits that could be achieved with the use of cover crops (Fig. 1). A



**Fig. 1.** Cereal rye (*Secale cereale* L.) cover crop as an integrated weed management strategy. Image depicts winter annual weeds thriving (left side of the image) in the absence of an established cereal rye cover crop stand (right side of the image) at an on-farm research study conducted by Werle et al. (2017) near North Platte, NE, United States.

major concern about the use of cover crops is their negative impact on the subsequent cash crop(s) in terms of yield reduction [26]. However, a quantitative summary of published studies has shown that if properly managed, the use of cover crops would not only suppress weeds but could increase the yield of subsequent crops [29]. Cover crops can provide weed suppression between and within growings; first as a living mulch, then as a residue after termination [30]. Weed suppression provided by cover crops could be through allelopathy, superior competition for resources, and physical alteration or impediment.

Allelopathy as a mechanism of weed suppression by cover crops has been widely discussed [31,32]. It has been assumed that certain toxins from cover crops inhibit weed seed germination and seedling growth. Allelopathic effects of cover crops are usually confounded with other mechanisms of weed suppression highlighted above. As a living mulch, cover crops provide weed suppression through competition for essential resources. This competition can greatly inhibit weed seed germination, seedling establishment and growth by reducing light quality, soil moisture and nutrients [33]. For example, quality of light getting to a weed seed and seedling through a cover crop leaf canopy could be greatly reduced due to selective absorption of red light by chlorophyll, thereby reducing the R:FR ratio to the extent of rendering the phytochrome inactive to regulate weed seed germination and seedling growth [34–36]. Ability of cover crops to outcompete weeds and provide suppression varies with cover crop species and residue composition; thus species selection is an important step towards obtaining successful weed suppression via cover crops [37]. Cover crops such as cereal rye (*Secale cereale* L.), common oat (*Avena sativa* L.), triticale (*Triticosecale rimpau* C. Yen & J.L. Yang [*Secale cereale* × *Triticum aestivum*]), wheat (*Triticum aestivum* L.), and hairy vetch (*Vicia villosa* Roth) have been shown to be competitive against weeds [30,34,38,39]. For example, a study showed that radish (*Raphanus sativus* L.) and cereal rye provided greater light interception (0.95 to 0.99 in fraction of soil cover) compared to lower fraction (0.86) provided by white lupin (*Lupinus albus* L.) [33]; this competitive effect could reflect excellent (86–100%) suppression of natural weeds by radish and cereal rye [33,40] compared to poor (42%) weed suppression by white lupin [33]. In another example, despite similar biomass accumulation by Persian clover (*Trifolium resupinatum* L.) and subterranean clover (*Trifolium subterraneum* L.) as cover crops, the former was more competitive against weeds with suppression of chickweed (*Stellaria media* (L.) Vill.) and annual bluegrass (*Poa annua* L.) biomass to 0.8 g m<sup>-2</sup>; compared to increased biomass (38 g m<sup>-2</sup>) of these weed species with the use of the latter [41].

After termination, as residue, cover crops can lead to physical alteration to weed emergence by influencing the environmental conditions at the site of weed seed germination [42,43]. Environmental conditions such as light transmittance, soil moisture and soil temperature have great influence on weed seed germination, and seedling survival. Studies have shown that cover crop residues can influence daily soil temperature amplitude and light transmittance [44] with consequential suppression of weed emergence [42]. The level and duration of weed suppression provided by residues is positively related to the cover crop residue biomass [30]. For example, it was estimated that 3000, 6000 and 10000 kg ha<sup>-1</sup> of cover crop biomass could provide 40, 60 and 80% weed biomass suppression relative to a weedy control, respectively [30]. A high amount and thick layer of cover crop residue not only suppress weed emergence by influencing the light, soil temperature and other environmental conditions, but also cause a physical barrier against upward growth as seedlings could exhaust their energy reserves before overcoming it [45–47].

Cover cropping has shown to be a valuable component of integrated weed management systems. Several studies have shown that integrating cover crops with the use of herbicides can minimize the development and provide better control of troublesome herbicide-resistant weed species [35,48,49].

### 3. Weed priming: a new paradigm in weed management

A common strategy to combat weeds is the application of PRE herbicides, which aim to prevent germinating weeds from establishing. This strategy helps controlling, to some extent, weed over proliferation but alone is not fully effective. To implement effective weed management using PRE herbicides, it is necessary to understand the biology of seed dormancy and germination. Seed dormancy is an essential developmental process during the life cycle of plants. It ensures the activation of hormones and molecular factors that prevent seeds to germinate when environmental conditions are not favorable [50]. During domestication, selection of crops with reduced levels of seed dormancy to guarantee homogeneous and higher germination rates have been prioritized. However, unlike domesticated crops, weed species show asynchronous and sometimes extended patterns of germination making weed management systems difficult to implement [51].

Induction of synchronous weed germination stimulants which we are going to refer as **weed priming**, could be a strategy to enhance weed management with PRE herbicides. In weeds, similarly as in crop plants, ABA and GA<sub>3</sub> are the major hormones that antagonistically control seed dormancy and germination [52]. At the molecular level, reciprocal transcriptional regulation of their metabolic genes controls the ABA/GA<sub>3</sub> ratio (Fig. 2). While ABA is the primary inducer of seed dormancy, GA<sub>3</sub> is the principal promoter of seed germination [53]. Recent findings suggest the collaborative activity of auxin with ABA in maintaining the repressed stage of seed dormancy [53]. Prior to seed imbibition (phase I), ABA and auxins repress germination preventing developmental progression and metabolic activation (phase II). Seed imbibition triggers a variety of biochemical and cellular events associated to DNA repair, increased levels of GA<sub>3</sub> and ethylene, and the active translation of newly synthesized mRNAs [54]. Previous studies showed that ethylene potentialized the effect of GA<sub>3</sub> while repressing ABA, suggesting that ethylene is involved in the regulation of seed germination and dormancy by increasing the seed germination rate [55,56]. After metabolic activation, the genetic

and transcriptional machinery is in place to induce radicle emerge (Phase III) and seed establishment. Exogenous application of phytohormones involved in the stimulus of seed germination combined with PRE herbicides might expedite and shorten the germination window of weed species allowing for better efficacy of PRE herbicides. Chemical or natural compounds with high levels of GA<sub>3</sub> and/or ethylene can prime weed germination providing synchronous emergence.

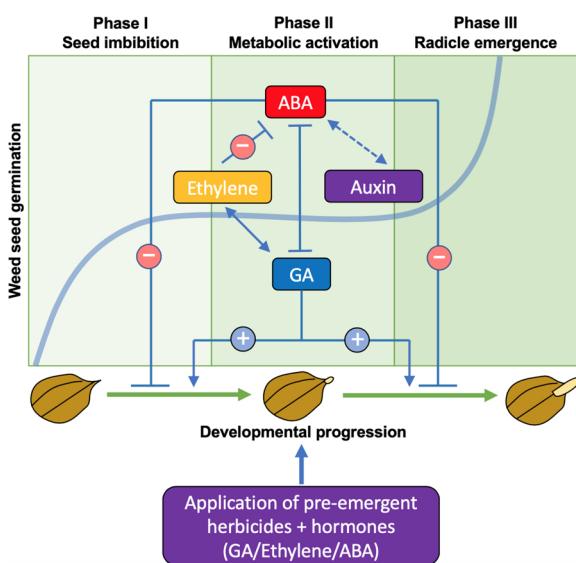
Most studies exploring the effect of hormones on weeds have been performed in laboratory conditions [57–60]. However, studies performed at the field scale have shown promising results. For instance, Stevens et al. [61] evaluated germination of nine agricultural weed species (*Arctotheca calendula* (L.) Levyns [Asteraceae], *Avena fatua* L. [Poaceae], *B. tournefortii* Gouan [Brassicaceae], *Bromus diandrus* Roth [Poaceae], *Echium plantagineum* L. [Boraginaceae], *Hordeum leporinum* Link [Poaceae], *Lolium rigidum* Gaudin [Poaceae], *Raphanus raphanistrum* L. [Brassicaceae] and *Sisymbrium orientale* L. [Brassicaceae]) treated with GA<sub>3</sub> and butanolide in laboratory condituiions [61]; for three of the species (*B. tournefortii*, *R. raphanistrum*, and *H. leporinum*) both compounds promoted similar germination, whereas the germination rate of the remaining six weed species was further enhanced with GA<sub>3</sub>. In addition, they also tested butanolide (2-20 g ha<sup>-1</sup>) under field conditions and reported increased germination rates of *A. fatua*, *A. calendula*, *B. tournefortii* and *R. raphanistrum*. While Stevens et al. [61] did not evaluate GA<sub>3</sub> under field conditions (butanolide only), results from their lab studies further warrant field evaluations of GA<sub>3</sub>. Even though additional studies are needed to confirm the applicability at the field scale, current evidence, while not conclusive, still provides a first step to understand the efficacy of using a combination of hormones and PRE herbicides as a potential integrated weed management strategy.

While the economic return of such strategy remains uncertain, GA<sub>3</sub> products registered for use as plant growth regulators in horticultural and agronomic crops are commercially available (e.g., Activol®, Berelex®, RyzUp®, RyzUp SmartGrass® are GA<sub>3</sub> based products commercialized by Valent BioSciences LLC, Libertyville, IL, USA) and we speculate it would become affordable if adopted at optimum biological rates on a large-scale.

Further dosage experiments will be necessary to determine the best ratio of application of GA<sub>3</sub> combined with PRE herbicides. Similarly, the application of GA<sub>3</sub> related compounds might effectively induce weed priming. The use of combinatorial PRE herbicides and plant growth regulators such as products with GA<sub>3</sub> as active ingredient could enhance germination inducing a homogeneous emergence of weeds species propitiating and potentializing the effect of PRE herbicides. Conversely, instead of promoting germination, PRE herbicides could be combined with repressors of germination, for instance, ABA. In *Arabidopsis*, it has been shown that a sulfonamide compound, quinabactin, induces ABA-like responses [62,63]. Even though quinabactin has been used to protect plants against drought stress, it could be used with PRE herbicides to decrease germination. Quinabactin activates ABA receptors which trigger transcriptional and signaling ABA-like responses.

### 4. Novel integrated weed management approach

Integrating cover cropping with PRE herbicide in a hormone induced weed emergence pattern is an innovative approach that would ensure early and effective control of weed species with reduced reliance for POST herbicides and other POST weed control strategies. Herein, we propose a novel approach towards integrated weed management in annual cropping systems using *Amaranthus* spp. as a target weed species and soybean as the target crop species. Given their biological attributes (e.g., small weed size, extended emergence window, and widespread resistance to herbicides), *Amaranthus* spp. have become the main species driving weed control decisions within agricultural communities in the Midwest and Midsouth United States and beyond [8,19,51]. Research has indicated that from an yield potential standpoint, soybeans can be compatible with properly managed cereal rye cover crop thus an ideal opportunity for the proposed system [29].



**Fig. 2.** Weed priming strategy to induce synchronous germination. Hormones regulate three stages of germination: seed imbibition (phase I), metabolic activation (phase II) and radicle emergence (phase III). ABA and GA<sub>3</sub> antagonistically inhibit and promote seed germination, respectively. Hormone crosstalk involving other hormones including ethylene and auxin accentuate and facilitate the regulation during seed germination. Application of hormones (GA/ethylene) together with pre-emergence (PRE) herbicides might speed up asynchronous weed germination allowing an effective action of herbicides. Alternatively, ABA-like compounds could be used to prevent germination. Continuous lines represent confirmed evidences. Dotted lines represent potential interactions.



**Fig. 3.** Established soybean crop amidst a stand of chemically terminated cereal rye (*Secale cereale* L.) cover crop residue at the University of Nebraska-Lincoln West Central Research and Extension Center, North Platte, NE, and at the University of Wisconsin-Madison Arlington Agricultural Research Station, Arlington, WI, United States. Combined with PRE-emergence herbicide and seed germination hormones, this would represent the “Priming Integrated Weed Management Strategy” proposed herein. a) Planting cereal rye. b) Planting soybean into cereal rye. c) Soybean growing into cereal rye. d) Soybean growing into cereal rye residue.

First, cereal rye, a popular cover crop species amongst United States’ farmers, is established in the fall prior to soybean cultivation. Secondly, soybeans are planted into cover crops (Fig. 3). Thirdly, cover crops are chemically terminated when enough biomass is produced to achieve effective levels of weed suppression ( $> 3000 \text{ kg ha}^{-1}$ ) [64]. For chemical cover crop termination, glyphosate is tank-mixed with herbicides containing soil residual activity (e.g., acetolactate synthase (ALS)-, long-chain fatty acids (LCFA), microtubule assembly, photosystem II or protoporphyrinogen oxidase (PPO)-inhibitors), and hormones (ABA or GA, seed primers). By promoting an unfavorable environment for weed establishment (presence of cover crop residue) combined with GA<sub>3</sub> to stimulate germination at the time PRE herbicides are available at the highest concentration in the soil will likely increase the likelihood of successful control of *Amaranthus* spp. and thus reduce the reliance on POST control strategies. GA<sub>3</sub> could potentially enhance crop establishment expediting canopy closure further suppressing weeds. Another approach is to combine PRE herbicides with ABA, a hormone that stimulates seed dormancy. Reducing weed germination is likely to reduce herbicide selection pressure and/or herbicide failure. Also, adding cover crops + ABA is likely to promote enhanced microbial activity that could lead to enhanced weed seed decomposition over time. Primarily, the impact of ABA on cultivated and non cultivated seeds should be investigated. Nonetheless, an integrated weed management approach with ABA could potentially increase sustainability by maintaining both crop and weed species in the agroecosystem.

Despite promising results under laboratory conditions [57,65], the fate and effectiveness of hormones under field conditions is not fully understood, particularly when combined with PRE herbicides and the presence of cover crop residue. Thus, this is a topic that truly warrants future research. Keeping a low infestation of the soil seedbank should be the main goal of any weed management program, particularly in an era where HR has become a major concern and threat to sustainable,

practical, and economical agricultural production. If successful, the approach proposed herein could enhance control of *Amaranthus* spp. while reducing reliance on POST weed control strategies and simultaneously increasing the sustainability of soybean production systems in the United States and beyond by leading to higher adoption of soil health conservative and less chemically dependent weed management strategies.

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